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THE DEVELOPMENT AND GROWTH OF *CIONA*

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(Text-figs. 1—3)

Ciona intestinalis (L.) is probably the most cosmopolitan species of ascidians and has long been of general interest. The adult morphology has been well described in monographic form by Roule (1884), the physiology of the heart and circulation by Heine (1902), Enriques (1904) and Wolf (1932), of the nervous system by Magnus (1902), Hecht (1918, 1926), Cate (1928), Haffner (1933), and Bacq & Florkin (1935), and of the digestive system by Yonge (1925). Developmental studies include that of the early embryology by Conklin (1905), problems of fertilization by Morgan (1945) and Damas (1899, 1900). In no work, however, has there been a presentation of the entire *Ciona* organism from the tadpole stage through the critical post-larval stages to the young cionid ascidian. The present account portrays this period of development, together with a discussion of some significant but relatively obscure aspects of adult structure.

EGGS AND THE REARING OF *CIONA INTESTINALIS*

While ascidians in general are difficult to rear to maturity under laboratory conditions, *Ciona* is relatively easy, and together with *Botryllus schlosseri* (Pallas) and *Diplosoma gelatinosum* (M.-Edw.) is liable to appear more or less spontaneously in large aquaria into which tadpoles may have been brought. Artificial fertilization is readily accomplished, and at almost any time of the year, since *Ciona* is sexually mature above a certain size and reproduction is seasonal only to the extent of the rhythm of the growth cycle. Normally eggs are set free spontaneously at dawn, although individuals kept in the laboratory may accumulate eggs and the oviduct become swollen. Eggs in good condition can usually be obtained from such forms at least for 2 or 3 days. In relatively young *Cionas*, with sexual maturity recently attained, eggs are greenish or yellow-green when viewed *en masse* in the oviduct. Later in the season a reddish tinge appears. The significance is not known.

Cionas usually are completely cross-fertile, the degree of self-fertility or sterility being more variable and the subject of extended investigations by Morgan (1945). Eggs sink in still water, but the buoyant 'floats' or outer follicle cells keep them in suspension with the slightest agitation. After fertilization the essential requirements for normal embryonic development are

the complete removal of excess sperm and oviductal fluid, by washing with fresh sea water, and above all, as Morgan (1945) has demonstrated, the use of glassware chemically and organically clean. Batches of abnormally developing *Ciona* embryo in apparently clean vessels are notoriously common. He has shown that abnormality is definitely due to a contaminating agent, traces of either cleaning fluid or organic and bacterial substances, and that washing in sea water and subsequent autoclaving is the best preventive procedure.

At usual room temperatures from 16 to 20° C., the eggs develop to the tadpole stage in about 24 hr. Hatching is effected by means of a proteolytic enzyme, the activity of which is inhibited below a pH of 7.0 (Berrill, 1929). Follicle cells and membranes may be artificially removed by crab-stomach juice diluted with sea water (about 1 part of 50) without significant damage to the fertilized egg or developing embryo (Berrill, 1932), and have been so removed by Morgan for experimental purposes.

The rearing of metamorphosed individuals to maturity is possible if a relatively large volume of water is employed. *Cionas* grow readily in an inverted bell jar with plunger, with the diatom *Nitzschia* used as the basic food, and a dark paper shield used to control the amount of light and accordingly the density of the diatom culture. The usual nutrient salts are added from time to time to maintain the culture.

TADPOLE LARVAE AND METAMORPHOSIS

The small tadpole larvae (Fig. 1 A) are at first positively heliotropic and later negatively heliotropic and positively geotropic, the average free-swimming period being 12 hr. or more. The structure is comparatively simple, and the tadpoles have a relatively elongated trunk. The general structure of the tadpole and the process of metamorphosis have been well described by Willey (1893). His observations are mainly confirmed, including the frequent though not invariable secretion of a gas bubble at the anterior end of the attachment area, causing individuals to float towards the water surface, a phenomenon that accounts for the profusion of *Ciona* frequently found attached to the undersides of ships and floating warp, buoys, etc. Some additional detail is available, especially with regard to the sensory vesicle and tail. Willey's and the earlier accounts paid little attention to cellular constitution, and it is of some interest to bring information on *Ciona* into conformity with what is known concerning tadpoles of other species that have received more recent attention. The nature of the otolith as a single-celled organ has been recognized from the first, but the ocellus has been assigned an indefinite and excessive number of both retinal and lens cells. Actually, there are three lens cells with more or less spherical lenses, surrounded proximally by pigment cells in the form of a cup, into which extend the distal parts of eight or nine receptor cells as seen in optical section. What is significant is that this constitution of the sensory

vesicle, otolith and ocellus together, is the standard equipment of the great majority of ascidian tadpoles no matter how elaborate they may be in other ways. In the same manner the tail shows the generalized ascidian condition, namely, a central notochord consisting of yolk-containing vacuolated cells,

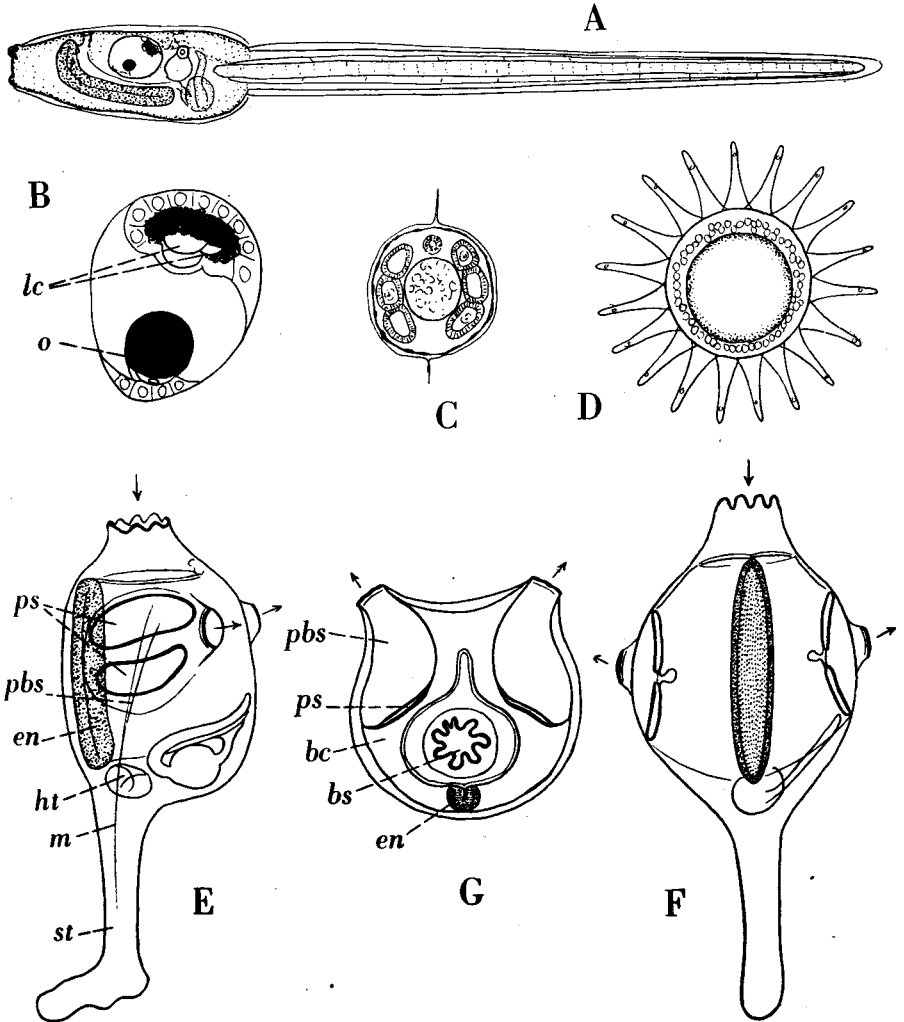


Fig. 1. *Ciona intestinalis*. A, tadpole larva. B, sensory vesicle of tadpole with unicellular otolith and ocellus with three lens cells. C, cross-section of tail showing central notochord, dorsal neural tube, and three muscle cells on each side. D, egg with characteristic 'floats' or outer follicle cells resting on membrane, and inner continuous layer of 'test' or inner follicle cells. E, first ascidian stage, with functional systems, from left side. F, the same from ventral or back side. G, the same from anterior or upper side. bc, branchial chamber; bs, branchial siphon; en, endostyle; ht, heart; lc, lens cells; m, longitudinal muscle; o, otolith; pbs, peribranchial sac; ps, protostigmata; st, stalk; ingoing arrow denotes the branchial siphon, outgoing arrows the two peribranchial siphons.

about forty in number, arranged in a single row, with a band of muscle tissue on each side, each consisting of three cells in cross-section, the cells having a central endoplasmic region containing the nucleus, and a cortical fibrillated contractile zone. The origin of the primary gill slits and the rotation of the main body axis during metamorphosis have been described in detail by Willey. The region between the endostyle and the anterior tip of the tadpole bearing the three adhesive papillae undergoes a marked extension, with a relative growth of the antero-dorsal epidermis. What Willey calls the pre-oral lobe becomes the stalk of attachment. It consists of epidermis, and the bilateral origin of the loose mesenchyme cells contained within it hardly justifies Willey's interpretation of them as homologues of pre-oral coelomic diverticula.

THE FUNCTIONAL ASCIDIAN

The completely metamorphosed individual has a beating heart, which shows the characteristic rhythmical reversal from the first, a pair of active protostigmata on each side, and a contractile longitudinal muscle extending from the trunk into the stalk. This stage has been previously illustrated, both by Willey (1893) and Berrill (1929), but its significance has been underestimated. It may well represent a primitive stage in ascidian evolution, no longer sexually mature, but retaining two features that later become greatly modified. One of these, the existence of protostigmata in place of the rows of definitive stigmata characteristic of the larger individuals, has long been the subject of description and speculation, the main emphasis having been upon the mode of origin of new protostigmata, and interpretation of tongue bars in an effort to establish homology with *Amphioxus* and *Balanoglossus*. The stage presents greater interest when the correlation of protostigmata with the persistence of the paired peribranchial siphons is recognized. The number of protostigmata on each side slowly increases with growth and extension of the branchial and peribranchial sacs until six are attained. This is a prolonged developmental phase that terminates more or less suddenly as a critical size is reached. During a relatively short developmental period, two changes occur together to give the typical ascidian condition. The six protostigmata each divide, first into two, then four, and finally eight definitive stigmata, while the pair of peribranchial siphons fuse to become the single mid-dorsal atrial siphon. On the one hand lateral peribranchial siphons and protostigmata are definitely correlated, and on the other rows of definitive stigmata and a single atrial siphon. A similar condition occurs in species of *Ascidia*, *Phallusia*, *Ascidiella*, *Corella* and *Diazona*.

Other changes take place during the growth period of what may be called the first ascidian form. While the number of protostigmata increases, the number of longitudinal muscle strands also increase from one to six or seven, the strands thereafter only increasing in individual thickness and not in number.

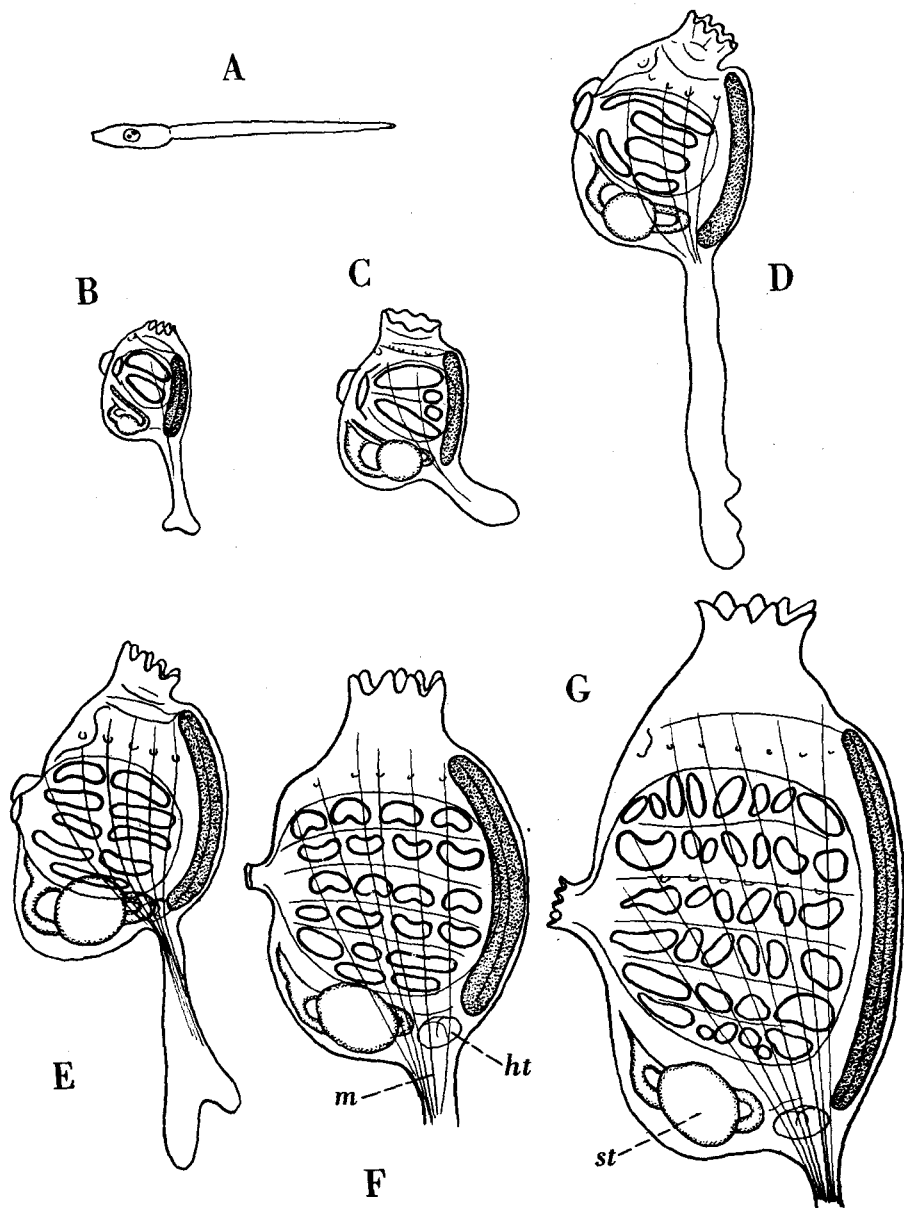


Fig. 2. *Ciona intestinalis*. A, tadpole drawn to same scale as remaining figures. B, C, D, three stages in growth of first ascidian stage with paired peribranchial siphons. E, F, G, three stages showing transition from first ascidian stage to second stage with single median atrial siphon, six rows of definitive stigmata, and final number of muscle bands. *ht*, heart; *m*, muscle bands; *st*, stomach.

HEART, EPICARDIUM AND PYLORIC GLAND

The heart develops, according to Damas (1899), as a mass of mesenchyme cells situated near the ventral endoderm and not derived from it, as thought by Willey. A pericardial vesicle is formed, one side of which invaginates to form the contractile tissue or heart proper. Willey concludes that the tunicate heart is not homologous with that of vertebrates since *Amphioxus* lacks a heart and pericardium, an assumption of a phyletic sequence not necessarily valid. Whatever the relationship may be, it is significant that the heart opens anteriorly into the subendostylar blood vessel, possibly homologous with and at least equivalent in position to the ventral aorta of other chordates, and that posteriorly it connects with vessels distributed over the alimentary canal. When first active it occupies the space between the bend of the intestine and the base of the endostyle. The heart itself, as in all tunicates, is structurally peculiar, apart from the characteristic rhythmical reversal. The blood flows, not through a true cardiac tube, but along a deep longitudinal invagination of the pericardial wall, a functional canal being formed by approximation of the lateral lips to form the 'raphe' of the heart.

With growth of the individual, and noticeable during the first ascidian stage, the heart and pericardium grow not only in absolute size but relatively to the distance between the intestine and endostyle base. The nature of this growth is complex and in it may lie the answer to the question, whether the cionid visceral topography is primitive or derived from the diazonid. Growth occurs from the middle region with the relative position of the two ends of the heart remaining unaffected. The posterior side of the pericardium grows ventrolaterally in such a way that the invaginated cardiac canal is drawn out as a V-shaped tube. It may be more correct to say that the cardiac tube extends between two fixed points and accordingly inevitably develops a V-shaped flexure, and that the pericardial wall necessarily keeps pace with its growth. On the other hand, the anterior wall of the pericardium grows no more than the distance between the intestine and endostyle, so that actually only the cardiac invagination becomes V-shaped.

The epicardia or perivisceral sacs have been variously interpreted. Their development is best described by Damas (1899). They appear during the first ascidian stage as broad posterior extensions of the pharynx, one on each side, enveloping the digestive canal and heart. As growth proceeds they extend posterior to the viscera to form large perivisceral cavities, one enveloping the heart, intestine, right side of the stomach, and the developing gonad, and the other mainly the left side of the stomach. They effectively form a pair of visceral body cavities, with the visceral organs suspended from one another or from the body wall by mesenteries consisting of two epicardial epithelia with enclosed mesenchymal tissue. The openings to the pharynx become narrower but remain as relatively large communications, and there can be no doubt of

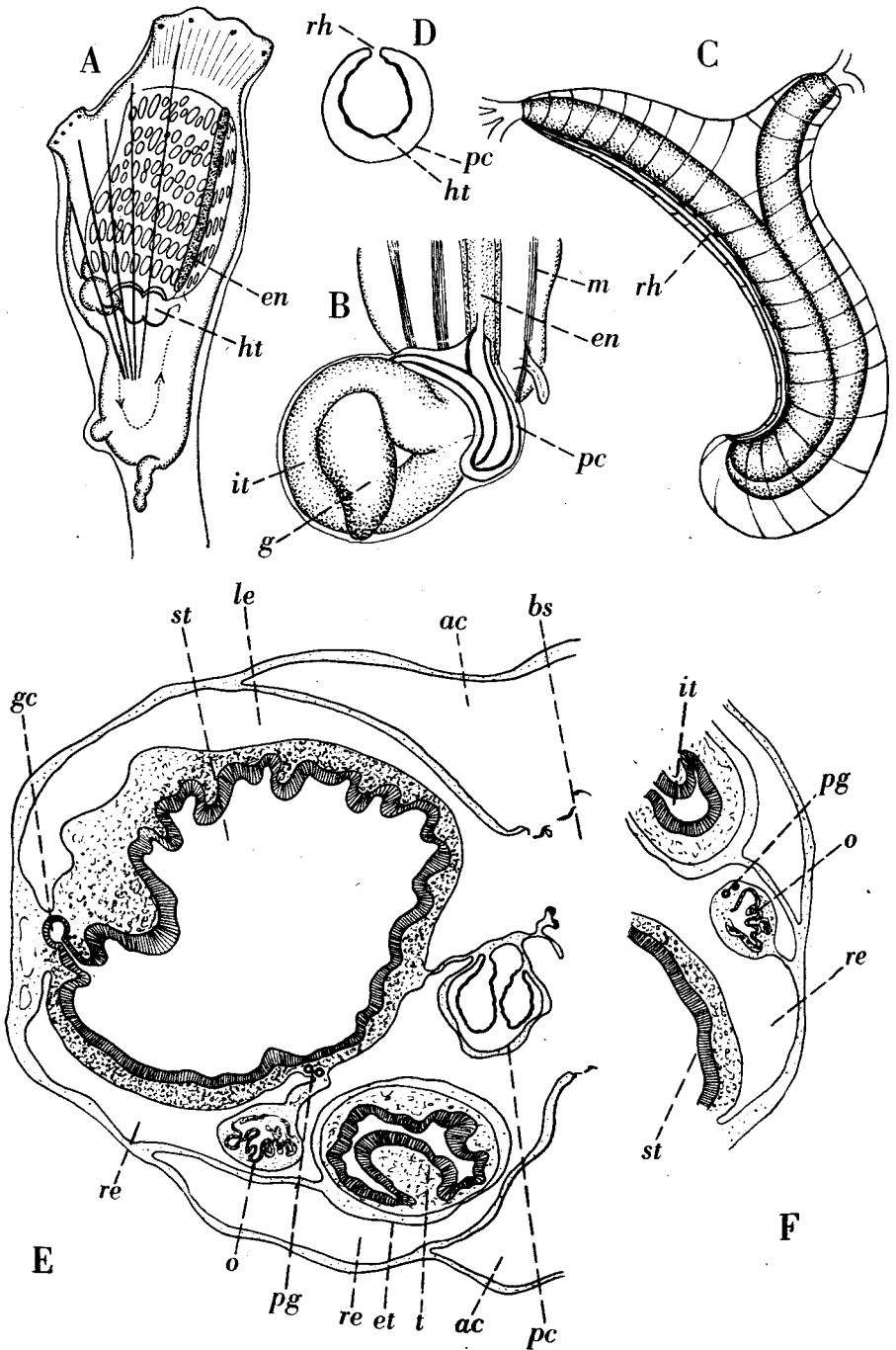


Fig. 3.

an exchange of fluid. Roule (1884) looked upon the perivisceral cavities as primitive but corresponding to the primary blastocoelic cavity of the larva. Newstead (1893) and Garstang (1928) recognize the homology with the epicardium of the other forms as suggested by Van Beneden & Julin (1886) but regard the latter condition the more primitive and the epicardium primarily a budding organ. Kupffer's original interpretation (1870) of the epicardial sacs as homologues of the vertebrate coelom, even though based on a faulty conception of their connexions, has never received adequate consideration and a strong case can be made out for this point of view.

Two other visceral structures are of some interest, the pyloric gland and the gastric caecum. Ascidians in general, together with the Thaliaceans, have a so-called pyloric gland of completely obscure function, arising as a single or bifurcating duct from the wall of the stomach near its junction with the intestine, which extends to and ramifies over the wall of the adjacent loop of the intestine as a system of fine canals. If the intestinal distribution is functional and not merely of topographic convenience, it suggests some sort of recovery process from the wall of the intestine into the cavity of the stomach. In any event, in view of its virtual universal occurrence among Tunicates, the absence of any description or illustration in Roule's minutely detailed and accurate monograph raised the question whether it did exist in *Ciona*, a point of some importance if *Ciona* should appear to be an ascidian prototype. Willey illustrates a young *Ciona* just after metamorphosis in which a typical pyloric gland appears, but no gland is discernible in a dissected adult. Sections of a 10 mm. individual, however, demonstrate its presence and the reason for its obscurity in the adult condition. The ducts leading from the stomach to the intestine are caught between two opposing sheets of the epicardium and are confined within the epicardial mesentery joining the stomach and the ovary, and the ovary and the intestine. The ducts are forced down into the tissue surrounding the ovarian epithelium and in certain sections appear to be structures isolated as part of the gonad itself. Willey calls the pyloric gland the caecum and concludes that the pyloric gland of ascidians and the hepatic caecum of *Amphioxus* are homologous. This may or may not be, but there is another diverticulum of the stomach wall usually and more accurately called

Fig. 3. *Ciona intestinalis*. A, later stage showing subdivision of six primary rows of definitive stigmata into double rows, relative growth of heart, and course of circulation in stalk. B, posterior part of mature *Ciona* removed from tunic, showing typical V-shaped heart and its relationship with the endostyle and intestine. C, enlarged view of heart and pericardium, showing V-shaped heart within nondivided pericardium. D, cross-section through heart near one end showing involution of heart within pericardium, and 'raphe' of heart. E, entire longitudinal section through posterior region of 10 mm. individual. F, partial section of same, several sections from E, showing passage of ducts of pyloric gland from stomach wall through gonad mesentery toward intestine. *ac*, atrial chamber; *bs*, branchial sac; *en*, endostyle; *et*, epicardial lining enveloping intestine; *g*, gonad; *gc*, gastric caecum; *ht*, heart; *it*, intestine; *le*, left epicardial cavity; *m*, muscle band; *o*, ovarian epithelium; *pc*, pericardium enveloped by epicardium; *pg*, ducts of pyloric gland; *re*, right epicardial cavity; *rh*, raphe of heart; *st*, stomach; *t*, typhlosole.

the gastric caecum that has an equal claim. It is shown in the post-larval stages of *Corella parallelogramma* (O. F. Müll.) figured by Hüss (1924), and can be seen in sectioned material of *Ciona* in young individuals as a tubular outgrowth from the posterior wall of the stomach, ending blindly as a small round sac. Its function is unknown and its homologies equally obscure.

During the course of later development of *Ciona intestinalis* f. *typica*, the post-abdominal stalk dwindles and individuals are attached by the base of the abdominal region. In the other two varieties, *longissima* and *gelatinosa*, the stalk survives, merely becoming relatively wide and, according to Arnbach & Brien (1932), is essentially equivalent to the post-abdomen of polyclinids, with the longitudinal body muscles extending to its tip and the epicardium of the left side extending into it between the afferent and efferent blood sinuses.

REFERENCES

- ARNBACH-CHRISTIE-LINDE, A. & BRIEN, P., 1932. Remarques au sujet du postabdomen des Cionidae. *Ann. Soc. Zool. Belg.*, T. LXIII, pp. 41-53.
- BACQ, Z. M. & FLORKIN, M., 1935. Mise en évidence, dans le complexe 'ganglion nerveux glande neurale' d'une ascidie (*Ciona intestinalis*), des principes pharmacologiquement analogues à ceux du lobe postérieur de l'hypophyse des vertébrés. *Arch. Internat. Physiol.*, T. XL, pp. 422-8.
- VAN BENEDEN, E. & JULIN, C., 1886. Recherches sur la Morphologie des Tuniciers. *Arch. Biol.*, T. VI, p. 237-476.
- BERRILL, N. J., 1929. Studies in tunicate development. Pt. I. General physiology of development of simple ascidians. *Phil. Trans. Roy. Soc. Lond.*, B, Vol. CCXVIII, pp. 37-78.
- 1932. Mosaic development in the Ascidian egg. *Biol. Bull.*, Vol. LXIII, pp. 381-6.
- CATE, J. T., 1928. Contribution à la question de la fonction du système nerveux de *Ciona intestinalis*. *Arch. Néerland Sci. La Haye*, T. XIII, pp. 391-401.
- CONKLIN, E. G., 1905. The organization and cell lineage of the ascidian egg. *Journ. Acad. Nat. Sci. Philadelphia* (2), Vol. XIII, pp. 1-119.
- DAMAS, D., 1899. Les formations épicaudiques chez *Ciona intestinalis* (L.). *Arch. Biol.*, T. XVII (1901), pp. 1-25.
- 1900. Étude du sac branchial chez *Ciona intestinalis* (L.). *Arch. Biol.*, T. XXXVI (1901), pp. 1-32.
- ENRIQUES, P., 1904. Della Circolazione sanguigna nei Tunicati (*Ciona intestinalis*). *Arch. Zool. Ital.*, Vol. II, pp. 11-17.
- GARSTANG, W., 1928. The morphology of the Tunicata, and its bearings on the phylogeny of the Chordata. *Quart. Journ. Micr. Sci.*, Vol. LXXII, pp. 51-187.
- HAFFNER, K. VON, 1933. Die überzähligen Siphonen und Zellen von *Ciona intestinalis* (L.). *Zeitschr. wiss. Zool.*, A, Bd. CXLIII, pp. 15-52.
- HECHT, S., 1918. The physiology of *Ascidia atra* Leseur. *Journ. Exp. Zool. Philadelphia*, Vol. XXV, pp. 229-99.
- 1926. The effect of exposure period and temperature on the photosensory period of *Ciona*. *Journ. Gen. Physiol.*, Vol. VIII, pp. 291-301.
- HEINE, P., 1902. Untersuchungen über den Bau und die Entwicklung des Herzens der Salpen und der *Ciona intestinalis*. *Zeitschr. wiss. Zool.*, Bd. LXXIII, pp. 429-95.

- HÜUS, J., 1924. Genital Organe und 'ganglio-genital Strang' bei *Corella parallelogramma* Mull. *Nord. Skr. Selsk. Kristianna*, No. 19, pp. 1-50.
- JUST, E. E., 1934. On the rearing of *Ciona intestinalis* under laboratory conditions to sexual maturity. *Yearb. Carnegie Inst. Washington*, 1933, 270 pp.
- KUPFFER, C. W., 1870. Die Stammverwandschaft zwischen Ascidiern und Wirbelthieren. Nach Untersuchungen über die Entwicklung der *Ascidia canina* (Zool. dan.) = *Ciona*. *Arch. mikr. Anat.*, Vol. VI, pp. 115-72.
- MAGNUS, R., 1902. Die Bedeutung des Ganglions bei *Ciona intestinalis*. *Mitt. Zool. Stat. Neapel.*, Bd. xv, pp. 483-6.
- MORGAN, T. H., 1938-42. The genetic and the physiological problems of self-sterility in *Ciona*. I-IV. *Journ. Exp. Zool.*, Vol. LXXVIII, pp. 271-334; Vol. LXXX, pp. 19-80; Vol. XC, pp. 199-228.
- 1945. The conditions that lead to normal or abnormal development of *Ciona*. *Biol. Bull.*, Vol. LXXXIII, pp. 50-62.
- NEWSTEAD, A. H. L., 1893. On the perivisceral cavity of *Ciona*. *Quart. Journ. Micr. Sci.*, Vol. xxxv, pp. 119-28.
- ROULE, L., 1884. Recherches sur les Ascidies simples. *Ann. Mus. Hist. Nat. Marseilles*, Zool., Vol. II, pp. 7-270.
- WILLEY, A., 1893. Studies on the Protochordata. I. On the origin of the branchial stigmata, preoral lobe, endostyle, atrial cavities, etc., in *Ciona intestinalis*, L., with remarks on *Clavelina lepadiformis*. *Quart. Journ. Micr. Sci.*, Vol. xxxiv, pp. 317-60.
- WOLF, E., 1932. Pulsation frequency of the advisceral and abvisceral heartbeat of *Ciona intestinalis* in relation to temperature. *Journ. Gen. Physiol.*, Vol. xvi, pp. 89-98.
- YONGE, C. M., 1925. Studies on the comparative physiology of digestion. III. Secretion, digestion and assimilation in the gut of *Ciona intestinalis*. *Brit. Journ. Exp. Biol.*, Vol. II, pp. 373-88.