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STUDIES ON THE ONTOGENY OF *TRITONIA HOMBERGI* CUVIER (GASTROPODA OPISTHOBRANCHIA)

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CONTENTS

Tritonia hombergi Cuvier is the largest British nudibranchiate mollusc; it is always found in association with the colonial cnidarian *Alcyonium digitatum*, in depths from 6 to 38 fm.

Cleavage and development up to gastrulation resemble the pattern described for other nudibranchs. The heavily yolked stereoblastulae gastrulate by epiboly. As the veliger form is assumed,

a slow movement of the anal cells (which predict the site of the proctodaeal invagination) represents the sole vestige of the morphogenetic processes of gastropod torsion and visceral flexure. This movement is completed before the differentiation of any recognizable muscle elements or of the organs of the visceral mass.

Hatching occurs 36 to 38 days (at 10 °C) after oviposition; the lecithotrophic larvae are at first negatively geotactic but after 1 or 2 days this is reversed and searching behaviour begins. Searching larvae could be induced to metamorphose only if a live healthy colony of *A. digitatum* was provided. The shell and operculum are cast, the velar lobes resorbed, and reflexion and differential growth of the mantle fold bring about a movement of the anal complex which reverses the embryonic torsion and visceral flexure. The adult dorsal integument is derived from the layer of the mantle fold which lined the embryonic mantle cavity. It is concluded that the tritoniid nudibranchs probably arose via stages in which the ancestral shell was enclosed by folds of the mantle. Both right and left embryonic midgut diverticula contribute to the formation of the adult digestive gland. Larval metamorphosis involves also concentration of the main ganglia and the enlargement of the rudiments of the definitive kidney and pericardium, while the larval retractor muscle and the metapodial gland begin to atrophy.

The young benthic stages browse on the tissues of the cnidarian. Thousands were reared in the laboratory for several months until, when some had reached a length of 3 cm, lack of sufficient food prevented their being cultured further. Shortly after metamorphosis, the histogenesis of the adult skin begins, the pleural ganglia make their first appearance, and the original embryonic otoliths are augmented. The larval kidney disappears and its functions are taken over by the definitive kidney. The ventricle and the auricles develop in the pericardium and, as the pallial branchiae are formed, the adult circulation of the blood is established. Changes in the radular dentition illustrate the taxonomic identity of *T. hombergi* Cuvier with *T. alba* Alder and Hancock. The primordia of the reproductive system are first detectable in post-larvae $1\frac{3}{4}$ mm in length.

The benthic stages feed exclusively on *Alcyonium*. Previous accounts of the feeding mechanism were incorrect in attributing to the jaws the function merely of prehension and in stating that the food is torn up by the radula. Digestion begins in the stomach after only a few minutes; the cnidarian spicules fall to the stomach floor from whence they are carried by cilia into the hindgut. In the juvenile *T. hombergi* partially digested matter is sucked into the lobules of the digestive gland by passive dilation following pulsating contractions of their muscle-sheaths; probably a similar mechanism exists in the adult tritoniid. In young animals absorption of the products of digestion, both particulate and liquid, takes place through the walls of the stomach, hindgut, and digestive gland. In older stages, particulate matter is taken up solely by the digestive gland, but fluids may be absorbed also through the stomach wall. Experiments with young individuals (in length 8 to 12 mm) at 15 °C showed that, while individual digestive cells pass through a sequence of changes, the complete digestive gland acts arhythmically under conditions of constant access to food.

The conclusion is reached that Pelseneer was in error when he stated that all opisthobranchs undergo during their development a torsion identical with that shown by prosobranchs. Only vestiges of gastropod torsion remain both in veliger and adult stages of many (perhaps most) living Opisthobranchia.

1. Introduction

Pelseneer (1891, 1894) believed the tritoniids to be the most primitive living nudibranchs. Primitive features are the lateral position of the anus and renal pore (indicating an early stage in the evolutionary detorsion which characterizes the Nudibranchia), the adult cerebral and pleural ganglia are relatively distinct, the right and left lobes of the digestive gland are distinct in some tritoniid species (Vayssière 1877), the position of the ventricle still shows signs of the ancestral asymmetry, the radula is large and broad, the kidney is only very slightly ramified, and the genital system is, to use Pelseneer's term, diaulic. Pelseneer constructed a family tree for the naked opisthobranchs which apparently showed modern Tritoniidae to be ancestral to the remainder; this view has been challenged by

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Evans (1914). Eliot (1910) has shown that *Tritonia* is in many ways a connecting link between the cladohepatic and holohepatic nudibranchs.

Tritonia hombergi Cuvier is the largest British nudibranch and is among the largest European gastropods. Specimens up to 15 cm have often been collected during the course of the present work, while Alder & Hancock (1845–55) state that adults up to 20 cm may occasionally be found. In view of this, it is a matter for surprise that so very little is known concerning the biology of this species, which, around British coasts, may be locally very abundant. The explanation is probably that it occurs in rather deep water, from 6 to 15 fm. (Odhner 1907), 16 to 30 fm. (Jones 1951), 'below the 30 fm. line' (Marine Biological Association 1957), 15 to 38 fm. (Miller 1961). *T. hombergi* is invariably associated with the cnidarian *Alcyonium digitatum*, on which it feeds; as Farran (1909) puts it, 'on every occasion when *T. Hombergi* has been taken, *Alcyonium digitatum* has also been found in the net'. It has been recorded from the Atlantic coasts of Europe, and there is a single mention (Pruvot-Fol 1954) of its occurrence in the Mediterranean. Apart from a record of the commensal copepod *Lichomolgus agilis* from the branchiae (Leigh-Sharpe 1935) and records of the presence of remains of *T. hombergi* in the alimentary canal of the dogfish *Scyliorhinus canicula* (Stephen, Rae & Wilson 1957; Thompson 1960), this is all that is known concerning the natural history of this species. Other species of *Tritonia* have been similarly neglected.

Cuvier's description (1803) of *T. hombergi* was accurate and clear; the monograph of Alder & Hancock (1845–55) gives a more detailed account of the structure of the adult tritoniid, and various gaps have been filled by Bergh (1880, 1880–92), Pelseneer (1894) and Odhner (1926). Nothing is known about the development of *T. hombergi*, but some observations on embryos and larvae of the related *Duvaucelia plebeia* have been made by Pelseneer (1911) and Vestergaard & Thorson (1938).

The present work on *T. hombergi* was begun in 1957. An account of the structure and mode of functioning of the reproductive organs (Thompson 1961) and some notes on defensive adaptations (Thompson 1960) have already been published. The present paper describes observations on the embryology, larval biology, metamorphosis and post-larval development. In particular, it was thought desirable to ascertain whether the mechanical process of torsion took place in *T. hombergi* in the way described by Pelseneer (1911) for a variety of nudibranchs, or in the way described for the dorid *Adalaria proxima* by Thompson (1958*a*). Secondly, there was the question of the preferences of the larvae during the searching phase of planktonic life, preferences which recent work on other invertebrates (for instance, Wilson 1956; Thompson 1958*a*; Ryland 1959; Crisp & Williams 1960) has shown may be highly specific. Thirdly, it was intended, in view of the supposed epipodial nature of the dorsal integument of the adult tritoniid (Garstang 1889, 1890; Herdman & Clubb 1892) to examine the fate of the larval shell and mantle in post-larval stages.

2. Material and methods

Living specimens were dredged from the *Modiolus modiolus* bed off the south-east coast of the Isle of Man, described by Jones (1951, p. 137). Adults were maintained in the laboratory in the fish- and lobster-rearing boxes used in the Port Erin Hatchery and described fully by Herdman (1908). Portions of spawn ribbons, produced in the laboratory or brought in from the field, were suspended by fine thread in 500 ml. beakers of sea water,

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in water-baths at $10 \pm 0{\cdot}5$ °C. The water was changed daily, taking precautions to avoid temperature shocks. No extra aeration was provided.

In the study of live embryonic stages, gently applied heat proved to be the best relaxing agent. Post-embryonic stages could, however, rapidly be narcotized by the addition of a little magnesium chloride.

Material for sectioning was fixed in the fluids of Perényi, Bouin (made up with sea water), Zenker (without acetic acid), Helly, Ciaccio (Dawes 1930), Lewitsky-saline (Baker 1958), or in neutral 5% formalin. Some fixatives were used hot when killing active developmental stages. The material was cleared with amyl acetate (Barron 1934), embedded in Hance's rubber wax (orientation being accomplished in the molten wax by means of heated needles), and sections were cut at from 5 to 15 μ.

The stains employed were Mayer's haemalum, safranin and light green, and the azan and the iron haematoxylin of Heidenhain. Many preparations were counterstained with eosin and alcian blue 8 GS (Steedman 1950). *Intra-vitam* dyeing with neutral red proved useful in the investigation of some structures. Radulae were prepared for microscopic examination in polyvinyl lactophenol using a technique previously described (Thompson 1958*b*). Drawings were made using a Leitz camera lucida.

Nomenclature of British animals referred to herein is according to the Plymouth Marine Fauna list (Marine Biological Association 1957); in the case of foreign species, the name used is that employed by the author cited.

3. Development of the embryo up to hatching (36 to 38 days at 10 °C)

The structure of the egg mass, the natural life cycle and the structure and mode of functioning of the reproductive organs have been described elsewhere (Thompson 1961).

Since the process of cleavage of the ovum resembles closely the pattern described in other nudibranchs (Casteel 1904; Pelseneer 1911; Thompson 1958*a*), no description will be given here of the development to gastrulation of *T. hombergi*; nor will cell-lineage be dealt with.

Five days after oviposition the somewhat flattened, heavily yolked, stereoblastulae begin to gastrulate, the blastopore being at first broad and shallow (figure 1*a*), later becoming deeper and more slit-like (figure 1*b*, *c*, *BL.*). Gastrulation is brought about by epiboly and no blastocoel or cleavage-cavity is present at any stage of the process.

The long axis of the blastopore-slit is coincident with the antero–posterior axis of the larva and of the adult. The axes of the developing organism will be described in terms of those of the adult.

A. *Assumption of the veliger form*

(i) *Stomodaeum and velum* (figures 1, 2, 9)

The blastopore closes briefly on the eighth day but a stomodaeal invagination (figure 1*g*, *STOM.*) is soon evident at approximately the point where the blastopore was last seen. This invagination deepens until contact is made internally with the developing organs of the posterior hemisphere of the embryo (figure 3, *STOM.*). The stomodaeum or foregut-rudiment has a lumen from the first, but not until the eighteenth day do lumina appear in the midgut; an open connexion then develops between foregut and midgut. By that

time the rudiment of the radular sac (figure 3, *RAD.RUD.*) has begun to develop, as a short, blind, posteriorly directed invagination in the ventral wall of the foregut just behind the mouth. Mesodermal elements which aggregate around this rudiment form the musculature of the buccal mass of later stages. Mucous goblet cells appear in the wall of the foregut.

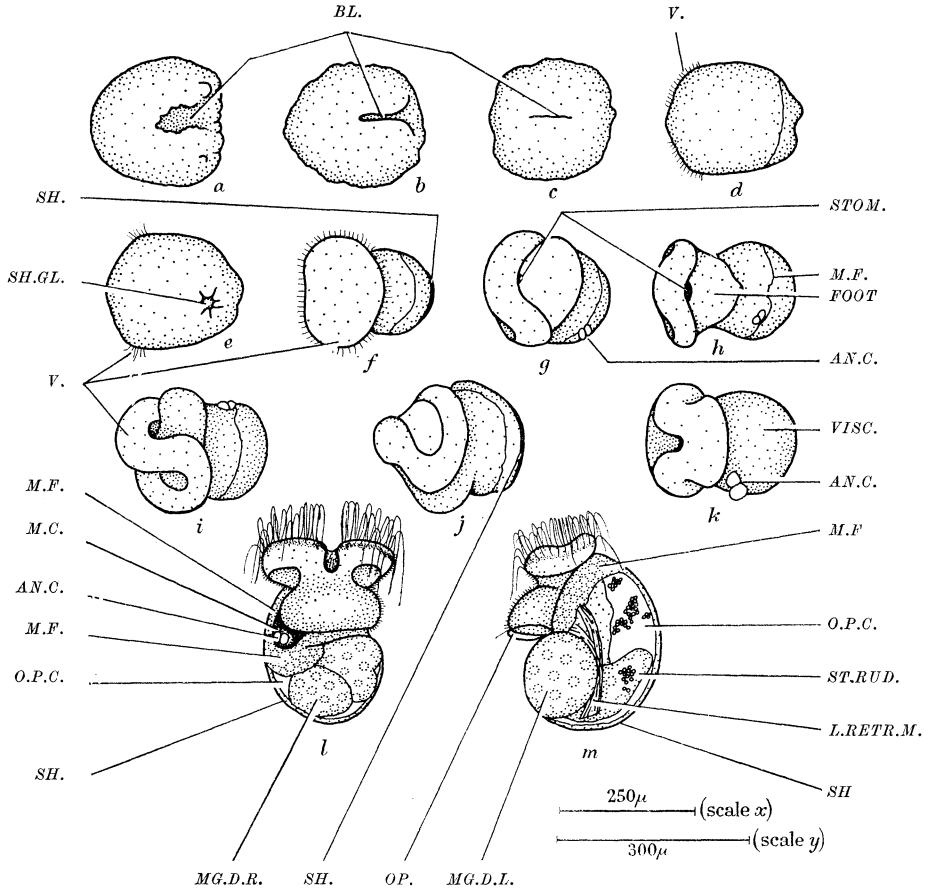

FIGURE 1. Embryonic development (drawings from life). *a*. Embryo at 5 days; ventral aspect. *b*. Embryo at 6 days; ventral aspect. *c*. Embryo at 7 days; ventral aspect. *d*. Embryo at 8 days; dorsal aspect. *e*. Embryo at 8 days; postero-dorsal aspect. *f*. Embryo at 9 days; dorsal aspect. *g*. Embryo at 9 days; right latero-ventral aspect. *h*. Embryo at 10 days; ventral aspect. *i*. Embryo at 10 days; right lateral aspect. *j*. Embryo at 10 days; left lateral aspect. *k*. Embryo at 13 days; ventral aspect. The shell now covers the entire visceral mass. *l*. Embryo at 15 days; ventral aspect. *m*. Embryo at 22 days; left lateral aspect. Cilia are not illustrated in *g* to *k*. Scale *y* applies to *l* and *m*, scale *x* to the remainder. (Abbreviations on p. 218.)

The anterior hemisphere of the embryo becomes ringed by the ciliated prototroch or velum (figure 1 *d* to *m*, *V.*) which curves ventrally to pass in front of the oral invagination. The velar band becomes raised on an antero-dorsal velar disk, shortly to become further differentiated into the paired velar lobes (figures 1, 9, *V.*).

(ii) *Shell-gland and mantle* (figure 1)

In a postero-dorsal position a deep but restricted invagination visible on the eighth day marks the first appearance of the shell-gland or rudiment of the mantle (figure 1 *e*, *SH.GL.*). Towards the ninth day this invagination disappears and flattened cells spread out from

it to cover the posterior hemisphere of the embryo (figure 1*h* to *k*). The leading edge of this spreading sheet of cells forms the mantle fold (figure 1, *l*, *m*, *M.F.*). The shell is secreted by these mantle cells; it is at first cap-shaped (figure 1*f*, *j*, *SH.*), but later, as development proceeds, cup-shaped (figure 1*k* to *m*, *SH.*), enclosing the whole of the embryonic posterior hemisphere (the visceral mass).

(iii) *Otocysts* (figures 7 to 9)

On the ninth day, the paired otocyst-sacs arise as invaginations on either side of the area which will form the foot. Origin of the otocysts by ectodermal invagination was not observed in *Adalaria* (Thompson 1958*a*). A calcareous spherical otolith appears in each otocyst-sac and may be observed in live embryos to rotate slowly under the influence of the lining cilia. The otocysts develop well in advance of any recognizable parts of the nervous system.

(iv) *Anal cells and hindgut* (figures 1, 5, 6)

Postero-ventrally, slightly to the right of the median plane of the embryo, a pair of prominent glistening cells make their appearance on the ninth day. These are the anal cells (figure 1*g*, *AN.C.*); their function is uncertain but they have considerable significance because in gastropods they mark the site of the area which will form the proctodaeal invagination (Raven 1958). Morphogenetic torsion in the typical gastropod always results in a shift in their position. During the ninth to thirteenth days the anal cells in *T. hombergi* move in an anterior direction (figure 1*g* to *k*, *AN.C.*) until they come to lie under the right side of the developing foot. This movement is the sole vestige of the ontogenetic mechanical processes of gastropod torsion and ventral ('ano-pedal') flexure. The mechanical cause of this change in the position of the anal cells is perhaps the spread of the mantle fold, carrying them on its anterior face to their final position.

The rudiment of the hindgut (which is endodermal) is not recognizable until the thirteenth day, after the completion of the anal cells' brief migration. In sections the terminal region of the hindgut (figure 5, *H.G.*) can then be seen to lie close beneath the anal cells. Several days elapse before a minute proctodaeal invagination gives rise to the anal pore. The inner walls of the hindgut become ciliated along the entire length. The hindgut was not observed to arise, as it does in many molluscs, as an outgrowth from the midgut.

(v) *Visceral mass* (figures 1, 4, 9)

Sections through 13-day embryos show no lumina or other spaces in the visceral mass; the midgut, midgut diverticula and hindgut all consist of solid masses of heavily yolked cells. As development proceeds, lumina appear in these organs and communications develop between them. The midgut diverticula are placed symmetrically on the antero-lateral faces of the midgut or stomach-rudiment. From the first, the left diverticulum is larger than the right and this proportional disparity increases later. The left diverticulum comes to consist of a relatively large number of small cells bounding a capacious lumen, while the right diverticulum remains composed of a few, larger cells and has only a small lumen (figure 4). As development proceeds, the yolk of both midgut diverticula is utilized; in the right diverticulum this results simply in a diminution in the size of the organ, while in the left diverticulum the volume of the constituent cells diminishes but the overall

dimensions of the organ remain unchanged. The inside wall of the stomach-rudiment becomes strongly ciliated. The hindgut communicates with the dorsal extremity of the latter and passes forwards, arching down to the anus, situated in a latero-ventral position on the right side of the embryo (figures 5, 9*c*, *H.G.*).

As visceral differentiation continues, blastocoelic spaces arise between the component organs and the perivisceral cavity (figure 1*l*, *m*, *O.P.C.*) is brought into being. The mantle becomes more transparent as its yolk is utilized and detail in the visceral mass can be seen with increasing clarity in live embryos. The mantle may become detached from the inside of the shell in various regions and a cavity (to be called the shell cavity) comes into being. The shell cavity (figure 9*a*, *b*, *SH.CAV.*) is open to the exterior at a later stage of embryonic development.

A small aggregation of cells near the anterior extremity of the rudiment of the hindgut is the rudiment of the larval kidney (figures 5, 6, 8, 9*c*, *K*2); by the fifteenth day it can be seen in sections that this organ opens to the exterior immediately dorsal to the anus (figure 5, *K*2). The tritoniid larval kidney is an unpaired structure; Heymons (1893) and Mazzarelli (1892) found paired rudiments in other opisthobranchs. Saunders & Poole (1910) give reasons for believing that this larval or secondary kidney is an excretory organ; they rightly dismiss the view of Lacaze-Duthiers & Pruvot (1887) that it is an anal eye.

The heavily yolked rudiment of the larval retractor muscle (figure 1*m*, *L.RETR.M.*), consisting of a small number of elongated cells, is recognizable in sections of 13-day embryos. Its position does not change during embryonic development, as it does in some prosobranch gastropods (Crofts 1937, 1955) and in *Aplysia* (Saunders & Poole 1910).

(vi) *Cerebral ganglia* (figures 2, 3, 7, 8)

Ectodermal proliferation in the area enclosed by the velum gives rise, on the thirteenth day, to a pair of tubular ectodermal invaginations (figure 2, *C.G.RUD.*). These reach into the embryo, passing one on either side of the foregut; they are the rudiments of the cerebral ganglia. A cerebral commissure, consisting of cells linking the two ganglion-rudiments, is soon established across the top of the foregut (figure 3, *C.C.*). The deep cerebral invaginations remain and proliferate nervous tissue until the eighteenth day. The ganglia become differentiated (figure 8) into an outer cortex of cell bodies and an inner medulla consisting mainly of nerve fibres. The cerebral commissure comes to consist mainly of nerve fibres (figure 3, *C.C.*).

(vii) *Control of the velar cilia*

At fifteen days the embryo has taken the form of a veliger of the usual type found in the Nudibranchia (figure 1*l*). The velum is markedly bilobed and bears a single row of long, compound, locomotory cilia. Subvelar and supravelar supporting cells bear short, continuously beating cilia. The velar locomotory cilia at first beat intermittently and arhythmically. From the eleventh day their beat is co-ordinated and orderly, with a laeoplectic metachronism (Knight-Jones 1954). It is not, however, until the fourteenth day that the beat of these locomotory cilia may be interrupted for longer or shorter periods. It may be of significance that the ability to halt and start the velar locomotory

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cilia follows closely on the appearance of the embryonic cerebral ganglia (13 days); Carter (1926, 1928) has shown these cilia to be under nervous control in the free veligers of some other nudibranchs.

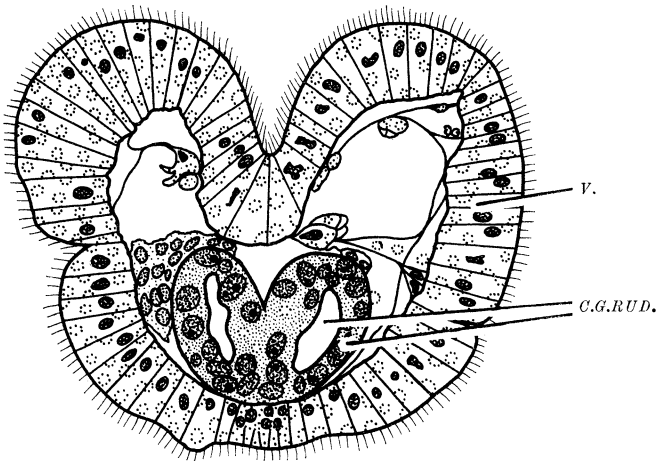

FIGURE 2. Transverse section through the cephalopedal mass of an embryo at 13 days (see figure 1*k*), passing through the velum and the intravelar invaginations which form the cerebral ganglia.

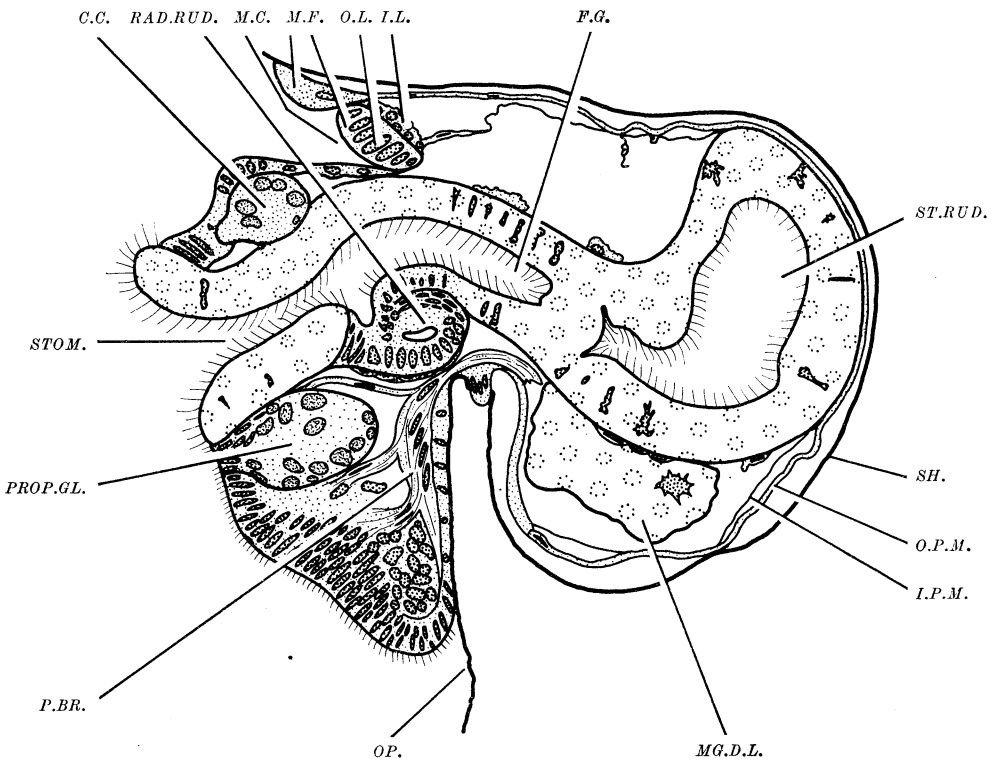

FIGURE 3. Longitudinal section, slightly to one side of the sagittal plane, of an embryo at 24 to 25 days, passing through the rudiments of the radular sac and left propodial gland.

(viii) *Foot* (figures 1, 3, 7, 8)

The embryonic foot, which gives rise to the metapodium of post-embryonic stages, is cloaked with fine short cilia which beat continuously. The posterior face of the foot lacks cilia but secretes a fragile, non-calcareous operculum (figures 1*m*, 3, *OP.*). As development proceeds, the yolk of the pedal ectoderm is utilized and the organ becomes less

granular and opaque. In the mid-line of the anterior face of the foot, however, a slightly elevated band of especially strongly ciliated yolk-laden cells persists for some days, running from the mouth down to the tip of the foot (figure 4, *MED.B.*). In other species

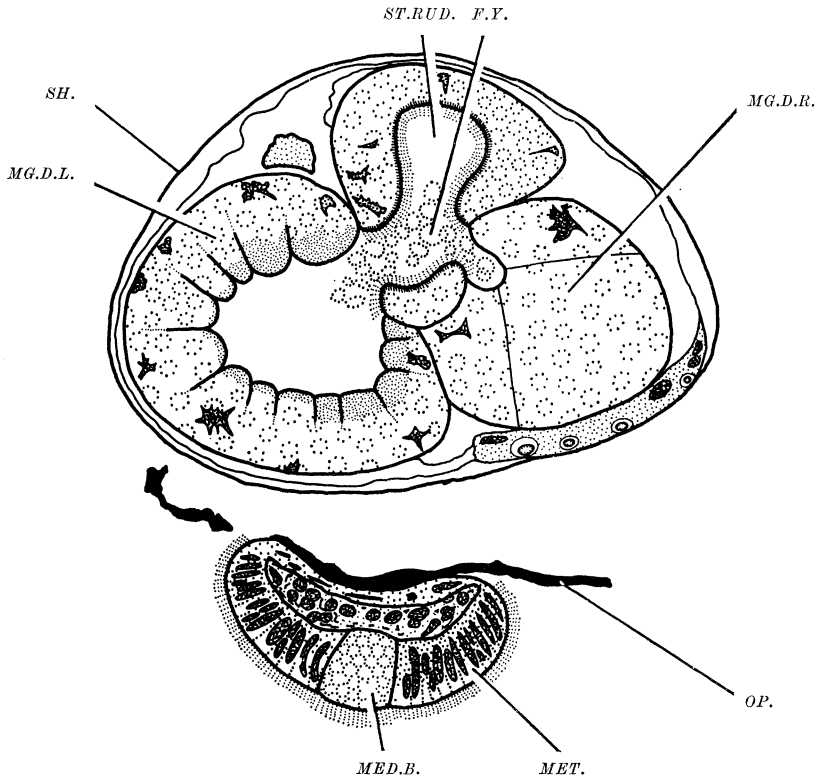

FIGURE 4. Transverse section through an embryo at 24 to 25 days, passing through the visceral mass and showing the relations between the midgut (stomach-rudiment) and the midgut diverticula. The plane of the section is posterior to the point of emergence of the hindgut from the stomach-rudiment. Dorsal is to the top of the page.

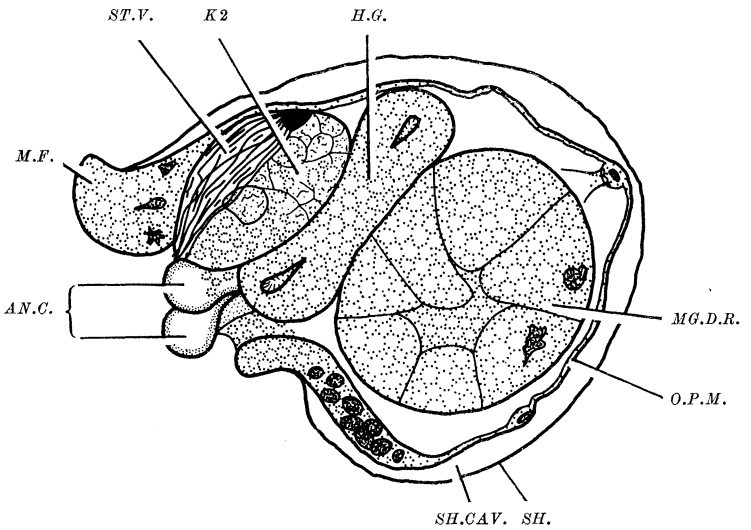

FIGURE 5. Longitudinal section through an embryo at 24 days, passing through the lateral extremity on the right side, and showing the anal cells, larval kidney and the terminal region of the hindgut. Anterior is to the left of the page, dorsal to the top.

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of nudibranchs (Thompson 1959) this pedal band plays a part in the larval feeding mechanism. In *T. hombergi*, as in the dorid *Adalaria* (Thompson 1958*a*), the median pedal band is obliterated by the development of the propodium long before hatching. A pair of subepidermal, multicellular metapodial mucus glands open to the surface approximately two-thirds of the way down the median pedal band. Numerous epidermal mucus glands open on the sides of the foot.

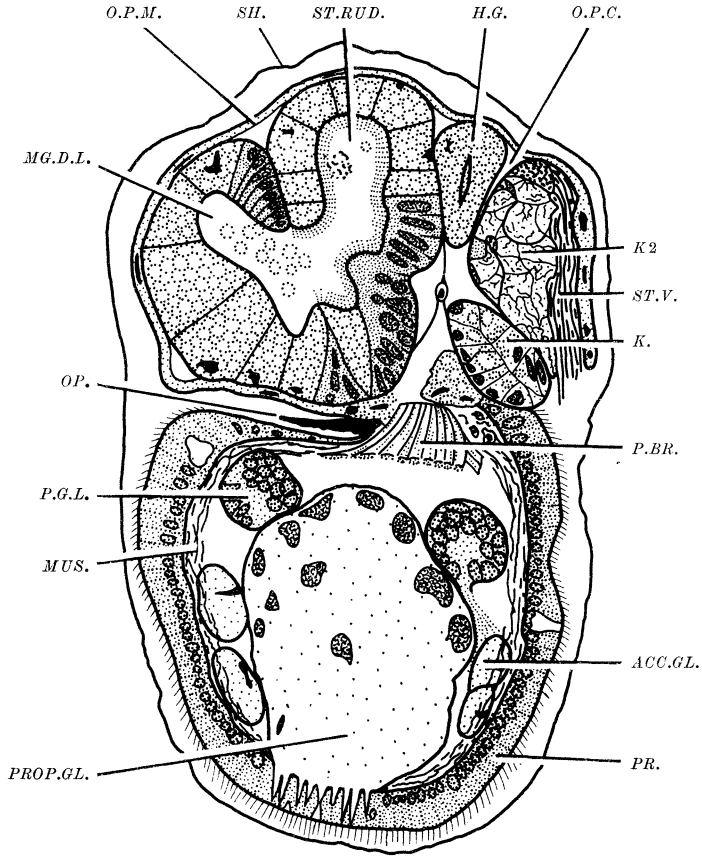

FIGURE 6. Horizontal section through a hatching embryo (36 to 38 days).

Cells are cut off inwards by the pedal ectoderm and come to assemble in two masses close to the respective otocysts. These masses form the rudiments of the pedal ganglia, visible first on the twentieth day (figures 6, 7, *P.G.L.*, 8, *P.G.R.*). The pedal ganglia become differentiated into the two layers, cortex and medulla.

(ix) *Mantle fold* (figures 1*h*, *l*, *m*, 3, 7, 9, *M.F.*)

The leading edge of the mantle, the mantle fold, becomes greatly thickened and forms a wide collar around the body immediately behind the velum. This collar is firmly attached to the mouth of the shell and secretes shell material until the twenty-seventh day of development. The shell itself consists of both calcareous and non-calcareous components.

The space between the mantle fold and the anterior body wall forms the mantle cavity (figures 1*l*, 3, 7, *M.C.*) which is widely open to the exterior. It is deepest and most capacious on the right side; in this region (figure 5) lie the anal cells, anus and the opening of the larval kidney. The face of the mantle fold lining the mantle cavity undergoes considerable

histological differentiation until by the twentieth day it has come to consist of an outer, more anterior, layer of columnar cells and an inner, more posterior, layer of rounded cells (figures 3, 7, 8, *O.L.*, *I.L.*). This change heralds the impending completion of shell secretion.

Early veliger stages are incapable of any muscular movement but by the fifteenth day small fibres of a developing cephalopedal muscle complex (figures 6, 7, 8, *MUS.*) are able to bring about twitching movements of the velar lobes if, for instance, alcohol is added to the water bathing an embryo. It is not until the eighteenth day that the larval retractor muscle (figure 1*m*, *L.RETR.M.*) is able to contract, even after such a violent stimulus as this, with sufficient strength to break the intimate shell mouth/mantle fold connexion and bring about retraction of the embryo into its shell. The connexion is broken naturally on the twenty-seventh day, shell-secretion being then completed.

B. *Later embryonic development*

The embryo of *T. hombergi* at 22 days (figure 1*m*) has attained a stage of development closely similar to that of the free larvae of most other species of nudibranchs. Hatching is, however, delayed for a further 14 to 16 days, during which time several significant changes occur.

(i) *Propodium* (figures 7, 8, 9, *PR.*)

Metapodial ectodermal cells immediately below the mouth multiply to produce the rudiment of the propodium. The median band of yolk-laden cells is at first interrupted at this zone of mitotic activity, and then obliterated completely as the developing propodium enlarges. At the same time a pair of propodial mucus glands (figures 3, 6, 7, *PROP.GL.*) develop from two restricted sites of proliferation postero-ventral to the mouth where ectodermal cells are cut off inwards and aggregate to form a pair of flask-shaped multicellular glands. Such an origin of the propodial glands was not observed in *Adalaria* (Thompson 1958*a*). Small multicellular accessory pedal mucus glands appear (figures 7, 8, *ACC.GL.*) beneath the pedal ectoderm. They open to the exterior by separate ducts all over the pedal sole.

(ii) *Optic ganglia* (figures 7, *OP.G.L.*, 8, *OP.G.R.*)

From paired regions of the intravelar ectoderm, close to the zones from which the cerebral ganglia arose earlier, cells are cut off which pass inwards to form discrete optic ganglia. They are not formed, as was found to be the case in *Adalaria* (Thompson 1958*a*), at the bases of ectodermal invaginations. At 25 days, optic pigment becomes visible in live embryos (figure 9*a*, *b*). The optic ganglia lie close above the respective cerebral ganglia (figure 13*a*).

(iii) *Completion of shell-secretion*

On the twenty-seventh day the mantle fold is detached and withdrawn from the mouth of the shell (figures 7, 8, 9, *M.F.*). No further shell-growth now occurs. The most obvious consequence of this withdrawal is that, in response to a mild mechanical stimulus, the embryo may now be retracted into the shell by contraction of the larval retractor muscle. This was hitherto impossible because of the restraining effect of the shell mouth/mantle

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fold connexion. By the twenty-seventh day the larval retractor muscle has differentiated to form a large number of elongated, yolk-free, contractile cells and its branches penetrate to all parts of the cephalopedal mass (figures 6, *P.BR.*, 7, *L.RETR.M.F.*). Co-ordinatory

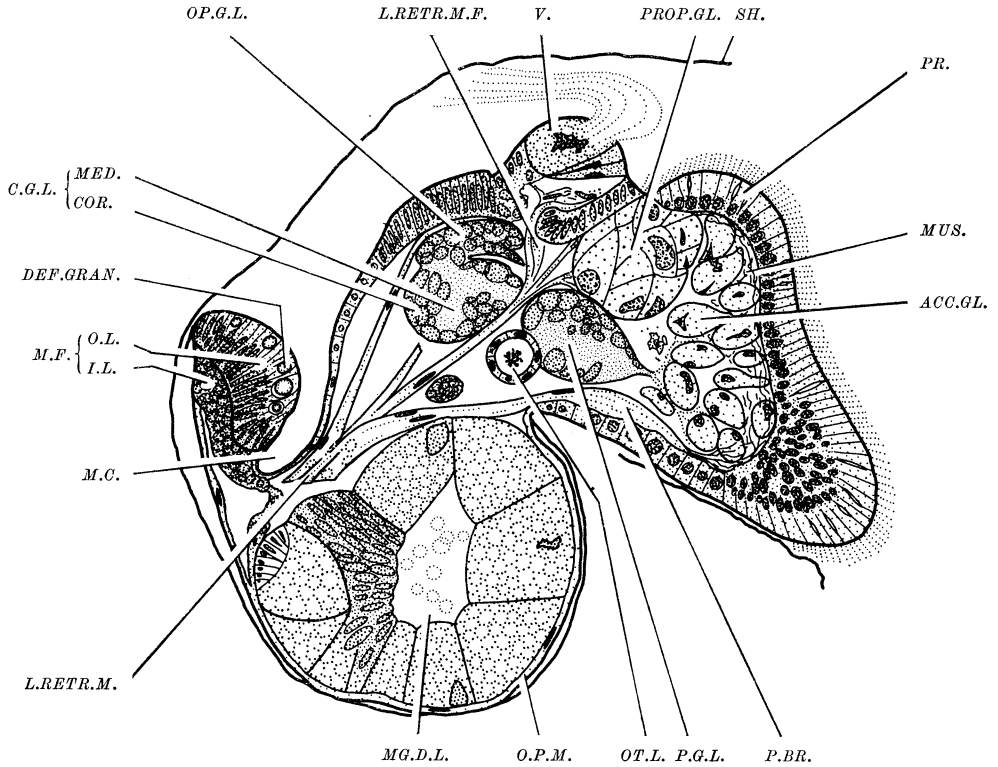

FIGURE 7. Longitudinal section (slightly oblique) through a hatching embryo (36 to 38 days), passing slightly to the left of the sagittal plane.

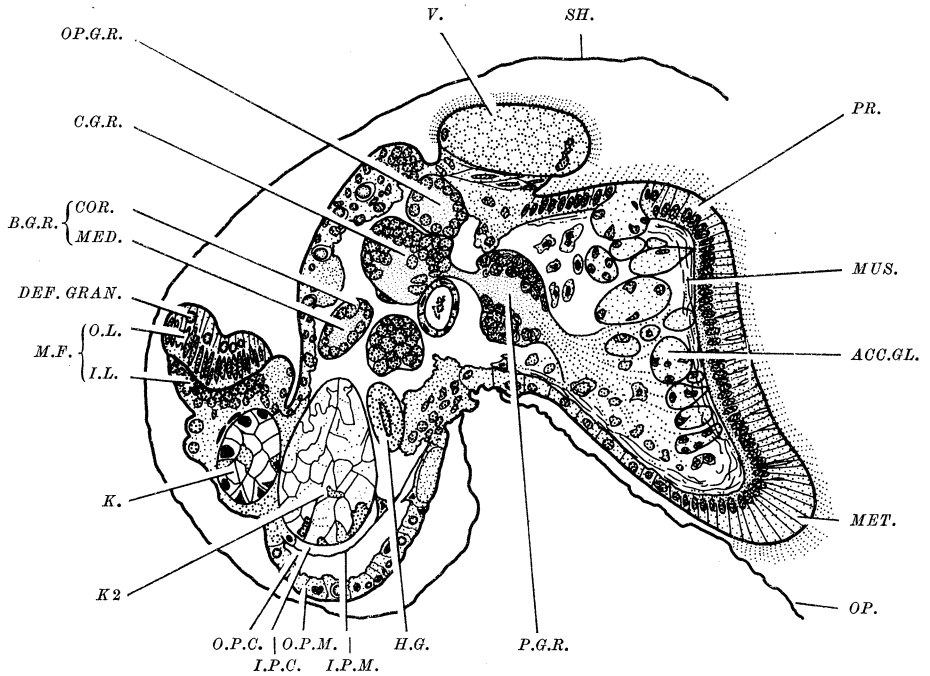

FIGURE 8. Longitudinal section (slightly oblique) through a hatching embryo (36 to 38 days), passing slightly to the right of the sagittal plane. The section is from the same series as figure 7.

systems of scattered muscle fibres (figures 6, 7, 8, *MUS.*) also play a part in retraction of the body. Pedal retractor muscles of the kind found in *Adalaria* (Thompson 1958*a*) do not develop at any stage in *Tritonia*.

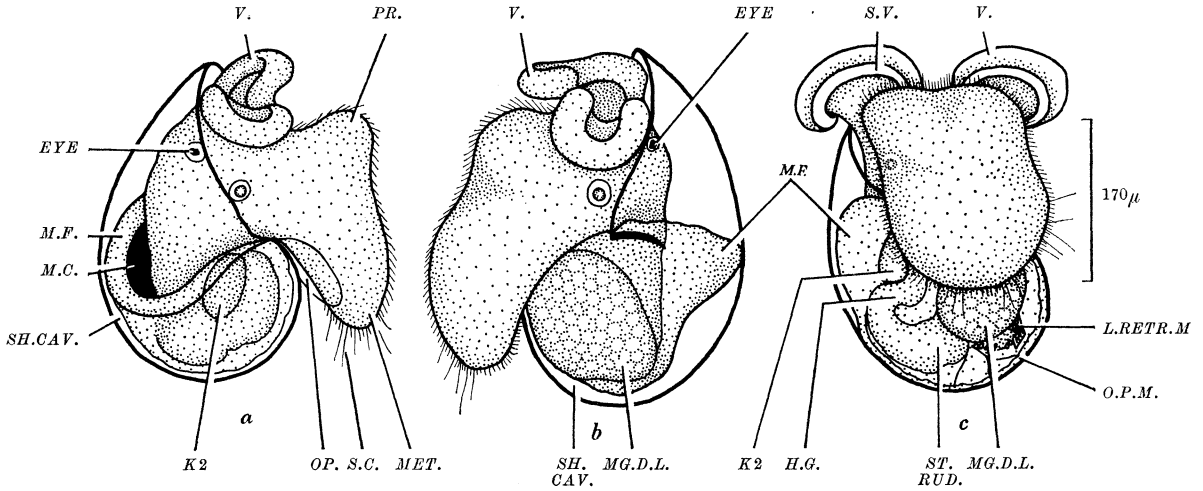

FIGURE 9. Early shelled larvae (drawings from life). *a*. Right lateral aspect. *b*. Left lateral aspect. *c*. Ventral aspect. The velar locomotor cilia are not illustrated.

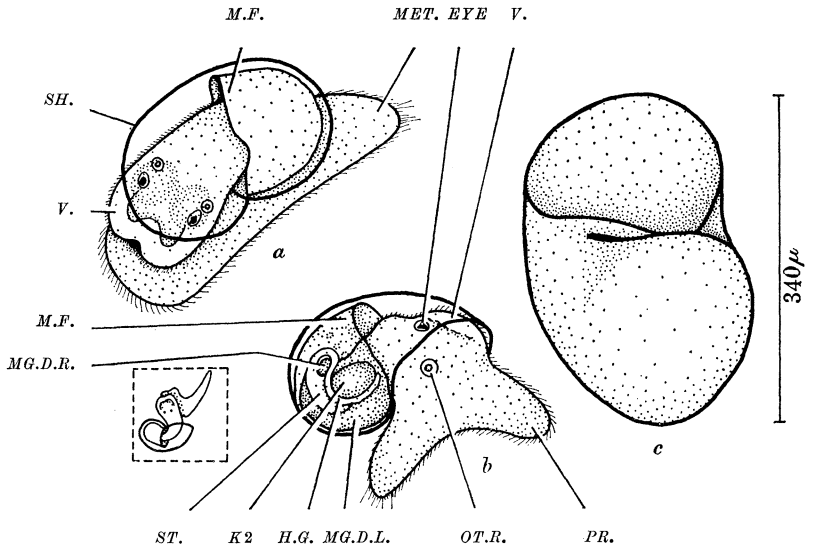

FIGURE 10. Metamorphosis. Drawings from life of animals removed from *Alcyonium* and partially narcotized with dilute magnesium chloride solution. *a*. Shelled stage (a few hours after settlement) creeping on glass; left latero-dorsal aspect. *b*. The same, lying on its left side; right lateral aspect. (Inset is a sketch of this animal endeavouring to right itself, showing the high degree of extensibility of the cephalopedal mass.) *c*. The shell; postero-ventral aspect. The scale applies only to *c*.

C. *Structure of the embryo at hatching* (figures 6 to 10*c*)

So close is the resemblance between hatching embryos of *T. hombergi* and *Adalaria proxima* (Thompson 1958*a*) that it is only necessary to describe the differences. The similarity is remarkable for the larvae of these two nudibranchs resemble one another far more than either resembles the larvae of its nearest relatives.

Points of difference between hatching embryos of *T. hombergi* and *A. proxima* are as follows.

(i) The larva of *T. hombergi* is slightly larger, having a shell (figure 10*c*) whose maximal dimension is 0·34 mm, compared with 0·28 to 0·30 mm in *A. proxima*.

(ii) The 'shell-peg' (Thompson 1958*a*, figure 31*A*, *P.*), which acts as a fulcrum for the base of the operculum during cephalopedal retraction, is a much wider structure in *T. hombergi* than in *A. proxima*.

(iii) Food-stores, mainly yolk-granules in the left midgut diverticulum, are present at hatching in *T. hombergi* but are usually absent in *A. proxima*.

(iv) The subvelar ridges are less prominent in *T. hombergi* than in *A. proxima*.

(v) The rudiment of the adult heart is identifiable in hatching larvae of *A. proxima* but not in *T. hombergi*.

(vi) In *T. hombergi*, that region of the larval kidney which is believed (Thompson 1958*a*) to function as a storage vesicle contains matter which in stained sections presents a coarsely striated appearance (figures 5, 6, *ST.V.*). In *A. proxima* the storage vesicle at hatching contains matter which, although coarse, is non-orientated.

(vii) The nervous system of the hatching embryo of the tritoniid does not include pleural ganglia, whereas rudiments of these organs are clearly identifiable in hatching *A. proxima*.

(viii) The mantle fold of *T. hombergi* contains numerous hyaline granules, each lying in a cytoplasmic vacuole (figures 7, 8, *DEF.GRAN.*). These granules may be traced through to the post-larval benthic stages and their homology with the adult epidermal defensive granules established. That they should make their appearance at so early a stage is perhaps surprising. Such granules are not present in *Adalaria*.

D. *Liberation from the egg*

It is difficult to obtain a reliable estimate of the exact day on which hatching would normally occur. Egg masses of *T. hombergi* maintained in still sea water in the laboratory never liberate their embryos; to obtain hatching the water must be stirred. The larvae are then liberated approximately 36 to 38 days after oviposition. The mechanism by which escape from the egg membrane is effected is not understood. The ovoid egg cases collapse after the departure of the embryos.

4. The larval phase

A. *Behaviour*

On hatching, the larvae swim strongly upwards and, in laboratory culture, become lodged in the surface film of the water. There they will remain indefinitely unless they are freed by dropping on to them a little sea water from a pipette. It was necessary to spray the meniscus of each culture at least at daily intervals to facilitate further development of the larvae.

Swimming is effected by the beat of the long velar cilia; when for brief periods they stop, the larva sinks passively. If the larva encounters any physical obstacle while it is swimming, it creeps over it in the usual gastropod fashion; except in certain circumstances (see later) swimming is soon resumed. If the larva is disturbed violently, retraction into the shell occurs and the aperture is closed by the operculum.

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B. *Nutrition*

The cilia of the cephalopedal mass set up a feeding current of the usual type found in nudibranch larvae (Thompson 1958*a*, 1959) but there is no evidence that food or other particles were ever ingested by the larvae while swimming. Certainly, food vacuoles were not detectable in the midgut diverticula after swimming larvae had been placed in a suspension of the diatom *Phaeodactylum tricornutum* Bohlin (kindly provided by Dr J. M. Kain). Larvae of *T. hombergi* will develop normally in boiled, filtered sea water. It may be concluded that these larvae are lecithotrophic. The midgut diverticula contain considerable reserves of yolk at hatching, contrary to the planktotrophic dorid nudibranchs.

During larval life the right midgut diverticulum diminishes in size (figure 10*b*, *MG.D.R.*). The left midgut diverticulum, which will give rise to the bulk of the digestive gland of post-larval stages, enlarges greatly and effects a slight rotation of the stomach which is corrected during metamorphosis.

C. *Analysis of the larval phase*

The larval phase can be divided into two distinct stages, the first occupying 1 or 2 days after hatching, the second of up to 10 days' duration. During the first, or obligatory phase of larval life, the larvae swim upwards and various internal changes occur. The beginning of the second or searching phase is signalled by a reversal of the original negative geotaxis. If, then, the larva alights on a favourable substratum, metamorphosis will occur. Searching involves alternating periods of creeping and swimming and may, in adverse circumstances, be prolonged for up to 10 days before the larva dies.

5. Settlement and metamorphosis

In culture conditions larvae of *T. hombergi* will settle and metamorphose only if a live, healthy colony of *Alcyonium digitatum* is provided, and there is every reason to suppose that in *T. hombergi* the larval requirements at settlement are as precise as in *Adalaria proxima*, whose larvae are able to distinguish accurately between numerous species of ectoproct Polyzoa (Thompson 1958*a*).

The following description of metamorphosis will refer to a larva which, on its descent from the surface layers of the culture medium, immediately encounters a live colony of *Alcyonium*.

The velar lobes and the upper part of the propodium are retracted into the shell and the larva (figure 10*a*, *b*) creeps actively over the surface of the alcyonarian. The velar cilia are never again employed in locomotion. Within a day, or two (depending on the turbulence of the medium), the shell (figure 10*c*) and the operculum are lost; within a further day metamorphosis is complete in the sense that adaptations to pelagic life have become transformed into those for benthic life, and the habits and appearance of the post-larvae have changed immensely (compare figures 9*a* to *c* and 11*d*, *e*).

A. *Changes in external form* (figures 10, 11)

When the shell and operculum are lost the post-larva takes on a flattened shape (figure 11*b*); the distinctions between the head, foot and visceral mass become indistinct (compare figures 10*b* and 11*e*) and the enlarging digestive gland comes to be contained partly within the foot. The length of the newly metamorphosed post-larva is approximately 0·40 mm.

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B. *Velum* (figures 10 to 12*c*)

The velar locomotory cells are resorbed as is the subvelar ridge and the whole apical area is reduced to a layer of single cell thickness (figure 12*c*, *OR.V.*) covering the antero-dorsal surface of the post-larva. This area becomes the oral veil of the growing slug (figure 11*d*, *e*, *OR.V.*).

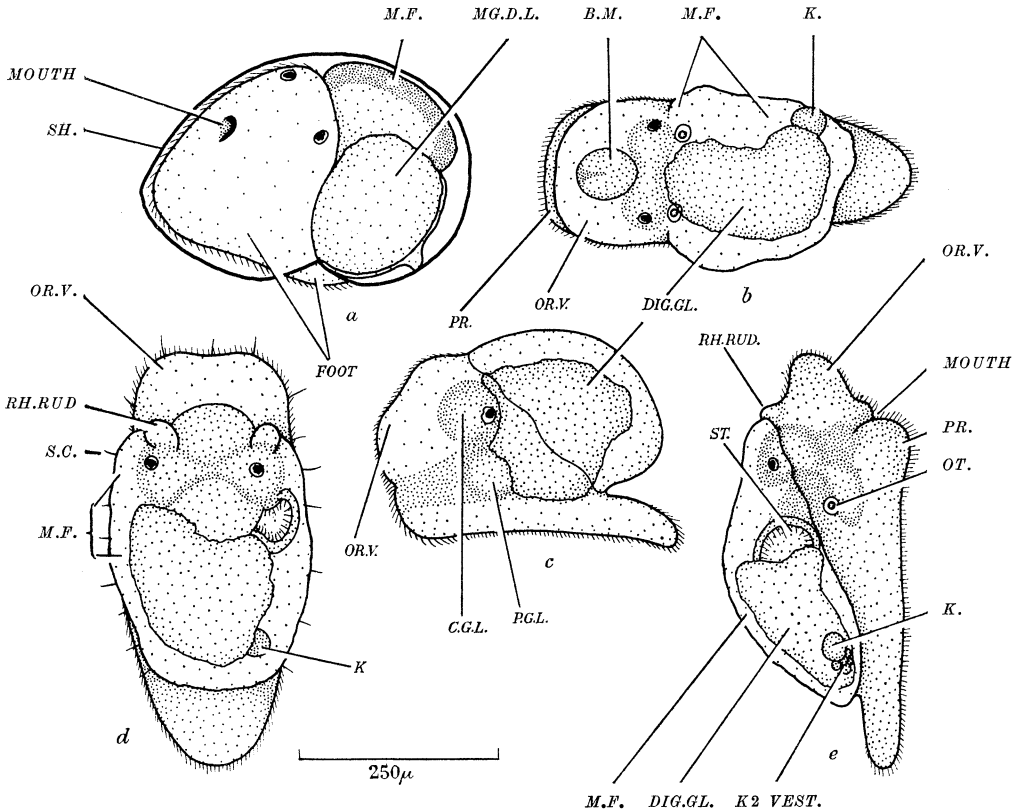

FIGURE 11. Metamorphosis. Drawings from life of animals removed from *Alcyonium* and partially narcotized with dilute magnesium chloride solution. *a*. Late shelled stage (mantle fold now reflexed); left lateral aspect. *b*. Shell-less stage, in length 0·41 mm; dorsal aspect. *c*. Shell-less stage, in length 0·36 mm; left lateral aspect. *d*. Shell-less stage, in length 0·50 mm; dorsal aspect. *e*. The same; right lateral aspect.

C. *Mantle* (figures 10 to 12)

The mantle fold becomes reflected back (figure 12*b*) and fuses with the outer peri-visceral membrane behind it (figure 12*c*). It is destined to form the dorsal integument of the post-larva (figure 11*d*, *e*, *M.F.*). The columnar layer of the fold, that is the layer which lined the mantle cavity of the veliger (figures 7, 8, 12*b*, *O.L.*), now forms the outer layer of the new dorsal integument (figure 12*c*, *M.F.*). Reflexion of the mantle fold results in the anus and the opening of the larval kidney coming to be directed laterally, instead of, as was earlier the case, anteriorly.

The columnar cells of the mantle fold now proliferate and spread rapidly over the dorsal surface of the post-larva. Granules of the repugnatorial glands appear in many of the new cells (figure 12*c*, *DEF.GRAN.*). Extending posteriorly, the mantle fold carries back with it along the right side the hindgut and larval kidney together with the

rudiment of the definitive kidney. The inner and outer perivisceral membranes become indistinguishable.

Anterior extension of the mantle is more rapid laterally than in the median plane; it extends forwards (figure 11 *b*, *M.F.*) over the area under which lie the eyes and partially

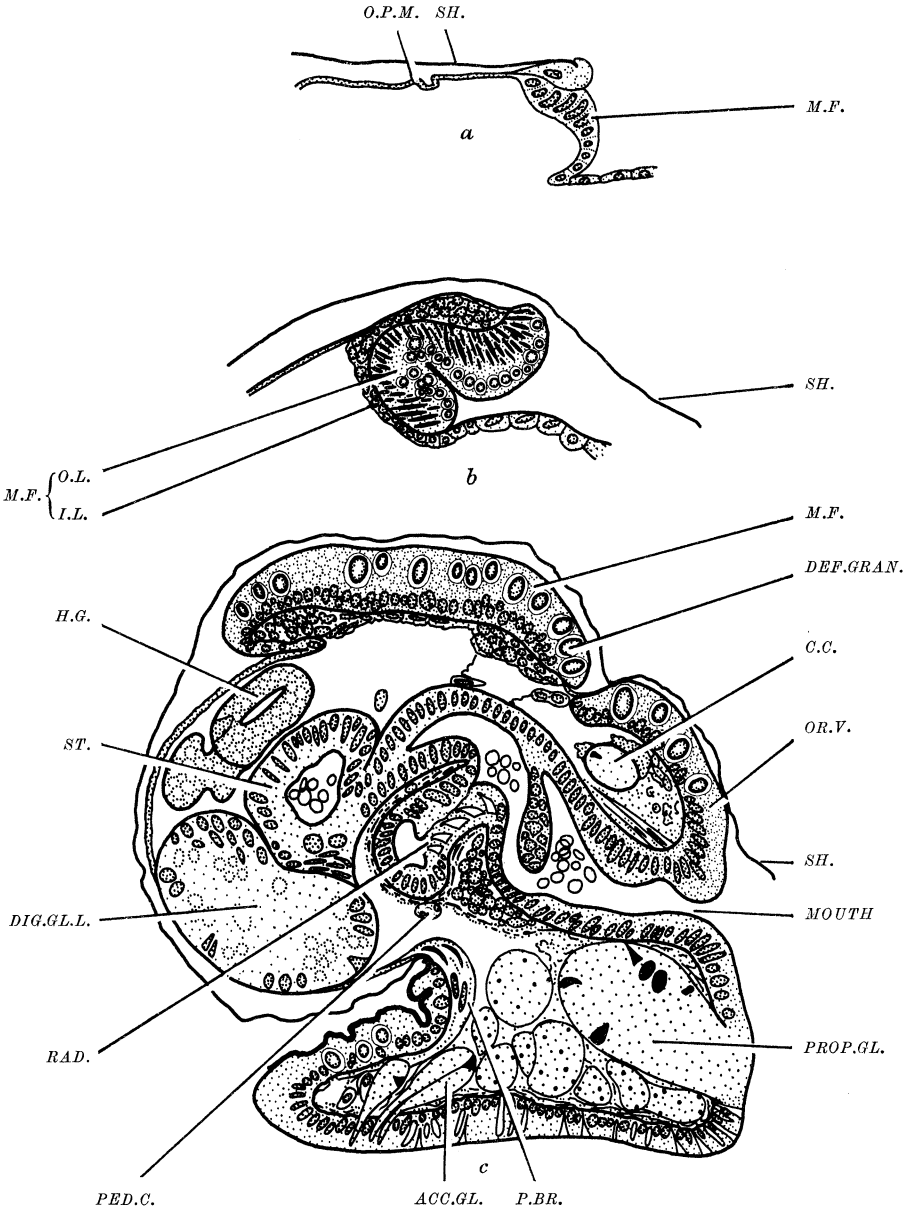

FIGURE 12. Sections showing the fate of the embryonic mantle fold. *a*. Portion of a sagittal longitudinal section showing the structure of the mantle fold in a 24-day embryo. *b*. Portion of a sagittal longitudinal section showing the structure of the mantle fold in a hatching embryo (36 to 38 days). *c*. Sagittal section through a metamorphosing stage, one day after having settled on *Alcyonium*. The mantle fold has now become reflexed back to cover the postero-dorsal surface of the body. Details of ciliation are not illustrated.

encircles (figure 11 *d*, *e*, *M.F.*) the bases of the paired prominences which are the rhinophoral rudiments (figure 11 *d*, *e*, *RH.RUD.*). The level of the rhinophores marks the anterior limit of the spread of the mantle.

D. *Pedal glands*

The metapodial glands disappear. The staining reactions of the propodial glands change at settlement, perhaps being connected with the production of some adhesive secretion; the glands cease to take up alcian blue and develop an affinity for azocarmine and for eosin.

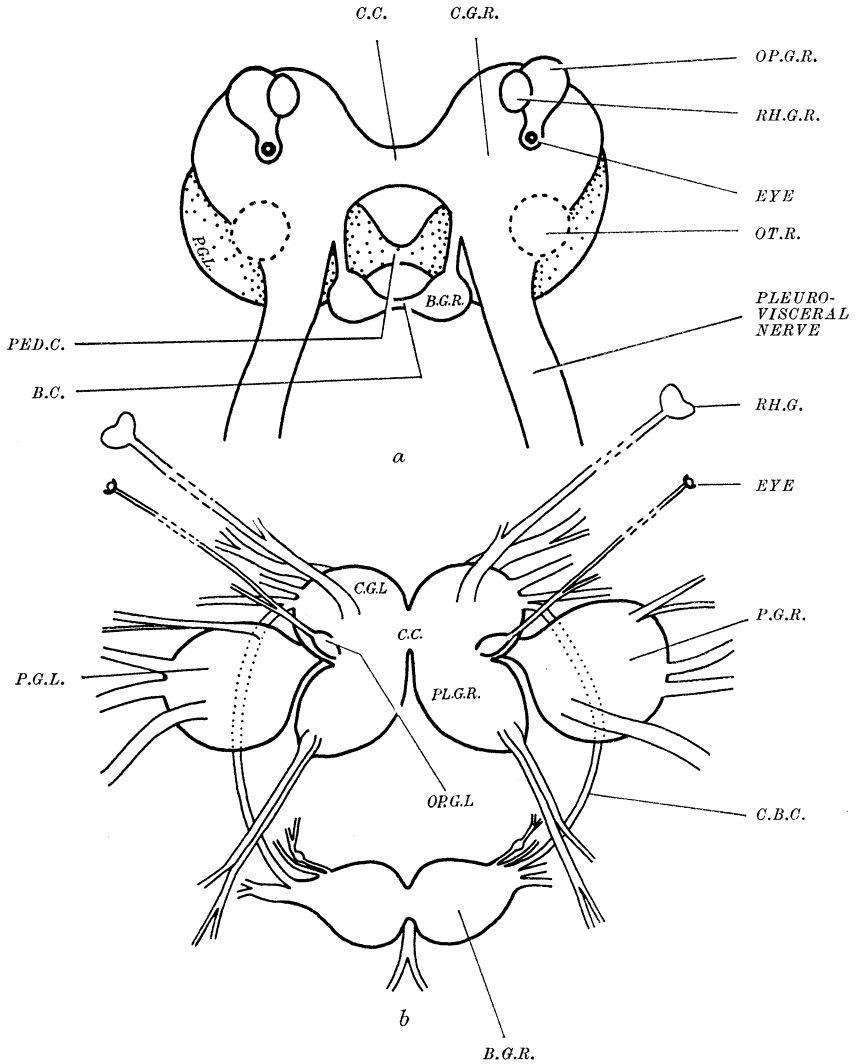

FIGURE 13. The nervous system. The main ganglia are shown, from the dorsal aspect, in, *a*, the earliest post-larval stages, for comparison with those of the adult, *b*. (In *b* the suboesophageal commissures are omitted.)

E. *Nervous system* (figures 12*c*, 13, 14, 22)

One of the results of the flattening of the body consequent on the loss of the shell and operculum is that the cerebral ganglia with their commissure are shifted posteriorly some distance (compare figures 12*c* and 22, *C.C.*). The pedal commissure comes to pass above the radula sac (figure 22, *PED.C.*) instead of, as was earlier the case (figure 12*c*, *PED.C.*), below it. The system of ganglia becomes more concentrated (figure 13*a*) and the commissures and connectives shorten. Proliferation of the optic ganglia from the intravelar ectoderm ceases. The eyes become separated from the cerebral ganglia but the optic

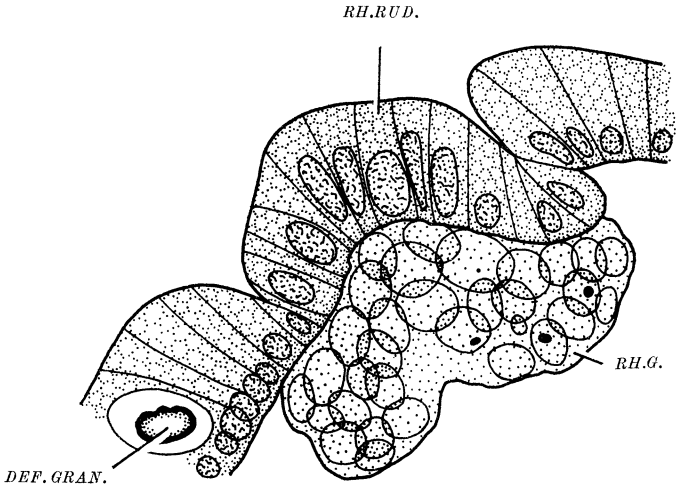

FIGURE 14. Portion of a transverse section through an early post-larva, showing the rudiments of the rhinophoral tentacle and ganglion of the right side.

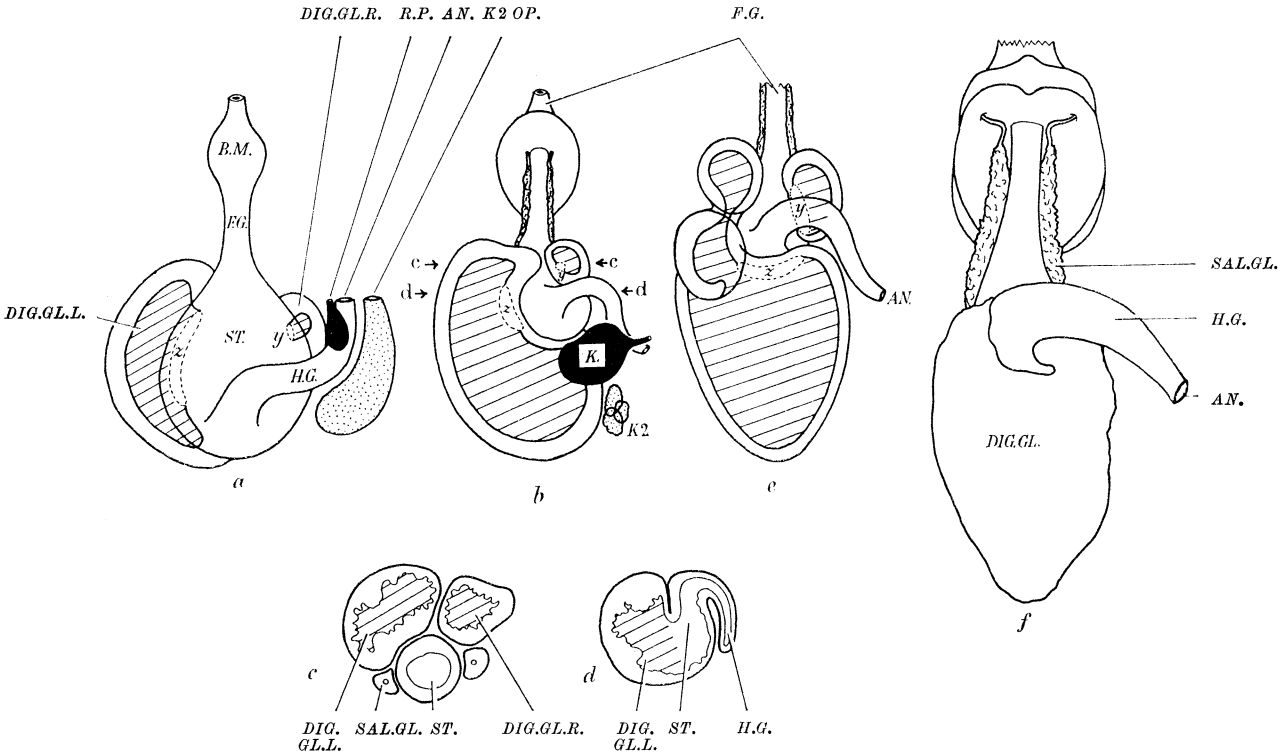

FIGURE 15. Diagrams, based on dissections and on reconstructions, showing some features of the disposition of the alimentary and renal systems. *a*. The alimentary and renal systems in the hatching embryo; dorsal aspect. *b*. The alimentary and renal systems after metamorphosis of the larva; dorsal aspect. *c*. Transverse section along the plane indicated in *b* by the letters c to c. Partially diagrammatic. *d*. The same, along the plane d to d. *e*. The alimentary canal in a post-larval stage, 1·4 mm in length; dorsal aspect. *f*. The alimentary canal in an adult, 12 cm in length; dorsal aspect. In *a*, *b*, and *e*, the interrupted line at *y* shows the position of the opening of the right digestive gland into the stomach; the interrupted line around *z* shows the position of the opening from the digestive gland of the left side. The approximate extent of the lumina of the lobes of the digestive gland is hatched in *a*, *b*, *c*, *d*, and *e*.

ganglia remain close to the antero-lateral faces of the latter. Stout optic nerves connect the eyes with the optic ganglia. Pelseneer (1894) gives a good illustration of the structure of the adult tritoniid eye.

The ectoderm above the eyes becomes thickened and cells are proliferated inwards to form the rudiments of the rhinophoral ganglia (figure 14, *RH.G.*). The thickenings themselves form the rhinophoral tentacles (figures 11*d*, *e*, 14, *RH.RUD.*). They are partially encircled by the anteriorly extending mantle fold; their origin from the oral veil (which is in turn derived from the velar ectoderm) is thus disguised.

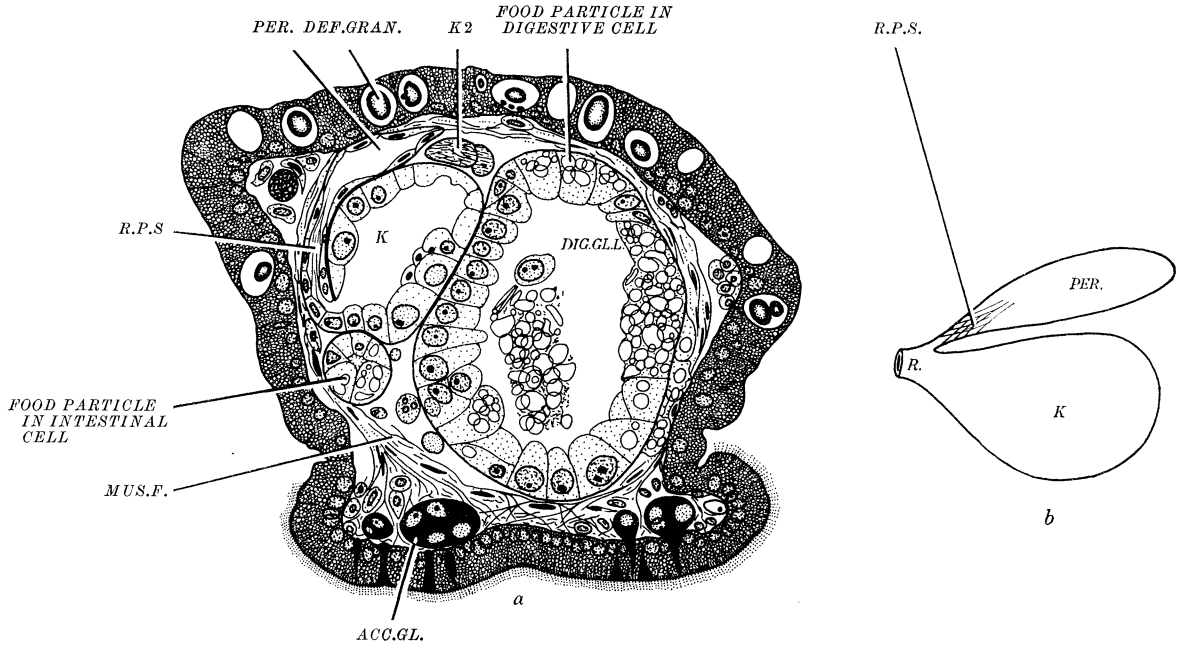

FIGURE 16. The reno-pericardial system. *a*. Transverse section through a post-larva, in length 0·5 mm, soon after metamorphosis, passing close to the level of the anal opening. Lewitsky-saline and azan. *b*. Diagram showing the relationship between the pericardium and the definitive kidney in the young post-larva. Dorsal is to the top of the page in both *a* and *b*.

F. *Muscle systems*

The larval retractor muscle becomes reduced in size and, soon after the shell has been cast, is scarcely detectable. At the completion of metamorphosis, only the pedal branch of the retractor muscle remains visible.

G. *Alimentary canal*

The buccal mass enlarges rapidly, the radular formula changing from 3–4 × 0.1.0 to 5–6 × 4.1.4 within a day after settlement. Jaws develop as chitinous thickenings of the lateral and dorsal walls of the foregut. Paired tubular salivary glands make their appearance, probably formed as outgrowths from the foregut. Feeding, by the use of the radula, commences soon after settlement and the stomachs of nearly all post-larvae were packed with alcyonarian material. This contradicts Berrill's (1931) view that a gastropod smaller than several millimetres in length might not have a functional radula.

During metamorphosis the position of the anus moves some distance towards the rear. This movement is a reversal of gastropod torsion and visceral flexure. Contrary to the

condition in the dorid *Adalaria* (Thompson 1958*a*), the anal complex never reaches a mid-posterior site but merely takes up a position on the right side (figure 15*b*, *e*, *AN.*).

The stomach becomes divided into an antero-ventral lobe which receives the foregut, and a postero-dorsal lobe which gives off the hindgut. The histological appearance of both foregut and stomach comes to resemble that of the adult *Tritonia*. It is a uniform non-glandular endothelium (figure 26) loaded with intracellular granules or globules like

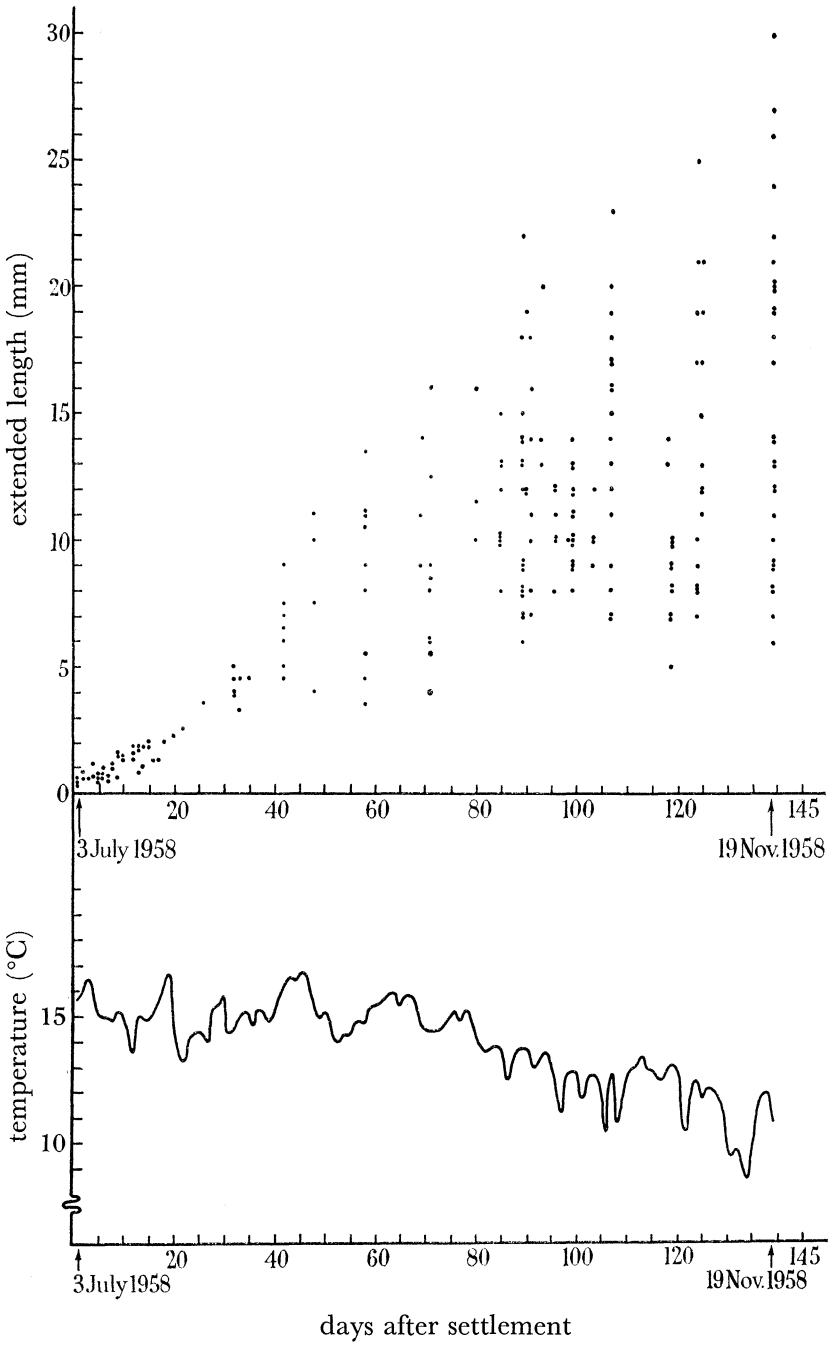

FIGURE 17. Growth rate of post-metamorphal stages during twenty weeks of laboratory rearing, under conditions of constant access to food (*Alcyonium digitatum*). Fluctuations in the temperature of the sea water are shown below.

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those described by Henneguy (1925) and Graham (1938) in many eolidacean nudibranchs. Both authors state that such cytoplasmic inclusions (resembling wheat-grains) are present also in tritoniid nudibranchs. This appearance in stained sections resembles closely the cytoplasm of the pedal epidermis and the epidermis of the sides of the body and the oral veil (figure 22); its significance is not agreed upon, but it is obviously correlated in some way with a cnidarian diet (Graham 1938).

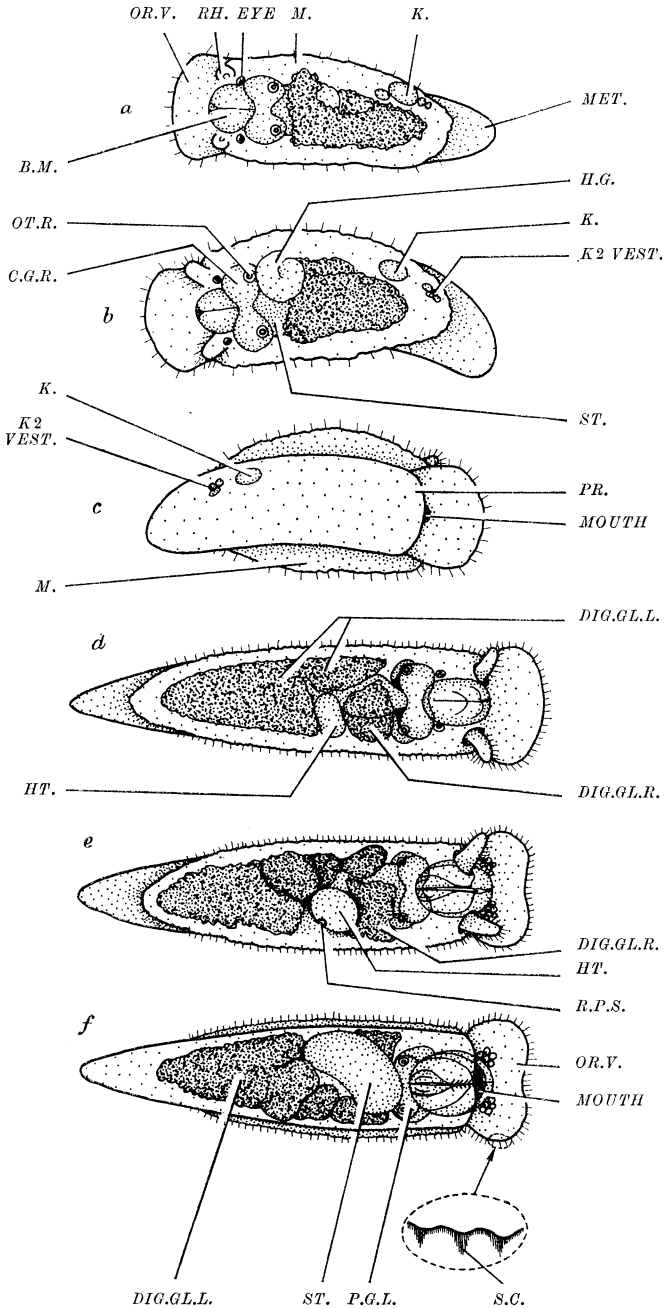

FIGURE 18. Post-larval development. Drawings from life of animals removed from *Alcyonium* and partially narcotized with dilute magnesium chloride solution. (Not to the same scale.) *a*. Juvenile 0·61 mm in length; dorsal aspect. *b*. Juvenile 0·70 mm in length; dorsal aspect. *c*. The same, from the ventral aspect. *d*. Juvenile 0·97 mm in length; dorsal aspect. *e*. Juvenile 1·75 mm in length; dorsal aspect. *f*. The same, from the ventral aspect.

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The two midgut diverticula enlarge and the left diverticulum comes to lie partially within the foot. They form the post-larval digestive gland. Each opens separately into the stomach (figure 15*b*). In the dorid *Adalaria* (Thompson 1958*a*) the right diverticulum disappears at metamorphosis; *Tritonia* resembles *Marionia* (Vayssière 1877) and *Eubranchus* (Fischer 1892) where both diverticula apparently persist.

H. *Renal system* (figures 11, 15*b*, 16, 18*a*, *b*, *c*)

The larval kidney begins to degenerate but is still visible (with its conspicuous storage vesicle) both in life and in sections, at the end of metamorphosis (figure 11*e*, *K2 VEST.*). The definitive kidney (figures 15*b*, 18*a*, *b*, *K.*) enlarges and can be seen to open to the exterior near the anus. The rudiment of the pericardium becomes detectable in sections through post-larvae towards the end of metamorphosis; the pericardium and kidney are linked (figure 16*a*, *b*, *R.P.S.*) by a short tube which contains numerous long flagella; this tube is the reno-pericardial syrinx.

6. Subsequent development

Post-larval stages fed on *Alcyonium digitatum* were reared in the laboratory for 20 weeks; at the end of this period (figure 17) many had attained a length of 3 cm. The difficulty of obtaining sufficient of their alcyonarian food rendered it impossible to rear them further. During this period many important changes occurred. Post-larvae in length $2\frac{1}{2}$ to 3 cm (figure 19*c*) differ from adults only in detail.

A. *Skin* (figures 12*c*, 14, 16*a*, 22)

The epithelium over the whole body remains of single cell thickness; all epidermal cells contain the peculiar 'wheat-granules' of Henneguy (1925) and of Graham (1938). The epidermis is ciliated, most strongly over the oral veil and pedal sole. In early benthic stages the mantle bears, in addition, numerous rather stiff compound cilia (figure 18*f*, *S.C.*).

Many of the epidermal cells of the mantle, oral veil, rhinophoral sheaths and branchiae (when such have been developed) contain granules lying in clear cytoplasmic vacuoles (figures 16*a*, 22, *DEF.GRAN.*). It is believed (Thompson 1960) that these granules (which may be discharged in a slimy brown fluid if the tritoniid is roughly handled) have a defensive function. They have some resemblance to the rhabdoids of flatworms, for instance *Polycelis cornuta* (Jennings 1957). Byne (1893) has stated that tritoniid slime may blister the human hands, but this has not been my own experience in handling many hundreds of these slugs. The staining properties of the supposedly defensive granules vary with the fixative employed. After fixation with Lewitsky-saline or with Zenker they stain red with azan and with safranin, black with Heidenhain's iron haematoxylin; after Ciaccio fixation they stain blue, purple or red with azan, black to grey with Heidenhain. Another type of epidermal gland cell, termed gland cell of type 1 by Thompson (1960), contains secretory globules whose staining properties are similarly variable; after fixation with Lewitsky-saline they are dyed red by azan, black by Heidenhain, while after Ciaccio fixation they stain blue with azan and not at all with Heidenhain.

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Initially, post-larvae have no pigment in the skin; the characteristic yellow to purple coloration of the adults begins to appear several months after metamorphosis, at a length of about 3 cm. According to Alder & Hancock (1845–55), around the Isle of Man the largest specimens are always of the yellowish variety. Dalyell (1853) states, however, that leaden grey or brownish specimens are invariably the largest.

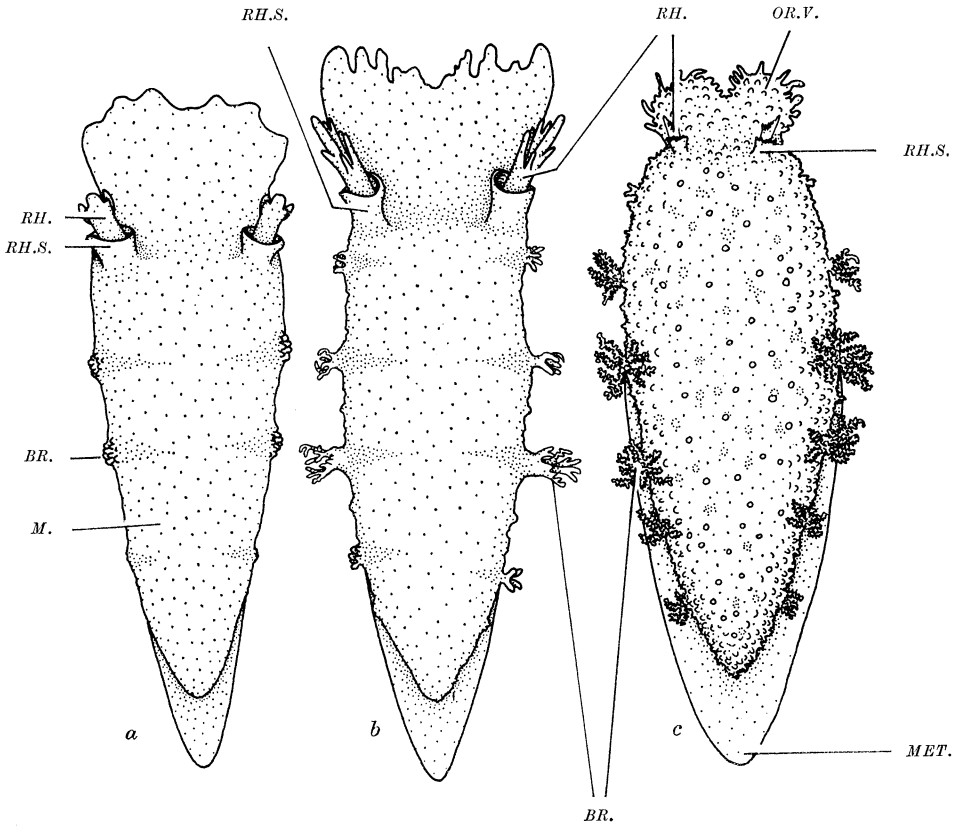

FIGURE 19. Post-larval development. Drawings from life of animals removed from *Alcyonium* and partially narcotized with dilute magnesium chloride solution. (Not to the same scale.) *a*. Juvenile 4½ mm in length; dorsal aspect. *b*. Juvenile 7½ mm in length; dorsal aspect. *c*. Juvenile 25 mm in length; dorsal aspect.

B. *Pedal glands* (figures 12, 16*a*, 22)

The propodial glands of the veliger (figure 12*c*, *PROP.GL.*) atrophy after metamorphosis and the lubrication of the pedal sole is then accomplished through a great multiplication of the larval accessory pedal mucus glands (figures 7, 8, 16*a*, 22, *ACC.GL.*). In later benthic stages these come to be especially numerous at the leading (propodial) edge of the foot.

C. *Nervous system* (figure 13)

The pleural ganglia begin to become distinct some weeks after metamorphosis; a transverse constriction comes superficially to divide each cerebral ganglion into two regions, the posterior of which is the pleural ganglion (figure 13*b*, *PL.G.*). This mode of development of the pleural ganglia differs radically from that described for *Fiona* by Casteel (1904) or for the dorid *Adalaria* by Thompson (1958*a*), where they originate from a pair of zones of ectodermal proliferation near the larval mouth.

The rhinophoral ganglia (figures 13*a*, 14, *RH.G.*) enlarge as the rhinophores themselves grow; the latter become branched (figure 19, *RH.*), protuberances developing which are at first digitate, later arborescent. Rhinophore sheaths (figure 19, *RH.S.*) (derived from the skin of the mantle) develop around the bases of the rhinophoral tentacles; the rims of these sheaths also develop digitiform projections.

The otocysts lie close beneath the cerebral and pedal ganglia. The larval otocysts each contained a single spherical otolith; after metamorphosis these spheres have a diameter of 0·013 mm. In later life further irregular otoliths are secreted in each otocyst; the original spherical otolith can be recognized in squash preparations for some weeks after metamorphosis. The numbers of otoliths in the two otocysts which each individual possesses are often widely dissimilar in early post-metamorphal stages. Some examples will serve to illustrate this. The two otocysts of a juvenile $4\frac{1}{2}$ mm in length contained, in addition to the spherical embryonic otolith, respectively two and four further otoliths. Counts made on other juveniles gave the following results: length of juvenile 6 mm, otoliths 1+7, 1+12; length of juvenile 7 mm, otoliths 1+12, 1+12; length of juvenile $7\frac{1}{2}$ mm, otoliths 1+7, 1+12.

D. *Muscle systems* (figures 16*a*, 26, 28, *MUS.F.*)

The larval retractor muscle, although still detectable in some post-larvae 0·5 mm in length, disappears shortly after metamorphosis. The adult musculature, consisting of a subepidermal sheath of bundles of transverse, circular and longitudinal fibres (figure 16*a*, *MUS.F.*), is derived from the embryonic and larval cephalopedal muscle complex (figures 6, 7, 8, *MUS.*). Discrete muscles develop in connexion with the rhinophores, the buccal mass and, in later stages, the anterior genital mass. In addition, delicate sheaths of muscle fibres (figures 26, 28, *MUS.F.*) come to surround the lobules of the digestive gland, the kidney, the genital tubules and various other organs.

E. *Reno-pericardial, respiratory and circulatory systems* (figures 15, 18 to 21, 26)

The larval kidney and its storage vesicle (figures 15, 18, *K*2) disappear within a week after the completion of metamorphosis. Casteel (1904) and Mazzarelli (1892) believed that the larval kidney in nudibranchs might develop directly into the adult kidney; this view is incorrect.

The definitive kidney enlarges and its prolongations extend around the stomach and completely surround the digestive gland. When the ovotestis develops, it encroaches upon the kidney so that the position and extent of the latter in the adult may be as shown in figure 20*a*, *K*.

In early benthic stages the kidney is lined by a simple renal epithelium of single cell thickness (figure 26, *REN.C.*). Later, the wall of the organ becomes folded and in some regions digitiform processes of the renal wall may project into the lumen. Each renal cell contains one or more large vacuoles in which lie the insoluble granules of excretory material. The fluid contents of the kidney (figure 20*c*) always contain numbers of these granules surrounded by a membranous covering presumably derived from the parent renal cell. In life the excretory granules are pale brown in colour and are insoluble in water or alcohol; after fixation with Ciaccio they are dyed blue by azan. Discharge of the

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renal fluid is brought about by contraction of the delicate muscle sheath (figure 26, *MUS.F.*) with which the organ is provided. The renal duct and pore (figure 20*a*, *R.*) are immediately above the anus, and may be closed by the contraction of a sphincter muscle.

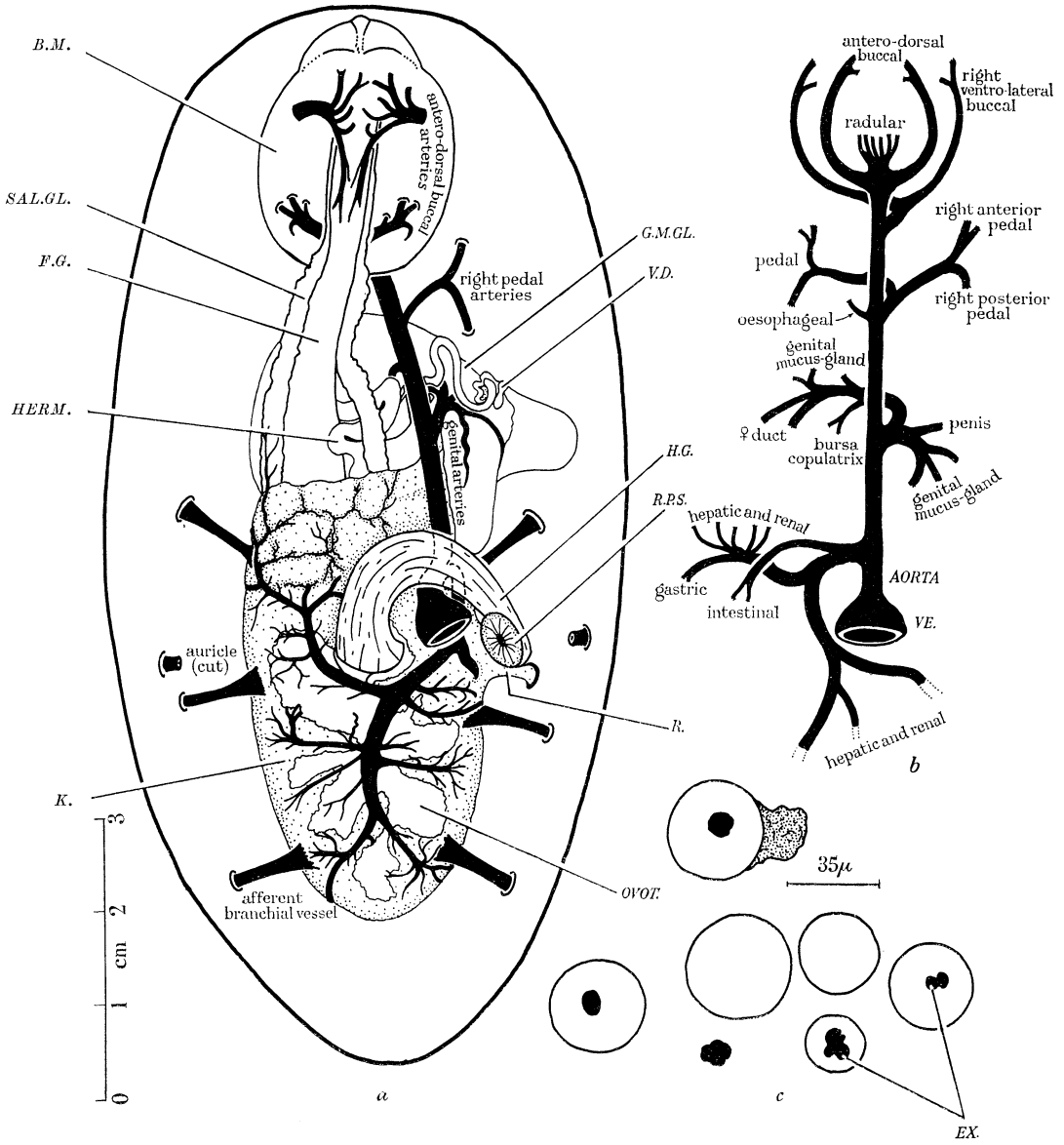

FIGURE 20. The reno-pericardial system. *a*. Dissection of a mature adult from the dorsal surface, to show the extent of the kidney and some features of the circulatory system. Renal tissue is dotted; blood vessels are shown black. *b*. Semi-diagrammatic representation of the branches of the aorta, based on dissections of injected specimens and on serially sectioned material. *c*. Contents of the renal fluid of an adult; unstained.

The kidney has an open connexion with the pericardium via the reno-pericardial tube which enters the pericardium at its right lateral extremity (figures 16*a*, 21*b*). At this point the reno-pericardial tube becomes dilated to form the pyriform reno-pericardial syrinx (figures 16, 20*a*, 21*b*, *R.P.S.*). The opening of the syrinx in the pericardial floor is lined by strongly ciliated folds by whose action a transfer of fluid from the pericardium

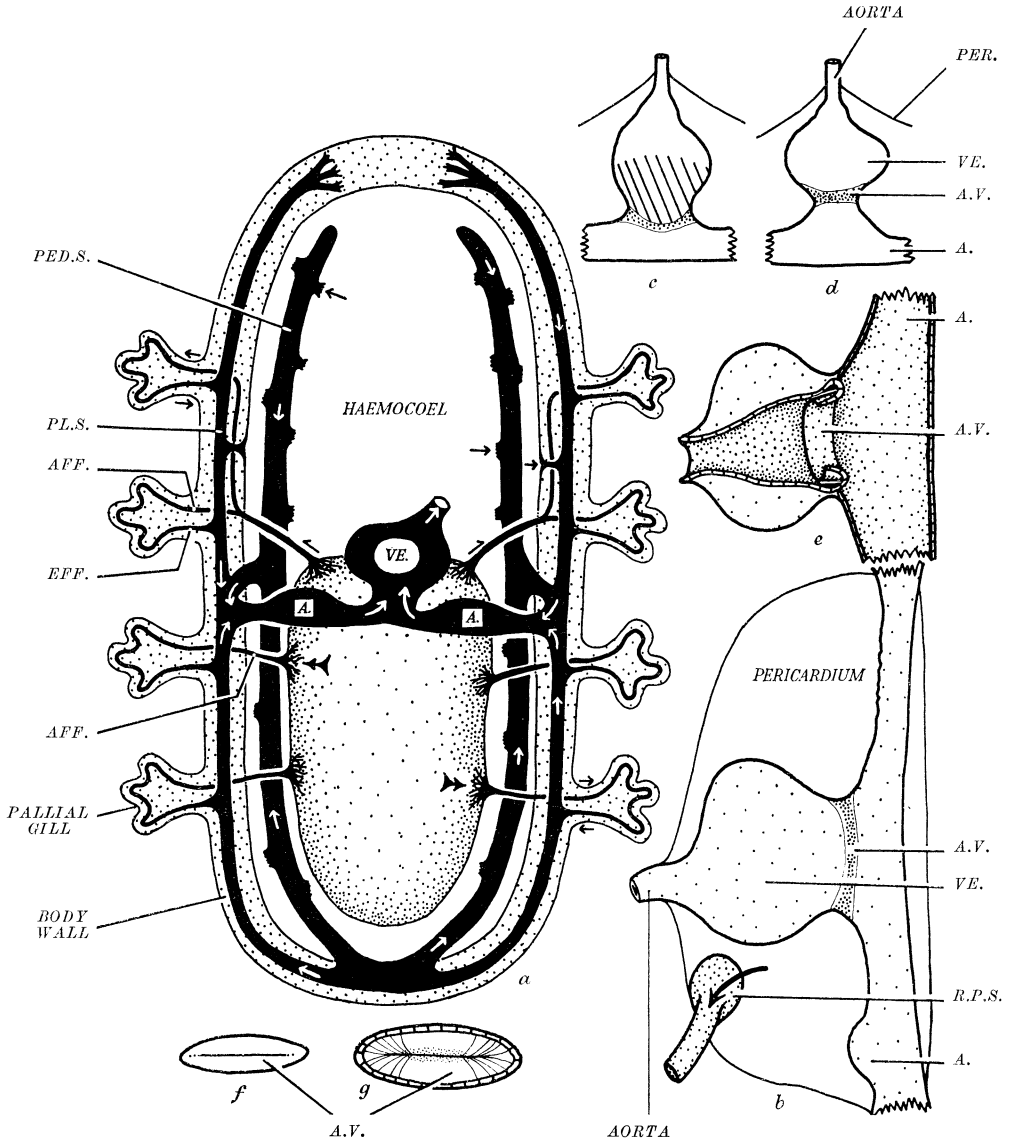

FIGURE 21. The circulatory system. *a*. Diagram showing the blood system, excepting the factors of the aorta (which are illustrated in figure 20); dorsal aspect. Based on dissections of injected specimens and on serially sectioned material. The arrows, which show the direction in which the blood travels in the various vessels, reflect the results of injection experiments with adult slugs. *b*. Dissection to show the heart from the ventral aspect. In this specimen the auricular expansion was absent on the left side; this is a rare condition. The arrow shows the direction in which particles were impelled following experimental injections into the pericardial cavity. *c*. The heart in a vivisected specimen, showing the shape of the ventricle during diastole; ventral aspect. The auriculo-ventricular valve is open. The region of the heart which contracts during ventricular systole is hatched. *d*. The heart in a vivisected specimen, showing the shape of the ventricle during systole; ventral aspect. The auriculo-ventricular valve is closed. *e*. Dissection to show the auriculo-ventricular valve, exposed by a slit in the ventral wall of the ventricle and the removal of parts of the auricles. Cut surfaces are shown hatched. *f*. The auriculo-ventricular opening viewed from the auricle. The valve is closed. *g*. The same, viewed from the ventricle.

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into the kidney is effected. This was established by injecting india ink into the pericardial and other cavities. Within the syrinx the much-folded endothelium is not ciliated, nor is it markedly glandular (this view conflicts with that of Hancock 1865).

Two days after metamorphosis the heart may be observed beating. The actual details of the mode of origin of the auricles and ventricle could not be established. At first (figure 18*d*, *e*, *HT.*) the heart lies wholly on the animal's right side, but, as post-metamorphal development proceeds, it comes to lie nearer the median plane (figure 21*a*, *VE.*). Ventricular systole involves the contraction of only a proportion of the wall of that organ (see figure 21*c*, *d*) and results in blood being driven into the aorta. Valves guard the auriculo-ventricular opening (figure 21*e*, *f*, *g*, *A.V.*) and the point of exit of the aorta from the ventricle. Puncture of the pericardium in a vivisected specimen impairs, but does not halt, the contractions and dilations of the ventricle; Krijgsman & Divaris (1955) suggest that the dilation of the molluscan ventricle at diastole can occur only if the pericardium exists as an intact closed sac. The weak contractions of the auricles serve only to fill the ventricle.

When the young tritoniids have reached a length of approximately 3 mm, accessory respiratory structures, the branchiae, begin to develop as outpushings, at first digitiform, later arborescent, of the lateral pallial ridges on each side of the body (figure 19). In my view, Herdman (1890) was wrong in discounting the possibility that these pallial outgrowths function as respiratory structures in *Tritonia*. Each outgrowth contains an efferent and an afferent blood vessel. The branchiae receive their afferent supply through closed vessels which travel from the digestive gland; the efferent vessels drain into the two longitudinal pallial sinuses which run in the dorso-lateral body wall. These pallial sinuses empty into the auricles, after receiving a branch on each side from the extensive pedal system of sinuses. At the posterior extremity of the visceral mass, pallial and pedal sinuses merge.

Most of the major components of the circulatory system are shown in figures 20 and 21. Although there are no true capillaries it can be seen that much of the system runs in closed vessels. In the supply of the digestive gland and of the branchiae both the afferent and the efferent blood travels in closed vessels. Much of the description of Alder & Hancock (1845–55), based on dissections of the blood system of adult tritoniids, was confirmed in the course of the present investigation.

F. *Alimentary system*

(i) *Structure*

The buccal mass (figures 12*c*, 15*f*, 22) increases in size throughout benthic life and so also does the radular ribbon. The buccal mass of the adult *T. hombergi* is, according to Alder & Hancock (1845–55), larger than that of any other nudibranch. During the progressive enlargement of the radula the shape of the teeth changes. In young post-larvae up to a length of approximately 2 cm the lateral teeth of the radula bear one or more subterminal sharp spines (figure 24*b*, *c*); in specimens larger than 3 cm these subterminal spines are absent (figure 24*d*). I have in my possession a preparation of the radula of a specimen collected in the field on 9 September 1958, 25 mm in length, which shows a stage transitional between the two types of dentition, with smooth laterals at the proximal (growing) end of the radular ribbon, while at the distal extremity are a number of lateral

teeth which possess typical subterminal spines. A further difference between the radulae of very small and those of larger specimens is that the central teeth of young specimens (up to a length of approximately 3 cm) bear numerous denticulations (figure 24*a*, *DENT.*) which are absent in adult radulae. Throughout life growth of the radula is more marked in breadth than in length. The adult radula and jaws are illustrated by Alder & Hancock (1845–55) and descriptions given by Cuvier (1803), Bergh (1880) and Eliot (1910).

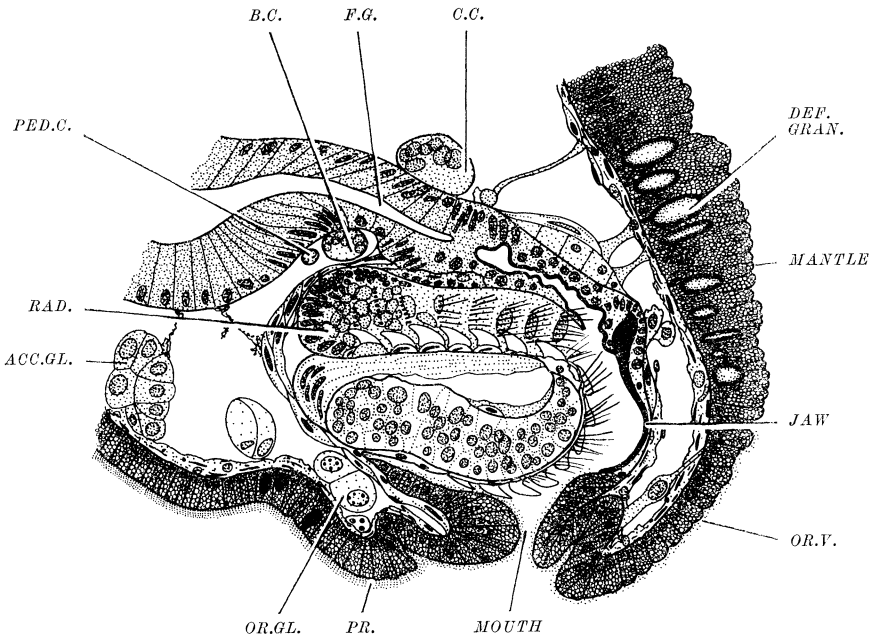

FIGURE 22. Portion of a longitudinal section through the anterior end of a juvenile 1·1 mm in length, showing the buccal mass and the ganglionic commissures.

With this information about the radula in benthic stages of *T. hombergi* it is clear that *T. alba* Alder & Hancock 1854 (see Alder & Hancock 1845–55, appendix vi, and Eliot 1910, pp. 93–4 and plate I, fig. 10) is merely a growth form of *T. hombergi* Cuvier. It is understood that Miller (1958) has made this discovery, which establishes the taxonomic identity of *T. alba* with *T. hombergi*, independently.

Large numbers of multicellular, flask-shaped oral mucus glands (figure 22, *OR.GL.*) develop around the mouth and discharge into the initial region of the alimentary tract, the oral canal (figure 23*a*, *OR.C.*). These glands correspond to the buccal glands described by Pelseneer (1894) for *Duvaucelia plebeia*. The salivary glands enlarge (figures 15*f*, 20*a*, *SAL.GL.*) and take on their characteristic histological appearance. The adult salivary gland contains secretory cells of two types. After fixation with Lewitsky-saline and dyeing with safranin and light green, the cells of one type, constituting the majority, have an irregularly shaped nucleus and pink cytoplasm, while the cells of the second type have an oval nucleus with prominent nucleolus and green cytoplasm.

Whereas in early post-larvae the stomach and the point of its communication with the hindgut lie on the right side of the body (figure 15*b*), differential growth changes later result in the return of these structures approximately to the median plane (figure 15*e*, *f*). As the digestive gland enlarges, both right and left lobes begin to be subdivided into

lobules (figures 15*e*, 18*e*, *f*). This process affects first the larger left lobe. For some time both lobes of the digestive gland retain separate openings (figure 15*e*) into the stomach. As lobulation becomes more complex, tongues of the wall of the stomach are drawn out

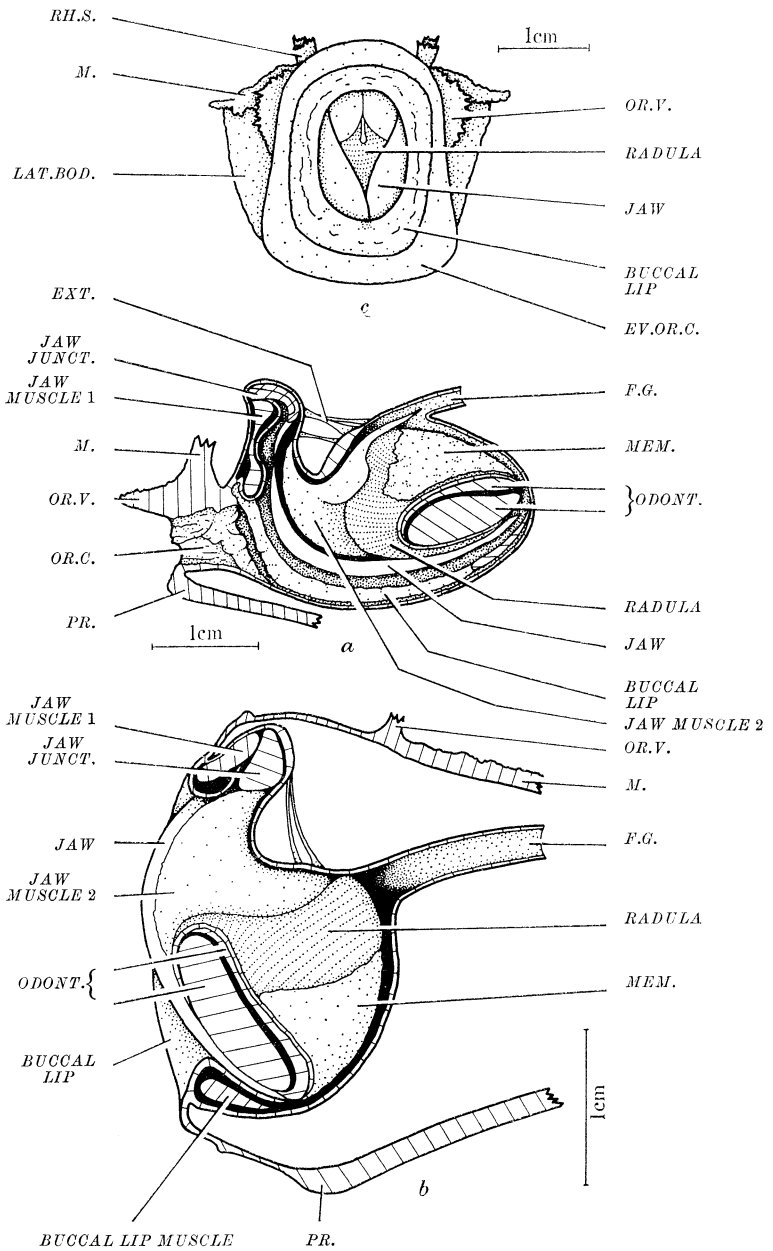

FIGURE 23. The adult buccal mass. *a*. Right sagittal half of the retracted buccal apparatus. *b*. Right sagittal half of the fully extended buccal apparatus. *c*. Front view of the extruded buccal mass. In *a* and *b* cut surfaces are hatched.

to form the ciliated conducting tubules (figures 25*a*, 27*a*, *COND.T.*) and this so complicates the picture that it soon becomes impossible to distinguish lobules of the original right lobe from those of the left. Similarly, the situation at the junction between the stomach and the conducting tubules becomes complex so that these appear to form, not the original two, but many hepato-gastric connexions (figure 25*a*, *COND.T.*). On death,

the stomach may so contract as to give the impression in preserved specimens that there existed a single huge hepato-gastric opening.

The appearance of the alimentary canal of the adult tritoniid is shown in figure 15*f*. The adult digestive gland appears to be a single structure, that is, it is holohepatic.

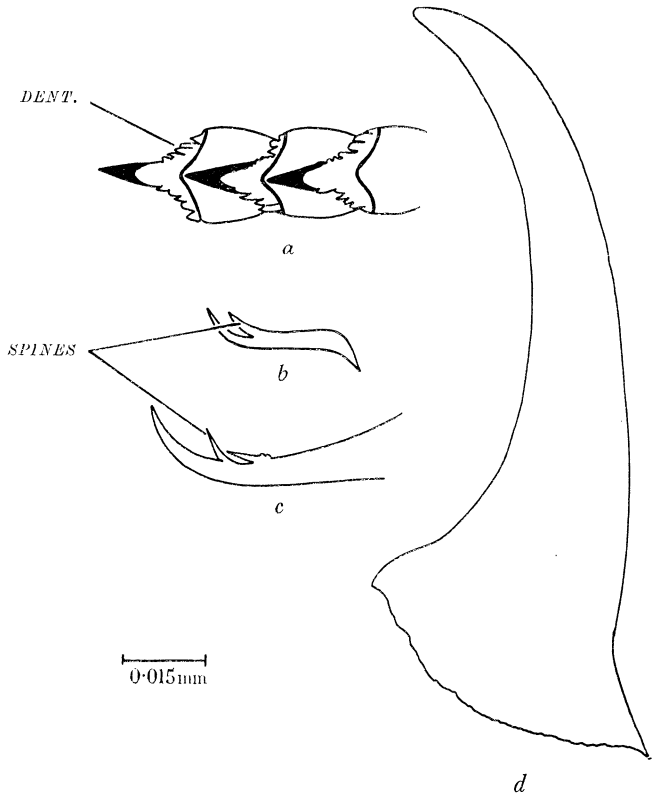

FIGURE 24. Radular teeth. *a*. Central teeth of a laboratory-reared specimen, 4½ mm in length. *b*. A lateral tooth from the same. *c*. A lateral tooth from a laboratory-reared specimen, 12 mm in length. *d*. A lateral tooth from a specimen from the field (October 1958), 30 mm in length.

A further feature of the ontogeny of *T. hombergi* to be dealt with here is the development, as a simple longitudinal endothelial fold, of a great typhlosole in the hindgut, after a body-length of approximately 10 mm is attained. This typhlosole becomes so massive (figure 25*a*, *TYPH.*) as to close off, except for a narrow slit, the hindgut from the lumen of the stomach. The terminal region of the hindgut forms, in juvenile tritoniids, a thinner walled dilated sac; in the adult this is not the case.

Some observations on the histogenesis of the walls of the stomach, foregut and hindgut have already been mentioned. An interesting feature is that, in common with many eolidacean nudibranchs (Graham 1938), in the stomach and hindgut of post-larval *Tritonia* there are no recognizable mucus glands; presumably the secretions of the oral, salivary and digestive glands are sufficient to lubricate the whole of the alimentary tract. This contrasts sharply with the sponge-eating dorid *Archidoris pseudoargus*, where lubricating glands are abundant throughout the gut (Forrest 1950, 1953).

The digestive cells (figures 26, 27) are very variable in shape, size and staining qualities, but there are no firm grounds for believing that more than one *type* of cell is present in the

digestive lobules. No lime-cells (Fretter 1941, 1943, 1948, 1951*a*; Graham 1938) were present in the digestive gland; if their function in other molluscs is connected with the maintenance of the pH of the digestive fluid (Fretter 1941) their absence in *Tritonia* is perhaps to be expected: live *Alcyonium digitatum* was found to contain 7 to 10% calcareous matter (spicules) by weight. None of the digestive cells is ciliated; cilia were carefully sought both in sectioned and intravitally stained material. Graham (1938) has called attention to the misconception of the mode of action of the digestive apparatus which may have led to the description, by various early workers, of a large number of types of digestive gland cells in some molluscs.

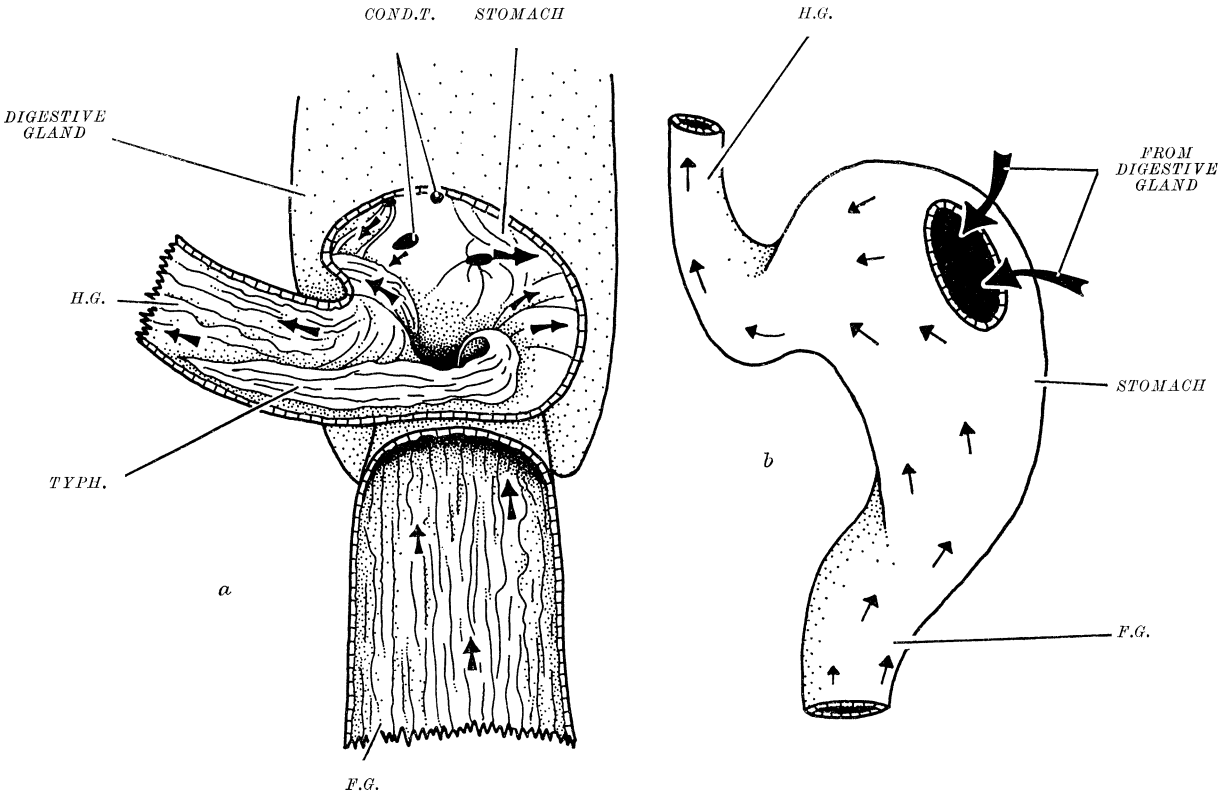

FIGURE 25. Ciliation of the stomach. *a*. Currents detectable in a vivisected adult after dorsal slits have been made to open up the hindgut and foregut; from the dorsal aspect. Cut surfaces are hatched. *b*. Diagram summarizing observations on the effects of the cilia of the foregut, stomach and hindgut. From the left lateral aspect. In both *a* and *b*, anterior is to the foot of the page.

(ii) *Feeding behaviour*

Post-larval stages of *T. hombergi* feed exclusively, both in nature and in the laboratory, on *Alcyonium digitatum*. This view is supported by the observations of Alder & Hancock (1845–55), Dalyell (1853), M'Intosh (1863), Hecht (1895), Walton (1908), Farran (1909), Hunt (1925), Odhner (1926) and Miller (1961). Stomach and hindgut contents of a large number of specimens brought in from the field at all seasons of the year consisted entirely of this prey, with some fragments of its epifauna and epiflora. *Alcyonium* contains numbers of calcareous spicules (in their longest dimension varying from 0·03 to 0·27 mm) which, although failing to prevent the attacks of larger tritoniids, severely restrict the feeding

activities of the young ones. Until the juvenile *T. hombergi* reach a length of 7 to 8 mm they are unable to ingest any of these spicules and feed simply by browsing on the living outer covering of the alcyonarian colony. Larger juveniles begin to bite slightly deeper and to ingest some of the spicules. These slugs do not select the openings of the zooids (which represent by far the 'meatiest' parts of the colony) but browse at random. (This was established by examination of faeces of small slugs; spicules of the zooid bodies are more elongated than those from interstitial areas and so can be easily identified.) As adult size is reached increasingly large pieces of the cnidarian may be bitten away; colonies of *Alcyonium* dredged in the spring and summer usually bore recognizable scars of such bites.

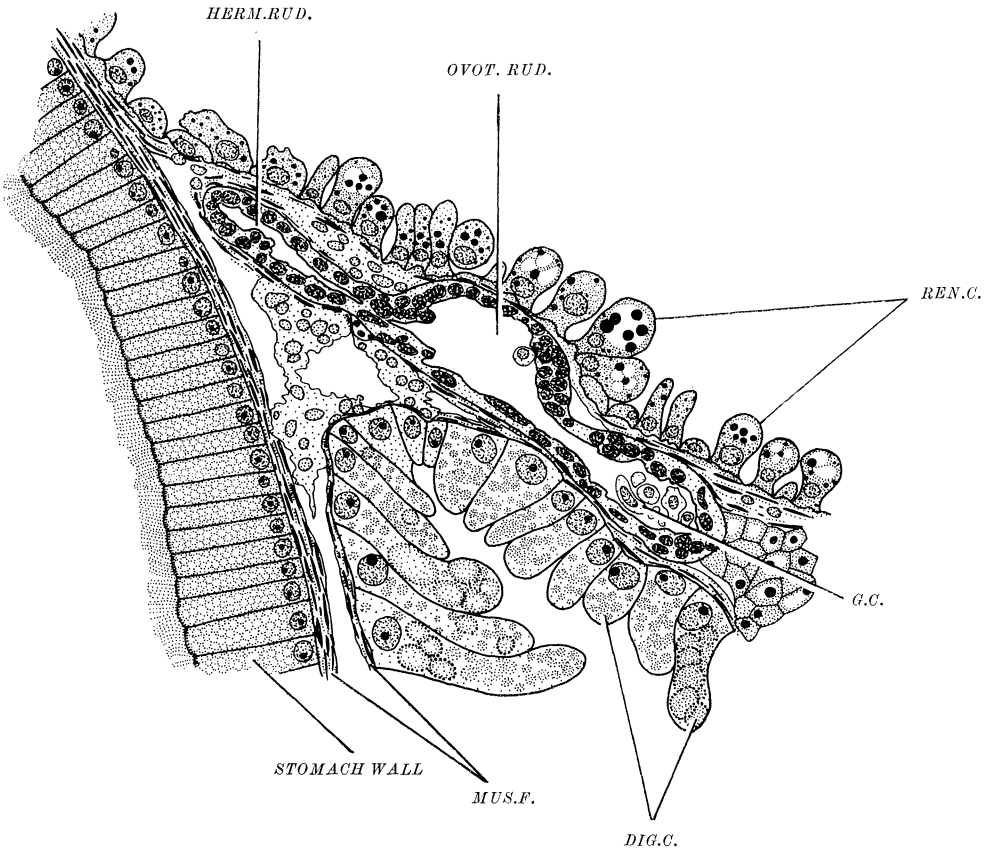

FIGURE 26. Portion of a transverse section through a juvenile 11 mm in length, passing through the stomach, digestive gland, kidney and the rudiment of an ovotesticular sac. The specimen was killed 3 h after food, following a period of starvation. Ciaccio and azan.

Both white and orange varieties of *A. digitatum* occur off the Isle of Man. It is interesting that only the former appears to be suitable as food for *T. hombergi*, contrary to a statement made by Dalyell (1853). The pigment resides mainly, if not entirely, in the spicules. If a tritoniid is given only an orange alcyonarian colony it will eventually eat it but the effect is a purgative one and soon afterwards semi-digested matter is extruded in the faeces. This effect was noted on a number of occasions; it never followed a meal of white *Alcyonium*.

The feeding mechanism is a remarkable process, during which the large buccal mass is extruded through the everted oral canal (figure 23*b*, *c*) and the oral veil is placed, like a hood, over a projecting region of an alcyonarian colony. The jaws are brought together

with some force and a piece of the prey is passed into the foregut by violent movements of the radula and contractions of the musculature of the buccal mass. The cutting edges of the jaws bear, in young slugs, numerous minute denticulations which are largely obliterated during adult life. A large tritoniid, in length 13 cm, was seen to bite off with a single movement a piece measuring $15 \times 10 \times 5$ mm. Several such pieces may be eaten within a few minutes, before a pause during which digestion begins. Another meal is often taken before the completion of the digestion of the last. Alder & Hancock (1845–55) and Eliot (1910) were incorrect in stating that the prey is torn up by the radula and in believing that the jaws function simply as prehensile organs.

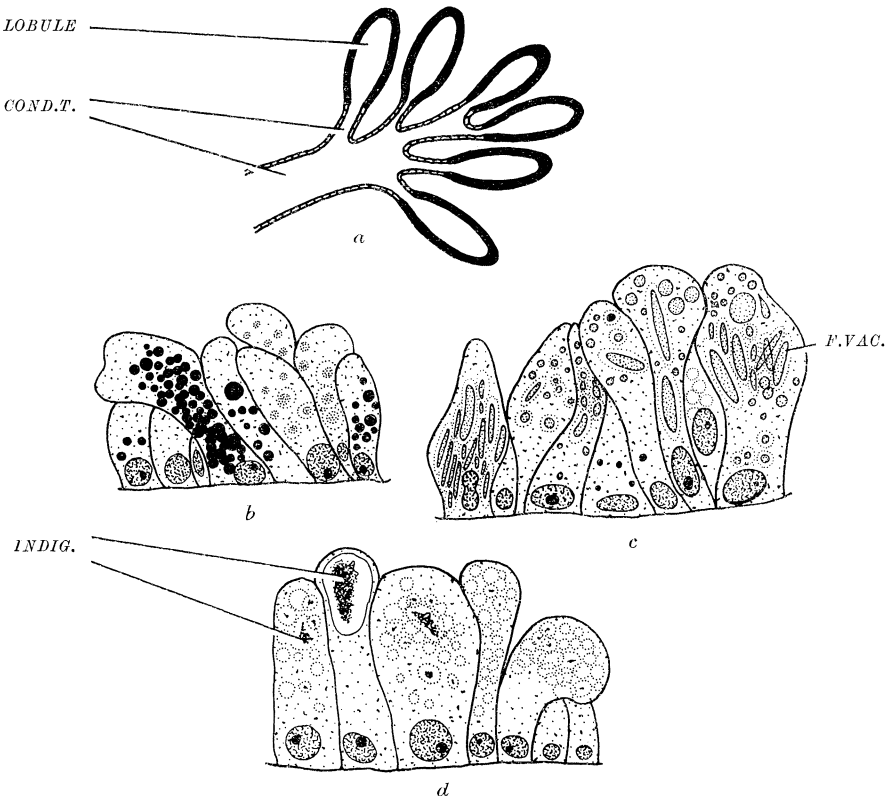

FIGURE 27. Histology of the digestive gland. *a*. Diagrammatic representation of the relations between the digestive lobules and the ciliated conducting tubules leading to the stomach. *b*. Portion of a section through the digestive gland of a juvenile 10 mm in length, which had been starved for 1 week. Lewitsky-saline and azan. *c*. Portion of a section through the digestive gland of a juvenile 10 mm in length, which had been starved for 3 days, allowed to feed for 1 h and then killed 4 h later. Lewitsky-saline and azan. *d*. Portion of a section through the digestive gland of a juvenile 10 mm in length, which had been starved for 4 days, allowed to feed for 1 h and then killed 24 h later. Lewitsky-saline and azan.

(iii) *Ciliation*

All regions of the alimentary canal are ciliated with the exceptions of the digestive lobules and of much of the buccal mass (which is 'chitinized'). All the cilia beat in effect towards the anus, in such a way that india ink, carmine powder or insoluble iron saccharate are transported straight through the gut, without ever entering the digestive gland. The ciliation of the stomach is shown in figure 25*a*, *b*; the currents all pass upwards towards

the opening into the hindgut. In addition to the hindgut typhlosole there are numerous minor ridges which tend to resist any tendency for heavy particles (such as alcyonarian spicules) to fall back into the stomach.

Such simplification of the molluscan stomach is (Graham 1949) often associated with the development of a macrophagous carnivorous habit and extracellular digestive processes, and is found in many pulmonates, tectibranchs and nudibranchs, as well as in some prosobranchs, for instance *Cerithiopsis tubercularis* which feeds on the sponge *Hymeniacidon* (Fretter 1951*a*).

(iv) *Transport of materials within the tract*

Pieces of *Alcyonium* are passed into the stomach by a combination of the action of the foregut cilia and of pulsating contractions of the muscle sheath of that organ. In the muscular stomach digestive breakdown begins and alcyonarian spicules begin to drop from the food in immense numbers. These come under the influence of the cilia lining the stomach and pass out across the hindgut typhlosole (figure 25*a*, *TYPH.*). The mechanism by which excessive loss of the actual nutritive content of the food is prevented clearly depends for its success on differences in the density and sinking rates of its different constituents. The efficiency of this mechanism is evidenced by the facts that food matter is never found in the faeces (after a meal of white *Alcyonium*) and that cnidarian spicules never enter the digestive gland.

Soon after digestion begins, food particles begin to enter the digestive gland and cytoplasmic food vacuoles are seen in histological preparations of the digestive cells. The mechanics of entry of particles and fluids into the digestive gland in molluscs has been a matter for controversy. Among the factors believed to be involved are the following.

(*a*) Contractions of the musculature of the stomach wall (assisted in some cases by contractions also of parts of the foregut) have been suggested for tectibranchs by Fretter (1939), for some sacoglossans by Fretter (1941), for *Archidoris pseudoargus* by Forrest (1950, 1953), for *Spiratella retroversa* by Morton (1954), for the fissurellid *Scutus* by Owen (1958), for lamellibranchs of the Petricolidae and Pholadidae by Purchon (1955*a*, *b*), for the basommatophoran *Otina* by Morton (1955*b*), and for chitons by Fretter (1937).

(*b*) Contractions of the pedal musculature, resulting in volumetric changes in the various components of the alimentary canal, have been suggested for the clam *Schizothoerus* by Patterson (1933) and for petricolid and pholadid lamellibranchs by Purchon (1955*a*, *b*). Purchon suggests that contractions of the adductor muscles may also make a contribution.

(*c*) Contractions of a smooth-muscle sheath of the digestive lobules themselves have been observed to be at least partly responsible in eolidacean nudibranchs by Alder & Hancock (1845–55) and Graham (1938), in the sacoglossan *Alderia modesta* by Evans (1953), in larval nudibranchs by Thompson (1959), in larval *Ostrea* by Millar (1955), and in some ellobiid pulmonates by Morton (1955*a*). Such a mechanism is known to be of great importance in the brachiopod *Lingula* (Chuang 1959).

(*d*) The contribution made by the action of the cilia lining the conducting tubules is difficult to evaluate. In the dorid nudibranchs *Archidoris pseudoargus* (Forrest 1953) and *Jorunna tomentosa* (Millott 1937) it is suggested that at least some of the cilia in these tubules beat effectively in the required direction. Similarly, some of the ciliary tracts in the eolid

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stomach may, according to Graham (1938), conduct particulate matter towards the digestive gland openings. In most molluscs (including *Tritonia*) such cilia as may be present in the conducting tubules of the digestive gland beat in such a way as to produce a current passing towards the stomach, that is, in the 'inappropriate' direction. Owen (1955) suggests that a counter-current may occur in the peculiarly shaped tubules of some lamellibranchs, but it seems unlikely that this theory has general applicability outside the Eulamellibranchia and Anisomyaria. In the main, it is probable that in molluscs ciliary mechanisms (unless periodic ciliary reversal is widely demonstrated in the way claimed by Forrest 1950) have little responsibility for bringing about the traffic of particles and fluids from the lumen of the stomach into the digestive gland.

In *Tritonia* it was possible to ascertain with clarity the means by which food particles are forced into the lumina of the digestive gland lobules of the juvenile benthic stages; the position with regard to the adult tritoniid is less satisfactory.

In young ones, approximately 1 mm in length, the whole process may be watched under the microscope; dilation following a contraction of the muscle sheaths of the right and left lobes of the digestive gland (figure 26, *MUS.F.*) may be seen to draw food particles into their lumina from the stomach. The two lobes may contract together or independently. Such contractions may be observed with ease during the early life of the tritoniid but after a size of approximately 1 cm has been attained the mantle becomes too opaque to permit this. In adult tritoniids there is little reason to doubt that a similar mechanism exists.

The movement of waste materials (chiefly the dissociated calcareous spicules of the prey) through the hindgut is brought about chiefly by ciliary action, aided no doubt by the slow muscular movements of the hindgut wall and typhlosole which have been observed to occur. Wastes form a diffuse mass in the terminal region of the hindgut and the loosely compacted faeces are expelled by muscular action from time to time.

(v) *Digestion*

No biochemical tests were made on the digestive enzymes, but the general pattern of digestion can nonetheless be made out. The secretions of the salivary glands and of the digestive gland act on food in the stomach in such a way as to coagulate and fragment the cnidarian mesogloea, liberating the calcareous spicules. The secretions of the salivary glands enter the tract through the ciliated salivary gland ducts; it is possible that this entry is aided by the contraction of the delicate muscle sheath of the glands. The digestive gland secretions are passed into the stomach by the beat of the cilia lining the conducting tubules, although again it is possible that contractions of the muscle sheath of the gland may assist.

In juvenile tritoniids up to a length of approximately 1 mm, cellular ingestion of food particles occurs in the walls of the stomach, hindgut and digestive gland, in all of which food vacuoles may be seen. In later benthic stages, however, where digestion is obviously brought about by both extracellular and intracellular means, the ingestion of food particles occurs solely in the cells lining the digestive lobules. (The role of amoebocytes in the lumen of the stomach is difficult to evaluate.) The absorption of fluids in the adult probably still occurs in addition through the wall of the stomach; coagulated densely staining masses may occasionally be observed in sections through the stomach wall at appropriate

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stages of digestion. Graham (1938) noted a similar phenomenon in some colidacean nudibranchs. The foregut and hindgut function, in adult tritoniids, simply as organs of conduction.

(*a*) *Pattern of feeding and digestion under natural conditions*

Sections through the digestive glands of tritoniids removed from an alcyonarian colony on which they had been browsing in nature, show a highly complex picture. Cells at all stages of the digestive process are visible and interpretation of the functional sequence of changes is impossible; this is a consequence of the fact that the slugs do not await the complete assimilation of one meal before beginning the next.

Faecal pellets of naturally browsing tritoniids nearly always contain large numbers of alcyonarian spicules (as do the faeces of the cypraeid *Simnia patula* (Fretter 1951*b*) which has the same diet). Also in the pellets customarily are a few broken radular teeth, a little finely particulate brown matter, some coarse brown vegetable matter (algal growth from the surface of the prey), often large numbers of small clear lipid globules and invariably vast numbers of bacteria (of two kinds, the majority being ovoid, while the remainder are rod-shaped). It is regrettable that, regarding the role played by these bacteria in the digestive processes of *Tritonia*, nothing can be said. It is not even certain whether they are part of the indigenous gut flora of the slug or are taken in with the food. A small number of pellets contain only large numbers of lipid globules and of bacteria; such pellets are physically unstable and may 'explode' at a touch.

(*b*) *Pattern of feeding and digestion in laboratory conditions*

Because of differences in the pattern of digestion correlated with temperature and with the size of the animal, the following account will relate throughout to tritoniids in length 8 to 12 mm maintained at 15 °C. Most of the experimental work was done using animals in this size-range because they are readily amenable to laboratory culture and, furthermore, they can be sectioned whole, thus preserving the natural relations of the various organs. It is important to note that the times required for the various phases of digestion are much longer in larger individuals; this is probably correlated with the relative surface area of the food-mass ingested.

To understand the digestive pattern it is necessary to study first the changes which occur if the animals are starved and then the changes occurring after renewed access to food.

Effects of starvation. During the first day after isolation from food the tritoniid produces faecal pellets of the usual type, containing numerous alcyonarian spicules. After this, pellets continue to be produced but they now contain only abundant colourless lipid globules, some fine brown matter, bacteria and a few radular teeth (which continue to be discarded from the radula even in starved individuals). The significance of the faecal lipid globules and fine brown matter can be illuminated by studying sections through the digestive gland of specimens starved for 1 week. In the cytoplasm of the digestive cells (figure 27*b*) and in the lumina of the conducting tubules are numbers of small spherules (after fixation with Lewitsky-saline these stain red with azan, black with Heidenhain's iron haematoxylin). These spherules resemble closely those described by Graham (1938) in the digestive cells and ducts of starved *Trinchesia glottensis*. It seems certain that they

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are identical with (or give rise to) the colourless lipid globules seen abundantly in the faeces of starved tritoniids; probably these globules are true excretory products for they are certainly not indigestible remains of a previous meal. These latter are in sections easily identifiable, lying in large cytoplasmic vacuoles in a small number of digestive cells; material liberated by the rupture of such vacuoles gives rise to the finely particulate brown matter found in the faeces. This brown matter represents what Forrest terms (1953, p. 228) the extrusion matter.

Response of starved animals to food. Animals starved for 1 week will begin to feed immediately they are permitted access to *Alcyonium*. Sections through the digestive glands of such individuals show the digestive cells (figure 26, *DIG.C.*) to be loaded with secretory globules, which stain blue with azan. These globules are liberated into the conducting tubules; sections and intravitally stained preparations show the conducting tubules filled with numbers of cytoplasmic spherules (probably the former tips of digestive cells) containing masses of secretory droplets. This semi-fluid secretion is carried into the stomach where evidently enzymes are liberated from the spherules since some breakdown of the tissues of the prey is manifest after only a few minutes' exposure to the gastric fluid.

It has been mentioned that various fractions of the rapidly dissociating food-mass are treated differently in the stomach, this being governed apparently solely by their density and sinking rate. The relatively heavy calcareous alcyonarian spicules are carried out of the stomach by ciliary tracts leading into the hindgut, while the lighter fractions of the cnidarian remain in suspension in the tritoniid's stomach. (No spicules were ever found in the digestive gland.) The possible means by which particulate food matter is passed into the digestive gland lobules have been discussed.

Three hours after the commencement of the meal, cytoplasmic food vacuoles are abundant in the digestive cells (figure 27*c*, *F.VAC.*). Fragments of coagulated cnidarian mesogloea are readily identifiable in sections through the digestive gland. During the following twelve hours sections show (figure 27*d*) a progressive diminution in the size of the food particles within the vacuoles. The vacuoles tend to coalesce so that brown, finely particulate indigestible matter is concentrated in one or two large vacuoles per cell (figure 27*d*, *INDIG.*). Their contents finally are discharged, over a period of many days, to be carried into the stomach and so into the hindgut. Excretory globules again appear abundantly in the digestive cells and the faeces once more begin to contain abundant colourless lipid globules.

In these paragraphs the digestive process has been reduced to the simplest situation. This might give the impression that there was a cyclical rhythm in the digestive gland which was manifest under natural conditions. This, if it implies a co-ordination of activity throughout all the digestive cells, is not the case. It is only after a period of enforced starvation that all the digestive cells are brought into step, as it were. When food is provided and the digestive cells begin their secretory and absorptive tasks, they soon become out of phase with one another, since individual cells may receive different proportions of the food-matter available and will therefore complete digestion at varying times. Sections through the digestive gland again soon show cells at all stages in digestion, extrusion and excretion. There is, however, generally more agreement of phase among the cells of a single digestive lobule than between adjacent lobules.

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The conclusion to be drawn is that, while individual digestive cells do pass through a sequence of changes during the discharge of their various functions, the complete digestive gland acts arhythmically under conditions of constant access to food.

G. *Reproductive system* (figures 26, 28)

The mature reproductive complex and stages in the maturation of the ovotestis have already been described (Thompson 1961); it is only necessary now to describe the earliest development of the genital organs. The system originates from two separate rudiments; one of these gives rise to the ovotestis (figure 26, *OVOT.RUD.*) and its hermaphrodite duct (figures 26, 28, *HERM.RUD.*), while the other (figure 28*a*, *ANT.GEN.RUD.*) develops into the anterior genital mass of the adult. The two rudiments first become detectable in post-larvae $1\frac{3}{4}$ mm in length (approximately 10 to 14 days after metamorphosis). The

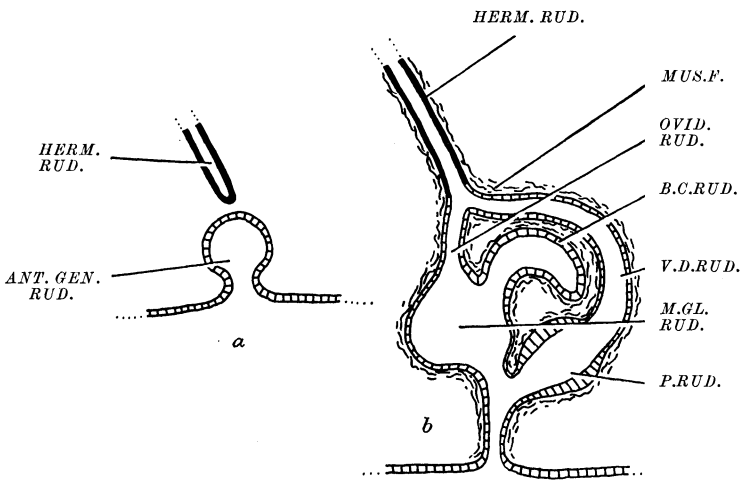

FIGURE 28. Diagrams illustrating the mode of development of the anterior genital mass. *a*. Diagrammatic section through a portion of the right side of a juvenile 4 mm in length. *b*. A similar section through a juvenile 11 mm in length. Parts of the genital system derived from the ectoderm are hatched.

ovotestis rudiment consists of a number (3 to 5) of small sacs lying against the outside of the digestive gland. These contain the primordial germ cells (figure 26, *G.C.*); they are linked to a strand of cells (soon to form the tubular hermaphrodite genital duct) which travels forwards through the haemocoel to end blindly close to the stomach.

The tubules of the anterior genital mass develop from an invagination (figure 28*a*, *ANT.GEN.RUD.*) of the antero-lateral body wall on the right side, a short distance anterior to the anus. This ectodermal invagination expands at its innermost extremity, forming a lobed chamber. Two of the lobes (the rudiments of respectively the oviduct (figure 28*b*, *OVID.RUD.*) and the vas deferens (figure 28*b*, *V.D.RUD.*)) extend into the haemocoel to meet and fuse with the nearby anterior extremity of the rudiment of the hermaphrodite duct. Two further lobes form the rudiments of the blindly ending bursa copulatrix (figure 28*b*, *B.C.RUD.*) and genital mucus gland (figure 28*b*, *M.GL.RUD.*). Mesodermal muscle fibres (figure 28*b*, *MUS.F.*) aggregate around the genital ducts and greatly thicken these originally fine, delicate tubes.

The later development of the gonad involves a great enlargement and multiplication of the original few ovotesticular sacs and an accompanying increase in the complexity of their annectent hermaphrodite ductule system. At the same time the organs of the anterior genital mass grow and take on their definitive histological and anatomical appearance (Thompson 1961).

7. Discussion

T. hombergi is closely associated throughout its life with the cnidarian *Alcyonium digitatum*. The resemblance to a parasite/host relationship is made striking by the ability of searching tritoniid veliger larvae to recognize alcyonarian tissues and to settle and metamorphose upon them. The benthic stages of the tritoniid life cycle are, in the laboratory, very sluggish in the presence of plentiful food, but may move with surprising speed if starved. Reasons have been given (Thompson 1961) for believing the life cycle to be an annual one, the adults dying towards the close of the protracted spring breeding period and being replaced by their progeny. It may be inferred that tritoniid larvae which settle on the natural *Modiolus* bed which was studied would have need to move about but little during their lives, since *Alcyonium* is abundant there.

The phylogeny of the naked Opisthobranchia is complex; there is little to be gained by taking up a rigid position on such matters, as was done, for instance, by Pelseneer (1891, 1894) who averred that the Sacoglossa had arisen from the eolid nudibranchs, which, together with the dorids, had sprung in turn from Tritoniidae. Another over-rigid view is put by Fretter (1939, p. 641): 'It is commonly accepted that the nudibranchs have arisen from the Bulloidea by way of the elysiomorphs.' My own view agrees closely with that expressed by Morton (1958) and is that the naked opisthobranchs are an assemblage consisting of the modern representatives of a variety of independent lines of evolution within the Gastropoda. In consequence, while it is still possible to speak of degrees of primitiveness in the nudibranchs and sacoglossans, it is incorrect to infer from this, as apparently did Pelseneer, that the 'primitive' modern forms are, were, or could be ancestral to the more 'advanced' ones. It is time that the polyphyletic origin of the modern sea-slugs was more generally recognized and such un-natural assemblages of convergent forms as the Cladohepatica, Aeolidacea, Holohepatica and Dendronotacea banished from the systematic literature. It is impossible to doubt, however, the truth of Pelseneer's (1891, 1894) conclusion, that the tritoniid nudibranchs show the basic condition of many of the trends detectable in the evolution of the naked opisthobranchs, and *Tritonia* may thus be considered to be among the most primitive living nudibranchs.

The most unequivocal statement concerning the phenomenon of developmental torsion in opisthobranchs was made by Pelseneer (1906, p. 78–9): 'It should be noted that in those Euthyneura which are detorted in the adult condition, the primitive torsion is manifest in the course of development, and in the larvae the pallial cavity is anterior and dorsal, the anus anterior, just as is the case in an adult Streptoneura.' In 1911 Pelseneer published the results of a study of the development of a number of species of opisthobranchs, results which appeared to bear out fully the conclusion to which he had come in his 1906 *Treatise*. At that time this conclusion was widely used to support the theory that the prosobranchiate condition in gastropods was ancestral to the opisthobranchiate, but, as has already been

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pointed out (Thompson 1958*a*), the invalidation of that conclusion in no way attacks this well-established theory.

Other views of torsion in opisthobranchs have come from several authors. Boutan (1899) at first claimed that opisthobranchs did not manifest any torsion during their development: all that happened was that, at an early stage, the anus and hindgut alone moved through approximately 90° from a midventral to a lateral site (this was his 'déviation larvaire'). He later stated (Boutan 1902), on the basis of his observations on developing *Philine*, that this movement involved not only the hindgut, but also the rest of the visceral mass. Pelseneer (1911) was then clearly correct in inferring from this that torsion in *Philine* must be homologous in essence (if not in degree) with the developmental twisting of, for instance, the diotocardian prosobranch *Haliotis*. (Boutan himself, however, did not take that view; as Yonge (1947, p. 483) puts it, 'It is difficult to summarize his unique views of torsion.')

In *Tethys* (Viguier 1898) and in *Fiona* (Casteel 1904) developmental torsion apparently does not involve a rotation of the whole visceral mass but only the movement of the anal complex (hindgut and larval kidney) from a ventral to a more lateral position. This movement is presumably brought about by differential growth processes. In *Aplysia* (Saunders & Poole 1910) the situation is similar except that the digestive gland and the so-called coelom (probably this is the blastocoelic inner perivisceral cavity—see Thompson 1958*a*) are also involved in some degree in the torsional movements; the change in the position of the attachment of the larval retractor muscle which these authors describe for *Aplysia* is difficult to interpret.

In *Tritonia hombergi* it has been shown that developmental torsion, accomplished by differential growth processes well in advance of the histogenesis of any muscular elements, takes place at such an early stage that it is complete before the visceral organs are differentiated. The anal cells, which are known to predict the site of the proctodaeal invagination, were observed to move a short distance during post-gastrulation development to take up their final position low down on the embryo's right side (figure 1*g* to *l*, *AN.C.*). This movement is the sole vestige in *T. hombergi* of the ontogenetic mechanical processes of gastropod torsion and ventral flexure.

In the dorid *Adalaria proxima* not even these small external signs of the process of torsion are visible; not only the viscera but also the anal cells are, at the time of their first appearance in the embryo, in their definitive larval positions (Thompson 1958*a*).

If it is accepted that Opisthobranchia are descended from prosobranchiate ancestors, then it is clear that along the series *Philine*—*Aplysia*—*Tethys* and *Fiona*—*Tritonia*—*Adalaria*, the embryonic external signs of the torsion of Prosobranchia diminish.

There is no doubt that Pelseneer (1906, 1911) was in error when he stated that all opisthobranchs undergo a torsion during their development which is identical with that of the prosobranchs. It is necessary only to glance at the living larvae of any nudibranch to be convinced that they have not undergone 180° torsion in the way described, for instance, by Crofts (1937) for *Haliotis*, since the positions of the anus and the greatest depth of the mantle cavity can be seen to be on the right side of the body, rather low down. While Pelseneer considered that the evolutionary detorsion which characterizes the modern opisthobranchs is brought about merely by *increased detorsion* in the young

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stages, it becomes clear that it results instead from *decreased developmental torsion*. Only vestiges of gastropod torsion remain both in veliger and adult stages of many (perhaps most) living opisthobranchs.

It is interesting to speculate on the way in which the shell-less condition has been attained in the evolution of many groups of the Opisthobranchia. Assuming the change

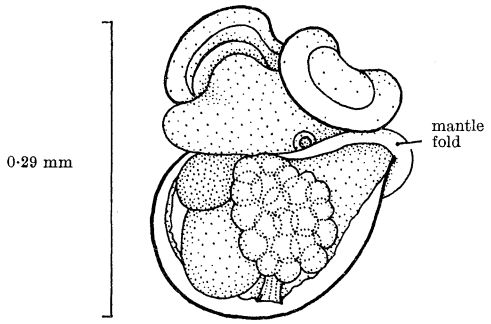

FIGURE 29. Late embryo, from the left lateral aspect, of *Berthella plumula*, showing the commencement of the reflexion of the embryonic mantle fold over the outside of the shell. Cilia are not illustrated.

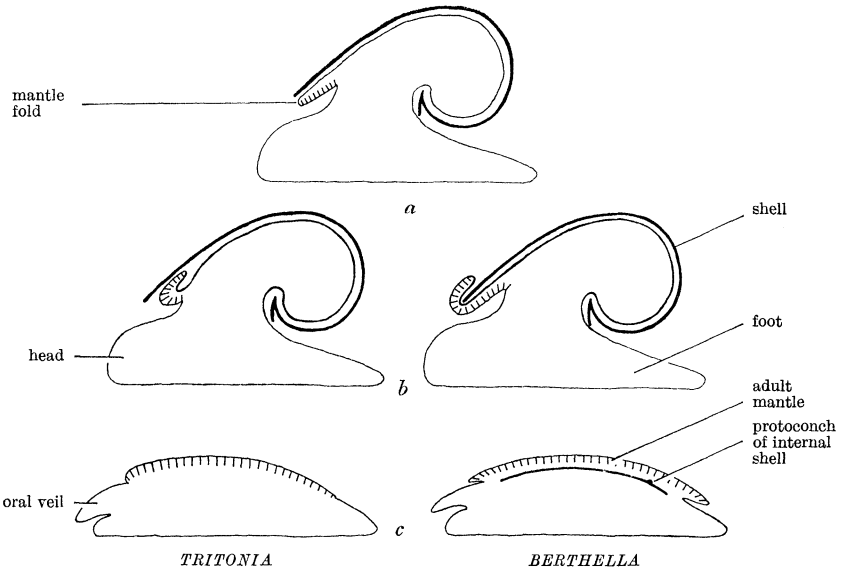

FIGURE 30. Diagrammatic sagittal sections showing the part played by the embryonic mantle fold in the adult body of, on the right, Notaspidea (e.g. *Berthellina* (Gohar & Abul-Ela 1957) and *Berthella*), and, on the left, dorid and tritoniid nudibranchs. *a*, embryonic stage; *b*, after hatching; *c*, adults.

to have been a gradual one, there are two ways in which the nudibranchs may have arisen from shelled ancestors; these are as follows.

(*a*) The shell may have become first enclosed, by lateral folds of the foot (like those of living *Natica* or *Akera*), subsequently to become reduced in importance and eventually lost altogether in the adult. The new dorsal integument would then be, in essence, epipodial.

(*b*) The shell may have become enclosed by folds of the mantle (like those of modern Cypraeidae, *Scutus*, *Philine* (Brown 1934), *Cryptochiton*, *Phlyctaenachlamys* and *Devonia*

(Popham 1939, 1940)), prior to its reduction and loss in succeeding generations. The new dorsal integument would then be derived from that layer of the mantle fold which lined the original mantle cavity.

Close study of the mode of origin of the dorsal integument in the post-larval tritoniid or dorid reveals that the second hypothesis is the only tenable one. Comparison between the post-veliger development of *Tritonia* and of the notaspideans *Berthella* and *Berthellina* may help to make this clear. During late development of the veliger of *Berthella plumula* (figure 29) and of *Berthellina citrina* (Gohar & Abul-Ela 1957) the mantle fold may be seen to begin to extend back over the outside of the lip of the larval shell. This process is continued until the shell in post-larval stages is wholly internal. In *Tritonia* (figures 12, 30), *Adalaria* and probably in *Discodoris erythræensis* (Gohar & Abul-Ela 1959) the mantle fold becomes reflexed in a very similar way so that, after metamorphosis, it may spread over the dorsum, but at no time is the shell in these nudibranchs even partially enclosed by the mantle. Figure 30 attempts to illustrate that the dorsal integument of these nudibranchs is exactly homologous with that of the Notaspidea; in my view there is every reason to believe that the evolution of these nudibranchs from shelled ancestors has followed in many essential features the ontogeny of modern *Berthella*. The manner in which the actual loss of the shell in each generation was pushed back further and further into development (until today the nudibranch larval shell is cast without passing through an internal stage) is an example of a familiar but little-understood ontogenetic phenomenon.

In conclusion, it must be emphasized that, while there is no longer any good reason for believing, with Garstang (1889, 1890) and Herdman & Clubb (1892), that the tritoniid and dorid dorsum is epipodial, the issue of the homologies of the various regions of the body of the Sacoglossa or of the eolidacean nudibranchs is still open to discussion. It may well prove that investigation of the metamorphosis of such forms would yield further evidence of the variety of the ways in which the modern sea-slugs have come into existence.

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auricle
.GL. accessory pedal gland
. afferent branchial vessel
anus
C. anal cells
T.GEN.RUD. rudiment of anterior genital mass
. auriculo-ventricular valve

buccal commissure
RUD. rudiment of bursa copulatrix
.R. right buccal ganglion
blastopore
. buccal mass
pallial branchia

C. cerebro-buccal connective
cerebral commissure
cerebral ganglion
.L. left cerebral ganglion
.R. right cerebral ganglion
.RUD. rudiment of cerebral ganglion
VD.T. conducting tubules
R. cortex

F.GRAN. defensive granule in cytoplasm
NT. denticulations
.C. digestive cell
.GL. digestive gland
.GL.L. left digestive gland
.GL.R. right digestive gland

. efferent branchial vessel
OR.C. wall of everted oral canal
excretory matter
T. extrinsic muscles

. foregut
AC. food vacuole
. free yolk in lumen

. primordial germ cells
I.GL. genital mucus gland

RM. hermaphrodite duct
RM.RUD. rudiment of hermaphrodite duct
. hindgut

HT. heart

I.L. inner layer
INDIG. indigestible extrusion-matter
I.P.C. inner perivisceral cavity
I.P.M. inner perivisceral membrane

JAW JUNCT. median junction of the two jaws

K. definitive kidney
K2 larval kidney
K2 OP. opening of larval kidney
K2 VEST. vestige of larval kidney

LAT.BOD. lateral body wall
L.RETR.M. larval retractor muscle
L.RETR.M.F. factor of larval retractor muscle

M. mantle
M.C. mantle cavity
MED. medulla
MED.B. median band of yolky cells
MEM. spongy membrane between the two lateral halves of the radula
MET. metapodium
M.F. mantle fold
MG.D.L. left midgut diverticulum
MG.D.R. right midgut diverticulum
M.GL.RUD. rudiment of genital mucus gland
MUS. muscle fibres of the cephalo-pedal muscle complex
MUS.F. smooth muscle fibres

ODONT. odontophore muscles
O.L. outer layer
OP. operculum
O.P.C. outer perivisceral cavity
OP.G.L. left optic ganglion
OP.G.R. right optic ganglion
O.P.M. outer perivisceral membrane
OR.C. oral canal
OR.GL. oral gland
OR.V. oral veil
OT.L. left otocyst
OT.R. right otocyst
OVID.RUD. rudiment of oviduct

OVOT. ovotestis
OVOT.RUD. rud

P.BR. pedal bran muscle
PED.C. pedal co
PED.S. longitudi
PER. pericardiun
P.G.L. left pedal
P.G.R. right peda
PL.G. pleural ga
PL.G.R. right ple
PL.S. longitudina
PR. propodium
PROP.GL. propo
P.RUD. rudimen

R. renal duct
RAD. radular sac
RAD.RUD. rudi
REN.C. renal cel
RH. rhinophoral
RH.G. rhinophor
RH.G.R. right rh
RH.RUD. rudim tentacl
RH.S. rhinophora
R.P. renal pore
R.P.S. reno-peric

SAL.GL. salivary
S.C. sensory ciliu
SH. shell
SH.CAV. shell ca
SH.GL. shell glan
ST. stomach
STOM. stomoda
ST.RUD. stomac
ST.V. storage ves
S.V. subvelar rid

TYPH. typhlosol

V. velum
V.D. vas deferen
V.D.RUD. rudim
VE. ventricle
VISC. visceral m

s
diment of ovotestis

nch of larval retractor

ommissure
linal pedal sinus
m
l ganglion
lal ganglion
anglion
leural ganglion
al pallial (pleural) sinus

odial gland
nt of penis

ac
iment of radular sac
lls
l tentacle
ral ganglion
hinophoral ganglion
nent of rhinophoral
cle
ral sheath

cardial syrinx

y gland
um or cilia

avity
nd (rudiment of mantle)

aeum
ch-rudiment
esicle of larval kidney
dge

ole

ns
ment of vas deferens

nass

List of abbreviations used in the figures

auricle
ACC. accessory pedal gland
AF. afferent branchial vessel
anus
AN.C. anal cells
ANGEN.RUD. rudiment of anterior genital mass
AV. auriculo-ventricular valve

B. buccal commissure
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PER. pericardium
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P.G.R. right pedal ganglion
PL.G. pleural ganglion
PL.G.R. right pleural ganglion
PL.S. longitudinal pallial (pleural) sinus
PR. propodium
PROP.GL. propodial gland
P.RUD. rudiment of penis

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RAD.RUD. rudiment of radular sac
REN.C. renal cells
RH. rhinophoral tentacle
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RH.G.R. right rhinophoral ganglion
RH.RUD. rudiment of rhinophoral tentacle
RH.S. rhinophoral sheath
R.P. renal pore
R.P.S. reno-pericardial syrinx

SAL.GL. salivary gland
S.C. sensory cilium or cilia
SH. shell
SH.CAV. shell cavity
SH.GL. shell gland (rudiment of mantle)
ST. stomach
STOM. stomodaeum
ST.RUD. stomach-rudiment
ST.V. storage vesicle of larval kidney
S.V. subvelar ridge

TYPH. typhlosole

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V.D.RUD. rudiment of vas deferens
VE. ventricle
VISC. visceral mass