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The Natural History, Embryology, Larval Biology and Post-Larval Development of Adalaria proxima (Alder and Hancock) (Gastropoda Opisthobranchia)

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THE NATURAL HISTORY, EMBRYOLOGY, LARVAL BIOLOGY AND POST-LARVAL DEVELOPMENT OF *ADALARIA PROXIMA* (ALDER AND HANCOCK) (GASTROPODA OPISTHOBRANCHIA)

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[Plate 1]

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Sparse and conflicting evidence concerning the mechanism and nature of developmental torsion and detorsion in the Opisthobranchia rendered necessary a full re-examination of these problems. Many contemporary general works either state or imply that during ontogeny opisthobranchs undergo 180° torsion (being identical with diotocardian prosobranchs in this respect) which is reversed in an unknown manner at some later stage of development. This theory is based on the work of Pelseneer (1911); the more detailed work of Saunders & Poole (1910) on *Aplysia* conflicts with Pelseneer's views, but nevertheless these views have found wide currency.

For this study, a species of dorid nudibranch, *Adalaria proxima*, was selected, for certain singular features of its biology rendered it possible to rear small populations through the complete life cycle in the laboratory. *A. proxima* has an annual life cycle, the adults in nature dying after spawning, and their place being taken by the new generation of juvenile dorids. Death after spawning is correlated not with exhaustion of the germ cells, but with exhaustion of the food reserves built up in the pre-sexual phases of the life cycle. The species feeds mainly on an encrusting polyzoan, *Electra pilosa*, the dorid buccal pump playing an important part in the feeding mechanism.

The spawn of *Adalaria proxima* shows the typical features characteristic of the egg masses of northern benthic invertebrates. The eggs are large, the number of eggs produced is small, the embryo hatches at a relatively advanced stage after a protracted embryonic period.

The early cleavage stages follow in all important respects the sequence and arrangement found in all dextrally organized gastropods. Gastrulation takes place by epiboly and the veliger form is rapidly assumed. Torsion in *Adalaria* is pushed back so far into development that it is no longer recognizable as a mechanical process. All the organs, as they become recognizable in sectioned post-gastrulae, are arranged in the post-torsional positions, although the process of torsion has been halted far short of the full 180° found in living Diotocardia.

The appearance of eyes, the development of the propodium and the structural and histological changes undergone by the mantle fold approximately 3 weeks after oviposition mark a departure from the pattern of embryonic development found in other species of dorid nudibranchs (Thompson 1957). Most of the organ systems are relatively greatly advanced in the hatching larva o *Adalaria*. The enlargement of the left midgut diverticulum brings about a slight rotation (continued during pelagic life) of the stomach; this is a process which is independent of torsion and was mentioned by Saunders & Poole, who, however, placed a slightly different interpretation upon it. Both dextral and sinistral components are present in the relations between the visceral and cephalopedal parts of the embryonic and larval body, but there can be no doubt that the dorids are truly dextral organisms. A complex arrangement of embryonic body cavities is present; it seems clear that the 'coelom' of *Aplysia* (Saunders & Poole 1910) corresponds to the inner perivisceral cavity of *Adalaria*, and that the term coelom in this connexion is a misnomer.

The pelagic phase is divided into two distinct stages, during the first of which the larvae swim upwards, this behaviour being reversed at the start of the second stage. Searching behaviour characterizes the second stage alone, the selection of a suitable substrate for settlement being governed by an elaborate and highly specific sensory mechanism. Metamorphosis will only occur on a live colony of the encrusting polyzoan *Electra pilosa*. The cephalopedal ciliary apparatus directs a feeding current into the mouth; normal further development will, however, take place even in sterile sea-

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water. The larval shell is a hyperstrophic one. Retraction of the larval body is brought about by the larval retractor muscle aided in a co-ordinatory capacity by contraction of muscular elements of the inner perivisceral membrane and of the cephalopedal subepidermal muscle complex.

Metamorphosis involves drastic changes, but no change in basic orientation. Detorsion and reversal of visceral flexure are brought about in two stages as the mantle fold first becomes inverted and then spreads over the dorsal surface of the post-larva. The widely stated view that the dorsal integument of the adult dorid is the product of the evolutionary enclosure of the shell by epipodial folds is contradicted by the embryological evidence. The larval retractor muscle disappears and the muscle complex of the adult is derived from the larval subepidermal muscle complex. The perivisceral cavities are obliterated and the adult haemocoel is derived from the larval cephalopedal subepidermal blastocoelic spaces. Calcareous spicules are laid down in the mantle. Concentration and fusion of the nerve ganglia result in a symmetrical arrangement, the embryonic system having shown traces of the ancestral streptoneury.

A discussion of the nature of torsion in opisthobranchs almost entirely disagrees with the views of Pelseneer (1911). All the available evidence implies that torsion in opisthobranchs is greatly modified and never approaches the full 180° twisting found in living Diotocardia. It is not, of course, suggested that Pelseneer's basic conclusion, that the prosobranchiate condition is ancestral to the opisthobranchiate, is in need of revision. Torsion in *Adalaria*, lying as it does at the extreme opposite end of the scale from the Diotocardia, is greatly modified and is no longer recognizable as a mechanical process; torsion in *Adalaria* does not occur for the same reasons as were important to the ancestral veliger, for in the dorids the larval mantle cavity does not serve to accommodate the head during retraction. The suggestion is made that the usual manner of referring to torsion during development as involving a movement of the pallial complex from a posterior to an anterior position, is inaccurate and results from any attempt to describe ontogenetical torsion from the study of the adult gastropod alone. Finally, attention is drawn to the paradox that, although the dorid nudibranchs are the most highly evolved gastropods living (speaking in terms of gross structure), the complex evolutionary steps which have led to the dorid have resulted in a secondary return, in many respects, to the original condition. In the adult dorid, only unimportant traces remain of the three most important steps in the structural evolution of the gastropods, visceral flexure, torsion, and bilateral asymmetry.

1. Introduction

All living gastropod molluscs either display the effects of torsion in the adult state, or reveal, both in their embryology and their finer adult anatomy, that they are descended from forms which did so. In the Opisthobranchia are placed a considerable number of those forms which have tended towards neutralization of some or all of the more marked effects of torsion on the adult gastropod; these tendencies are towards reduction or loss of the shell in the adult and reversion to the ancestral bilateral symmetry. Both tendencies, with their several correlated changes such as loss of the true ctenidium and concentration of the nervous system (euthyneury) reach their culmination, among living forms, in the body of the adult dorid nudibranch. In the adult dorid, only relics remain of the three great gastropod evolutionary steps, visceral flexure, torsion and coiling of the visceral mass.

Although in recent years there have been several important contributions to knowledge of the embryology and life histories of members of various subdivisions of the Gastropoda, the Opisthobranchia have tended to be neglected. The reason for this is plainly that it was considered potentially more rewarding to study the embryological sequences of the most primitive living gastropods. Certainly, the results of work on the embryology of diotocardian prosobranchs (Crofts 1937, 1955; Smith 1935; Robert 1902) have justified this expectation, and have contributed a great deal towards a more comprehensive understanding of the gastropod body.

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Superficial descriptions of a wide variety of opisthobranch embryos and larvae are recorded in the literature, but of these only the works of Saunders & Poole (1910) on *Aplysia*, and Pelseneer (1911) on a wide variety of species, make clear statements about the process of embryonic torsion in opisthobranchs. Unfortunately, these statements pose more questions than they answer, and the contradictions between them rendered necessary a full re-examination of the problem.

After preliminary observations on a variety of nudibranch opisthobranchs, a species of dorid, *Adalaria proxima* (Alder & Hancock) 1854, was selected for this study, for the following reasons: (*A*) It was found to have a precise distribution on the shore, rendering collection of material no problem; (*B*) It was found to have a relatively simple life cycle; (*C*) It was found possible to maintain small populations of this species in the laboratory, the natural food being abundant.

Because the investigation would lose much value were not all the stages from egg to adult covered, it was decided to make observations on a natural population of the species selected, in order that conclusions drawn from such observations might be used in the development of suitable culturing methods. This mode of approach to the problem proved successful, and it was later found possible to rear populations of *A. proxima* through the complete life cycle under laboratory conditions and even to extend the study into the second generation. These observations on laboratory-reared populations were a useful supplement to data derived from the study of the species in nature.

2. Material and methods

All observations were made on animals from the *Fucus serratus* zone of a rocky, sheltered shore extending into the first gulley on the west side of Church Island, in the Menai Straits separating the island of Anglesey from the mainland of North Wales.

Embryonic stages were examined alive, before clearing in glycerol after acetic acid fixation (Wilson 1904), or sectioning. Of the fixatives employed for spawn material, best results were obtained with Perényi's fluid and Bouin's fixative (made up in sea water); both were used cold for early embryonic stages, but the more active stages were preserved in a state of greater extension if the fixatives were used hot.

Larval and post-larval stages were fixed in hot Perényi's fluid, with or without preliminary narcotization. In the study of the development of the spicules, young post-larvae were fixed in neutral 5% formalin, before clearing and mounting in balsam.

Material for sectioning was cleared with amyl acetate (Barron 1934), methyl benzoate, or methyl benzoate plus 1% celloidin (the method of Peterfi). Smaller stages were coloured with aniline blue (W.S.) to facilitate orientation in the molten wax. The embedding medium employed was Hance's rubber wax (Gurr). Sections were cut at from 2 to 15 μ in thickness.

Stains employed included Heidenhain's iron haematoxylin, Masson's trichrome stain, Mayer's haemalum, Mallory's triple stain, Delafield's acid haematoxylin, and, as counterstains, eosin, orange G, and alcian blue 8 GS (Steedman 1950).

All drawings, except where specified otherwise, were made with the aid of a Leitz camera lucida. Mental reconstructions of serial sections were made.

A technique devised by R. E. Drinnan (1954) was employed, with certain modifications, in the investigation of the larval feeding mechanism.

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3. The natural history of *Adalaria proxima*

A. *Habitat*

On the shore dealt with, *A. proxima* lives predominantly in the *Fucus serratus* zone. Rarely, individuals may be found lower down the shore, among the Laminariae, but never higher than the upper limit of the *F. serratus*.

F. serratus, on this gently sloping, rocky shore, exceeds all the other seaweeds in the variety and abundance of the animals which encrust on the surface of its fronds. Various encrusting Polyzoa are among the most abundant members of this epifauna; the commonest species were:

Electra pilosa (L.) 1767
Flustrella hispida (Fabricius) 1780
Hippothoa hyalina (L.) 1767
Alcyonidium polyoum (Hassall) 1841

More rarely found in this zone of the shore were *Membranipora membranacea* (L.) 1767 (which is much more abundant on Laminariae), and *Schizoporella unicornis* (Johnston) 1847.

B. *Feeding behaviour*

Adalaria proxima has been observed to feed on four species of encrusting Polyzoa. Of these, *Electra pilosa* forms the staple natural diet, and the others, *Membranipora membranacea*, *Flustrella hispida* and *Alcyonidium polyoum* are attacked only if *Electra pilosa* is absent or sparse in the vicinity of the dorid.

The feeding behaviour was investigated by allowing dorids to browse on colonies of *E. pilosa* growing on glass plates. The process was observed through the underside of the plate with the aid of a stereoscopic microscope. The feeding mechanism is not a precise one; no fixed pattern of attack was evident. During the initial stages of attack on a zooid, the radula is not used continuously, but in short bursts of from three to eight strokes, followed by a pause before resumption of activity. The effective rasping stroke is the posterior to anterior one, during which the large spines of the lateral teeth point forwards.

When a breach has been made in the outer, membranous covering of the zooid, the lips of the dorid are applied to it and the soft parts sucked out. Sucking is achieved by pulsating dilations of the dorid buccal pump, but is rhythmically assisted by the lips, which press on the zooid membrane and compress the contents of the cell. Action of the radula is continued and the more solid parts of the zooid body are carried up into the dorid's mouth on the radular teeth.

On occasions, the predation of the dorid brings about the complete detachment of the outer membrane from the zooid cell; dorids turned over while feeding frequently have such membranes adhering to the under side of the foot (figure 1).

C. *Parasites and predators*

Although commonly present on other dorid species from the Menai Straits, neither *Splanchnotrophus* nor *Lichomolgus* (Copepoda) was ever observed to infect *Adalaria proxima*.

The population of *A. proxima* which was studied never became seriously depleted at any time, and no predation upon the adults was ever observed.

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D. *The life cycle*

Figure 2 is a representation of observations made on the population over a period of two years. Maximum and minimum sizes encountered on each occasion are considered; no estimate of mean length was possible without some scheme of quantitative sampling of the population.

It is clear that *A. proxima* is an annual organism, breeding in early spring; the breeding generation then dies and is replaced by its progeny. This interpretation is fully confirmed by laboratory observations.

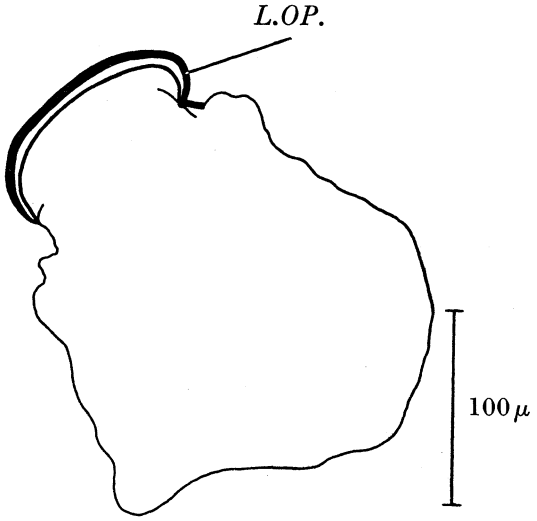

FIGURE 1. Membranous covering of a zooid of *Electra pilosa*, detached from the underside of the foot of *Adalaria proxima*.

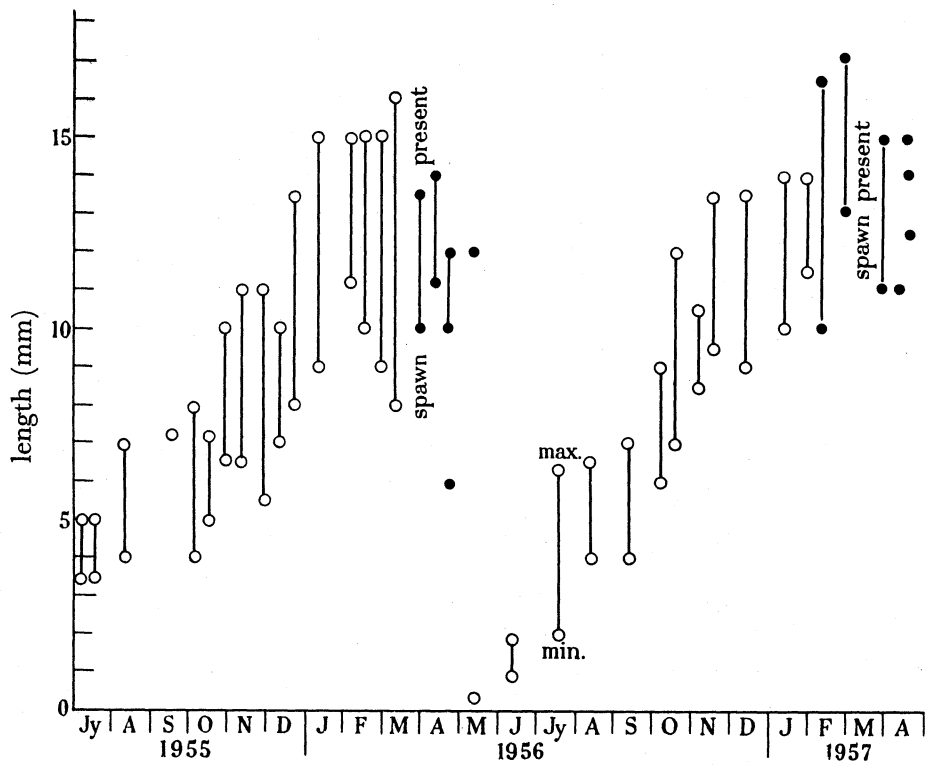

FIGURE 2. The life cycle of *Adalaria proxima*, reconstructed from collecting records.

At various times of the year, animals were preserved and sectioned, so as to follow the development of the reproductive organs. In the 1956–57 generation, the ovotestis began to differentiate in early November. At the same time, the rudiments of the organs of the anterior genital complex appeared. By mid-December, spermatozoa were visible in sections through the gonad and the maturation of the ova had begun. The ova were enlarging and their cytoplasm was becoming charged with yolk. By the time that breeding activity had commenced in 1957, the gonad was closely packed with large, heavily yolked oocytes and the seminal vesicle filled with spermatozoa.

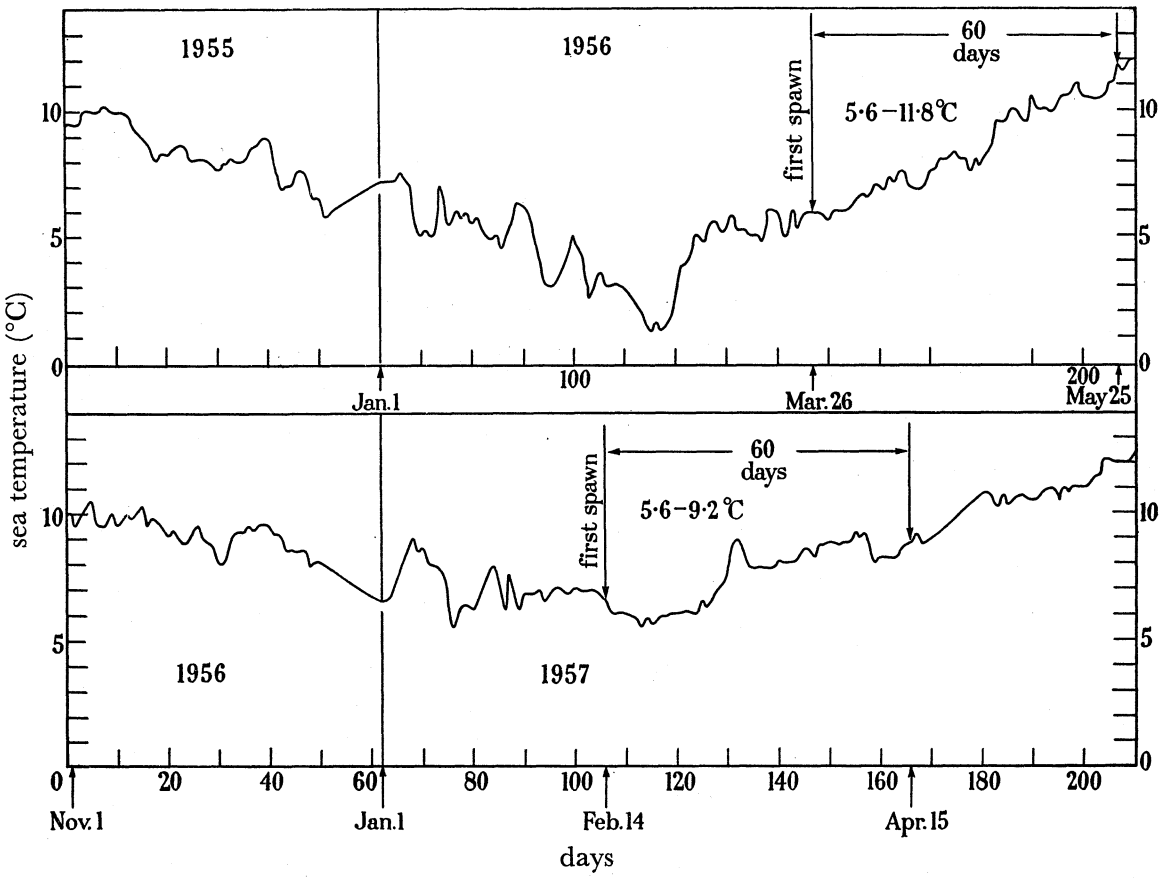

FIGURE 3. The relationship between the 1956 and 1957 breeding periods of *Adalaria proxima* and the sea water temperature in the Menai Straits.

In 1956 the breeding season began at the end of March and lasted approximately 8 weeks. In 1957 it began in the middle of February and had a similar duration. Daily sea temperatures during the months preceding these two breeding seasons are represented in figure 3, which shows a relationship between the spawning season and the temperature of the sea water. (It must be borne in mind that, as the *Fucus serratus* zone of the shore is exposed at certain phases of some tides, the temperature of the air may be important. Air temperature records for the area are, unfortunately, not available.) The severely cold winter and early spring of 1955–56 apparently delayed the onset of spawning until mid- or late March, i.e. until the water temperature had risen to approximately 6 °C. In the winter and spring of 1956–57, however, the water temperature rarely fell below this figure and *Adalaria proxima* probably began breeding as soon as the correct state of maturity

was reached. This interpretation is well supported by the results of a series of laboratory experiments to define the optimal temperature range for the achievement of the maximum reproductive potential of the species (Thompson 1957).

Adult dorids were observed copulating throughout the breeding season; they are, in common with all opisthobranch gastropods, hermaphrodite, and fertilization is reciprocal. A single mating suffices for the deposition of up to four spawn masses, of decreasing size. Feeding takes place during the season, but is not continuous and is frequently interrupted by breeding activity. There is evidence that feeding intensity begins to decline some months before the onset of spawning (Thompson 1957).

Towards the close of the breeding season, the dorids become smaller, and less active. In nature, most are washed away from the shore as soon as their vitality becomes diminished. Sections through such moribund animals show, rather surprisingly, that large numbers of eggs and reserves of spermatozoa are invariably present. The digestive gland, however, is much reduced in size, while the intestine, kidney and heart are often either absent or in a vestigial condition. The anterior regions of the alimentary canal are usually intact, but often displaced by the great development of the anterior genital mass; the ganglia of the nervous system, although similarly displaced, present a healthy appearance. A noteworthy feature is that large numbers of multicellular glands differentiate around the bases of the anal branchiae; they are present at no other stage of the life cycle. The mantle becomes thin and spicules and tubercles may be lacking in the middle of the back.

Senescence and death in this species are obviously not correlated with exhaustion of the germ cells, but with the consumption of all the reserves of food built up during the pre-sexual phases of the cycle.

4. The structure of the egg mass

Figure 4*A* shows an adult in the act of spawning. During oviposition, the spawn is slowly extruded, as a ribbon-shaped mass, through the genital aperture which is situated antero-laterally on the right side of the body. The spawn ribbon is shaped by the slit-like genital aperture as it emerges and passes back between the mantle and the side of the foot. At this time the jelly of the egg mass is of a sticky consistency, and pressure of the mantle and foot upon it causes it to adhere to the substratum. As the full length of the spawn ribbon is slowly extruded and pressed down, the adult moves in an anticlockwise spiral (when seen from above). The completed ribbon thus has this form (figure 4*C*). Oviposition of even a short ribbon takes several hours.

Up to three full turns may be present in the spiral of any ribbon. The spawn is flared out from its line of attachment to the substratum. In nature and in culture, these spirals of spawn frequently harbour various small animals (gammarids, polychaetes, etc.) which have never been observed to attack the eggs. In nature, spawn is deposited almost exclusively on fronds of *Fucus serratus*. In laboratory culture, however, the majority of egg masses are laid on the walls and floor of the vessel, even when fucoids are present; no confident explanation can be offered for this.

The matrix of the egg mass is a colourless transparent jelly, which in sections will take up mucus stains. In the field, egg masses are difficult to find owing to the transparency of this jelly. A delicate membranous tube spirals through the jelly and encloses the ova; this

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secondary membrane was found to be characteristic of the spawn of a wide variety of nudibranchs. The ova themselves are enclosed in primary egg cases; figure 4*E* and figure 50*A*, plate 1, show a single ovum with its two envelopes. The primary cases are ovoid and measure from 0·24 to 0·37 mm in their longest dimensions. Minute flaws are often present in them. Each case contains a single ovum; twin embryos are rare. The ova are heavily yolked spheres varying in diameter from 0·17 to 0·20 mm. The colour varies from yellow to white. (The number of eggs per spawn ribbon, the density of the eggs within the spawn and the colour and size of the eggs are influenced by temperature and other factors and will be dealt with elsewhere.) There are always a few empty egg cases of variable size and shape at each end of the ribbon; this is a common phenomenon in nudibranchs and was noted in a wide variety of species.

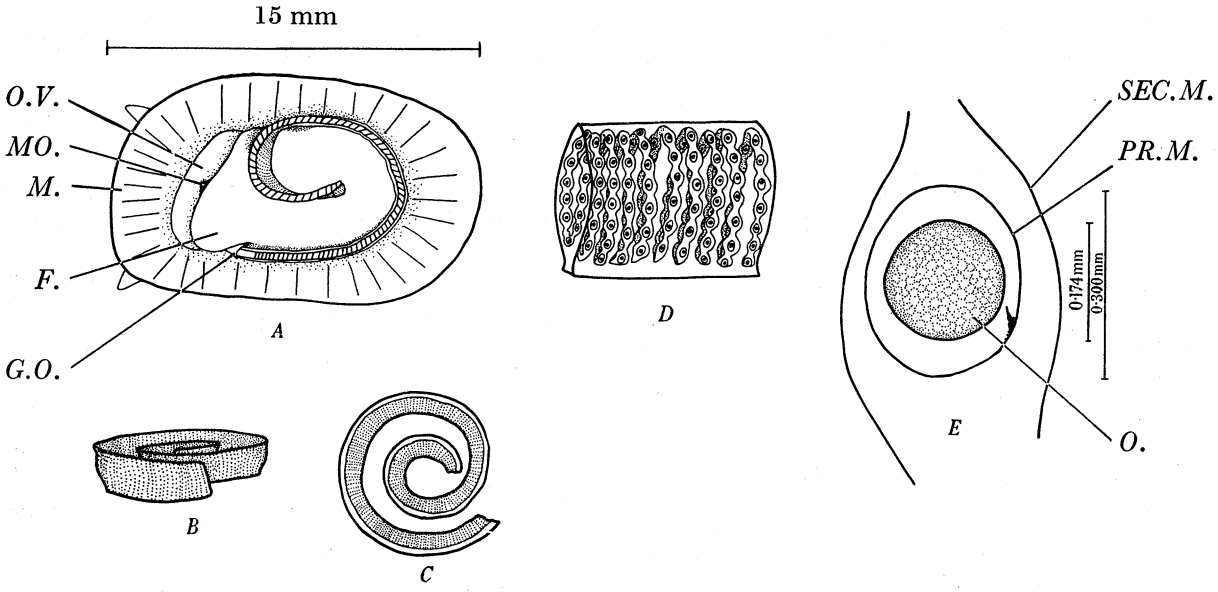

FIGURE 4. Oviposition in *Adalaria proxima*. *A*. Mature adult in the act of spawning on a glass plate; viewed from the underside. (The hatched region of the spawn ribbon is attached to the substratum.) *B*. Completed spawn ribbon, lateral aspect. *C*. Completed spawn ribbon, dorsal aspect. *D*. Enlarged portion of spawn ribbon (diagrammatic). *E*. Single ovum with its envelopes.

Table 1 shows a comparison of various features of the spawn mass of *Adalaria proxima* with those of various other species of dorid nudibranchs found in North Wales. The size of the uncleaved egg is much greater in *A. proxima* than in any other species of dorid investigated, with the exception of *Archidoris tuberculata*. Similarly, the embryonic period is longer in *Adalaria proxima* than in any other species investigated, except *Archidoris tuberculata*. (Further research is planned into the embryology of *A. tuberculata*, for the large size of the egg and the protraction of the embryonic period are correlated in this species not with the production of a relatively more complex larva, but of a simple larva of great size.) The number of eggs produced per spawn mass is far less in *Adalaria proxima* than in other dorids, as also is the density of distribution of the eggs within the spawn. (No accurate observations were made on the numbers of eggs in the spawn of either *Archidoris tuberculata* or *Jorunna tomentosa*, for they are so great as to render an exact numerical comparison superfluous.)

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Orientation of the embryos within their primary egg cases. The embryos are quite free within their primary egg cases throughout embryonic life; this is particularly evident during the gastrula stages. During gastrulation, that hemisphere of the embryo in which the blastopore is situated is heavier than the other; hence the embryos always exhibit the opposite pole to the observer. Compression of the eggs with a cover-glass, of course, allows examination of the blastopore.

TABLE 1. A COMPARISON OF THE EGG MASSES OF VARIOUS SPECIES OF BRITISH DORIDS

	Onchidoris muricata	*Onchidoris fusca*	*Archidoris tuberculata*	*Jorunna tomentosa*	*Goniodoris nodosa*	*Polycera quadrilineata*	*Adalaria proxima*
colour of ova	white-cream	white	white-pale cream	cream	white	white	white-yellow
prevalence of twin ova	none seen	rare	*ca.* 45 %	abundant	none seen	none seen	rare
duration of embryonic period in days from oviposition	14 at 9 to 10 °C	15–16 at 9 to 10 °C	36–40 at 8·5 to 9·5 °C	23 at 9 to 10 °C	no data	18–20 at 8·5 to 9·5 °C	36–42 at 9 to 10 °C
diameter of ova:							
single ova	0·08–0·10 mm	0·08–0·10 mm	0·16–0·165 mm	0·08–0·09 mm	0·09–0·10 mm	0·07–0·09 mm	0·17–0·20 mm
twin ova	—	—	0·16–0·17 mm	0·08–0·085 mm	—	—	—
longest diameter of primary egg cases:							
single	0·11–0·13 mm	0·14–0·17 mm	0·24–0·27 mm	0·14–0·20 mm	0·14–0·20 mm	0·10–0·11 mm	0·24–0·37 mm
twin	—	—	0·32–0·38 mm	—	—	—	—
density of distribution of eggs in the spawn:							
length of sample spawn ribbon	16 mm 33	70 mm	no data	no data	37 mm	24 mm	13 mm 65
average no. of ova per mm length of ribbon	153 261	1781	—	—	374	898	14 38
total no. of ova in the ribbon	2500 8613	124670	—	—	13838	21550	180 2470

Later, when the embryos reach mid- and late veliger stages, they become too large to move freely inside their cases. They are forced to lie with their long axes coincident with those of the ovoid primary cases. In consequence, because of the arrangement of the cases in spiralling rows, any given portion of a late spawn ribbon will generally exhibit embryos all orientated in the same way. This fact was used with advantage in selection of a suitable portion of the spawn for sectioning.

5. DEVELOPMENT OF THE EMBRYO UP TO HATCHING

Development within the egg case occupies from 36 to 39 days, at 9 to 10 °C (with rare exceptions).

A. *Cleavage*

The first few cleavage divisions will be described in order to emphasize the fundamental dextrality of organization of the dorid nudibranchs.

Soon after deposition of the spawn, maturation of the ova is completed and two polar bodies are extruded. They each contain a few yolk granules. The first cleavage takes place within a day after oviposition and results in two equal cells. Immediately after the division has been completed, they meet over only a relatively small area of their surfaces (figure 5*A* and figure 50*D*, plate 1); within an hour they come to be more closely associated together and their initially spherical shape becomes modified accordingly (figure 5*B* and figure 50*E*, plate 1). The polar bodies lie in the groove between the two cells. This, and succeeding early cleavages, is marked by the clarity with which the nuclear changes and the

inclination of the cleavage spindles with regard to one another can be observed in live material (figure 50*C* to *I*, plate 1).

After a resting stage lasting several hours, the second cleavage division begins. Cleavage is preceded by nuclear changes, and the cleavage spindles may be seen to be inclined with regard to one another. Division of the two members does not always occur simultaneously, so that transitory three-cell stages are occasionally observed (figure 5*C*). As in the first cleavage, the resulting daughter cells at first stand away from one another (figure 5*D* and

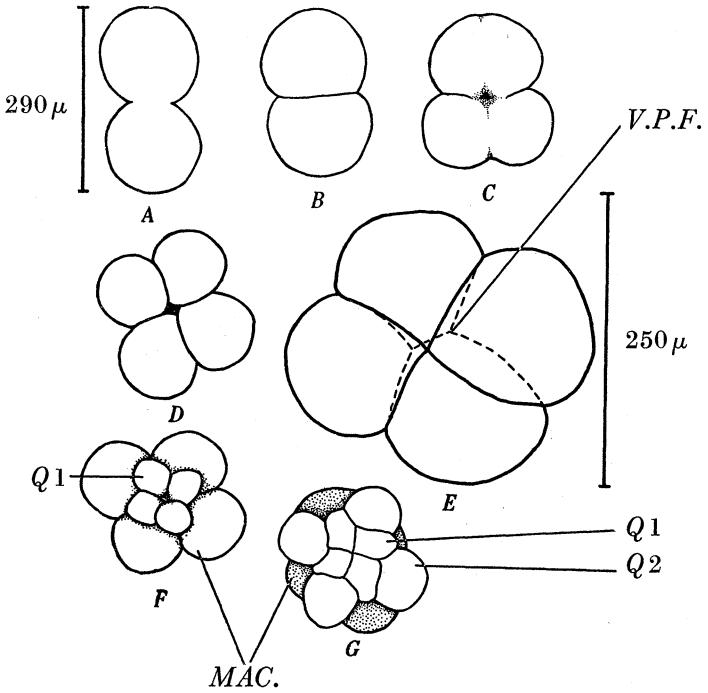

FIGURE 5. Cleavage stages of *Adalaria proxima*. *A*. Early two-cell stage. *B*. Late two-cell stage. *C*. Transitory three-cell stage. *D*. Early four-cell stage. *E*. Late four-cell stage, at greater magnification. (The interrupted line represents the interfaces at the lowest focal level.) *F*. Eight-cell stage; from the animal pole. *G*. Twelve-cell stage; from the animal pole.

figure 50*F*, plate 1), then soon become intimately associated (figure 5*E*). The second cleavage results in four cells of equal size. A ventral polar furrow is present, but dorsally the cell interfaces meet at a common point (figure 5*E* and figure 50*G*, *H*, plate 1).

Inclination of the cleavage spindles in preparation for the third cleavage is most clearly evident in live embryos (figure 50*I*, plate 1). Before division commences, the dorsal interfaces may be seen to bulge (figure 50*H*, plate 1) some time before the cells of the first quartet of micromeres are budded off. Division again may not affect all the parent cells synchronously, so that transitory five-, six- and seven-cell stages may be seen (figure 50*J*, *K*, plate 1). The direction of inclination of the cleavage spindles, the bulging of the dorsal interfaces and the final arrangement of the micromeres in relation to their respective parent macromeres (figure 5*F* and figure 50*L*, plate 1), all show this division to have been a dexiotropic one. Clockwise displacement of the cells of the first quartet continues even after nuclear division is complete.

Soon after the completion of the third cleavage division, the macromeres again divide, this time with the cleavage spindles inclined in a laeotropic manner; the four daughter cells

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budded off (the second quartet) are much larger than the cells of the first quartet (figure 5*G* and figure 50*M*, plate 1). By the laeotropic manner of their production, they effect a partial reversal of the dexiotropic rotation undergone earlier by the cells of the first quartet; the first quartet cells in consequence now come to overlie their respective parent macromeres (figure 5*G*). The embryo now consists of twelve cells, arranged in three quartets, each quartet differing from its neighbours in size.

Division of the cells of the first quartet follows shortly (figure 50*N*, plate 1), and cleavage continues in a series of alternately dexiotropic and laeotropic divisions. Cell lineage was not followed beyond the sixteen-cell stage; Casteel (1904), in his paper on *Fiona*, treats the subject in some detail.

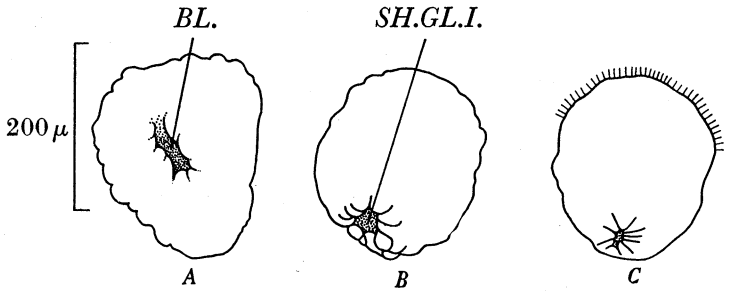

FIGURE 6. Gastrulae (drawings from life). *A*. Ventral aspect of embryo at 6 days. *B*. Postero-dorsal aspect of embryo at 7 days; blastopore closed. *C*. Postero-dorsal aspect of embryo at 8 days.

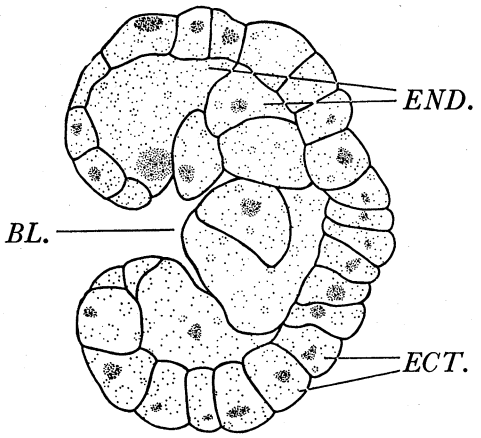

FIGURE 7. Section through embryo at 6 days (see figure 6*A*).

B. GASTRULATION AND ASSUMPTION OF THE VELIGER FORM

Gastrulation begins in 5 to 6 days after oviposition (at 9 to 10 °C). The 6-day embryo has assumed the characteristic 'heart' shape (figures 6*A*, 7). As gastrulation proceeds, by epiboly, the blastopore becomes more restricted and, at 7 days, is a narrow slit, showing the usual slight asymmetry. At the same time, the heart-shape characteristic of earlier stages becomes masked, the embryo assuming a more ovoid shape. The blastopore soon closes completely.

The shell gland invagination then appears at the opposite end of the embryo, a deep pit, bordered by large prominent cells (figure 6*B*, *C*, *SH.GL.I.*). The pit disappears with the eversion of the gland which then slowly spreads over the posterior hemisphere of the embryo (figure 8, *SH.GL.*).

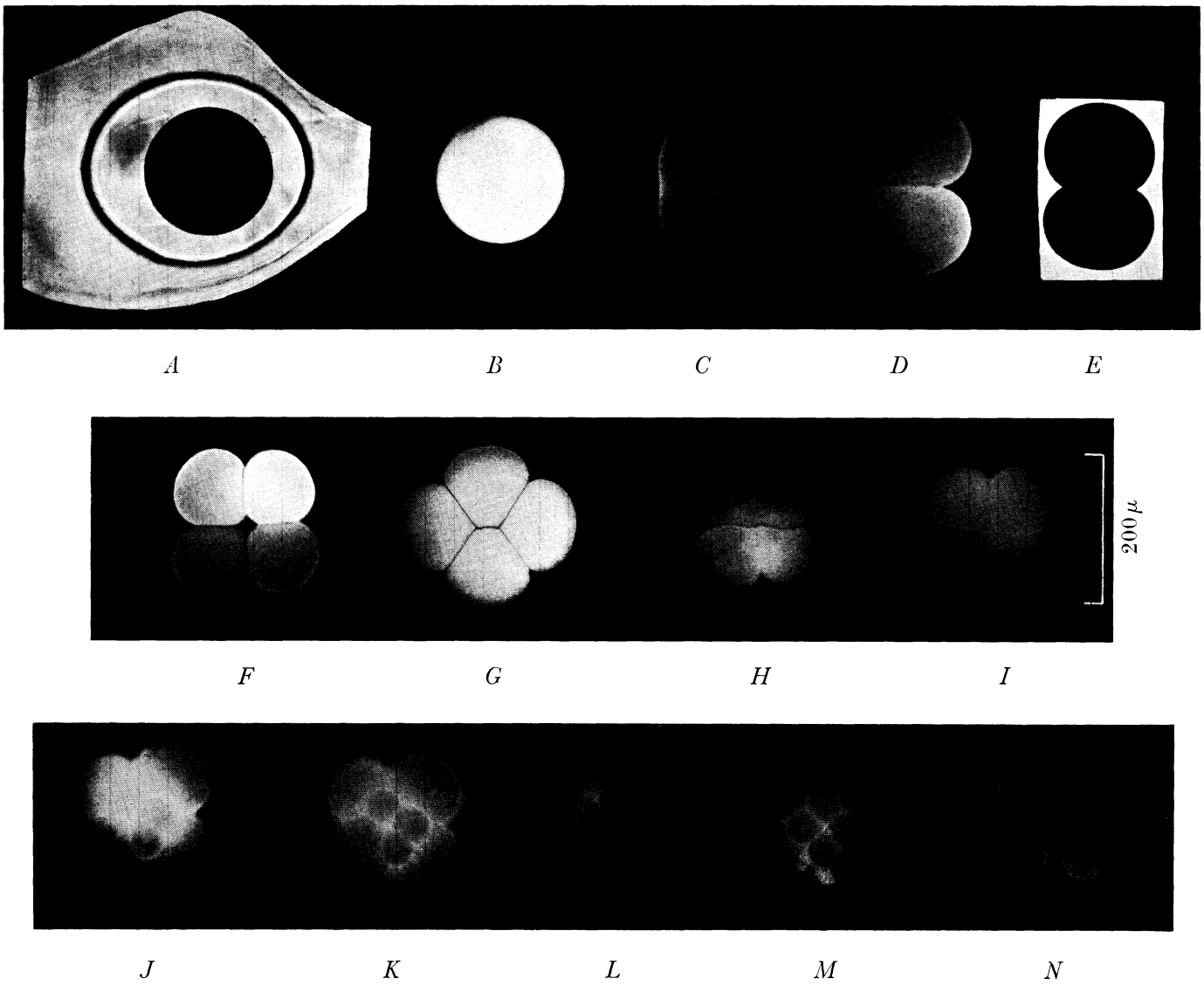

FIGURE 50. Microphotographs of cleavage stages of *Adalaria proxima*. All are of live embryos.

A. Uncleaved egg with its envelopes; direct lighting.
B. Localized translucency prior to extrusion of first polar body; dark ground illumination.
C. First cleavage beginning; dark ground illumination.
D. Early two-cell stage; dark ground illumination.
E. Slightly later stage; direct lighting.
F. Early four-cell stage; dark ground illumination. From animal pole.
G. Later four-cell stage; dark ground illumination. From vegetative pole.
H. The same, from animal pole; dark ground illumination.
I. Beginning of third cleavage division; nuclear spindles visible; dark ground illumination. From animal pole.
J. Transitory six-cell stage; dark ground illumination. From animal pole.
K. Transitory seven-cell stage; dark ground illumination. From animal pole.
L. Embryo after completion of third cleavage; dark ground illumination. From animal pole.
M. Embryo after production of second quartet of micromeres; dark ground illumination. From animal pole.
N. Embryo after division of first quartet (sixteen-cell stage); dark ground illumination. From animal pole.

(*Facing p.* 12)

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No cleavage cavity is present at any time before or during gastrulation (figure 7); shrinkage induced by various combinations of reagents may, however, give the effect, in sectioned material, of spaces between the cells.

Approximately 2 days after the closure of the blastopore, the mouth appears as a shallow pit, in a position corresponding to that at which the blastopore was last seen. The apical area of the embryo becomes finely ciliated (figure 6*C*), and soon these cilia can be seen to become aggregated into two broad areas, one on either side of, and anterior to, the mouth (figure 8*A* to *E*). As the cilia become longer, and, accordingly, more powerful, the embryo

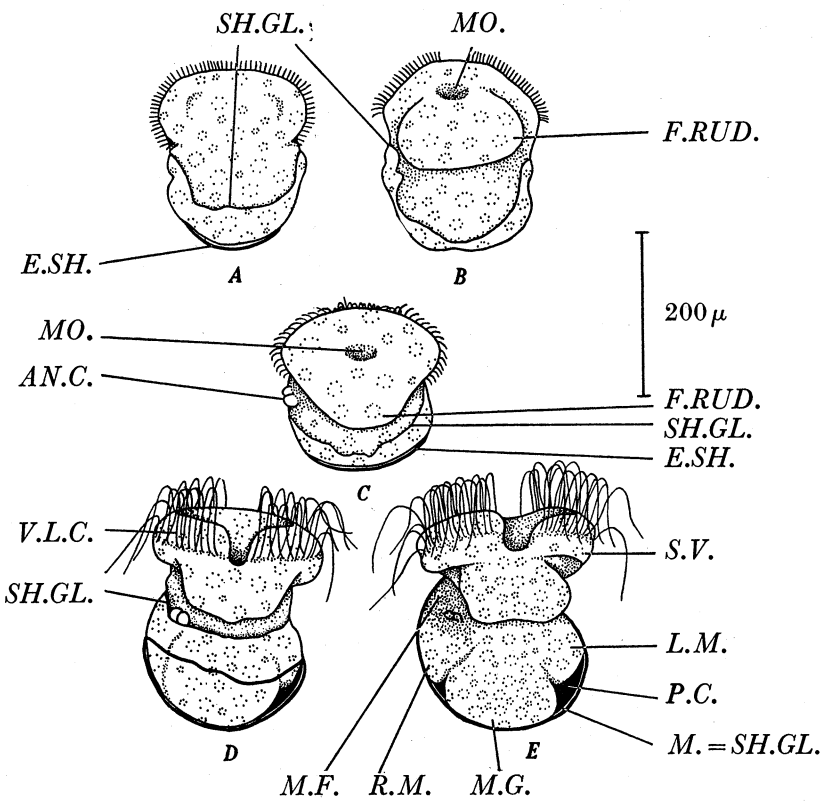

FIGURE 8. Transition to the veliger form (drawings from life). *A*. Embryo at 9 days; dorsal aspect. *B*. Embryo at 9 days; ventral aspect. *C*. Embryo at 10 to 11 days; antero-ventral aspect. *D*. Embryo at 12 days; ventral aspect. *E*. Embryo at 13 days; ventral aspect.

begins to move within the primary egg case, under the influence of their beat. These cilia, the precursors of the velar locomotor cilia of later stages, beat intermittently and almost at random; no metachronal mechanism as yet governs their activity. The apical area becomes flattened and gradually constricted into two saucer-shaped lobes, along the edges of which the cilia become especially strongly developed (figure 8*E*). A metachronal rhythm begins to govern the beat of these long velar cilia within 1 week of the closure of the blastopore. Although this rhythm at first may be observed to break down occasionally, and often does not affect all the velar cilia, it soon becomes perfect, and results in a wave of action which passes, if the embryo be viewed from the front, in a clockwise direction around the velum.

Shortly after the appearance of the mouth, the rudiment of the foot appears, as a single broad prominence below the mouth (figure 8*B*, *C*).

The anterior limits of the shell gland, when it has extended over the whole visceral sac or posterior hemisphere of the embryo (taking from 5 to 6 days to do this) form a collar or fold behind the cephalopedal mass. This is the mantle fold (figures 8*E* and 11*A*, *B*, *M.F.*); it extends further forwards as it secretes more and more substance at the mouth of the shell. The glistening embryonic shell first makes its appearance posteriorly (figure 8*A*, *E.SH.*), and soon becomes cup-shaped, enclosing the whole visceral sac (figure 8*E*).

The anal cells (figure 8*C*, *D* and *E*, *AN.C.*) are not prominent and first become visible in live embryos shortly after the appearance of the mouth. From their first appearance they are situated latero-ventrally on the right side of the embryo; they maintain this relative position until they finally become indetectable at a late stage. The anal cells mark the site of the anal opening when such is developed. Their function is not understood.

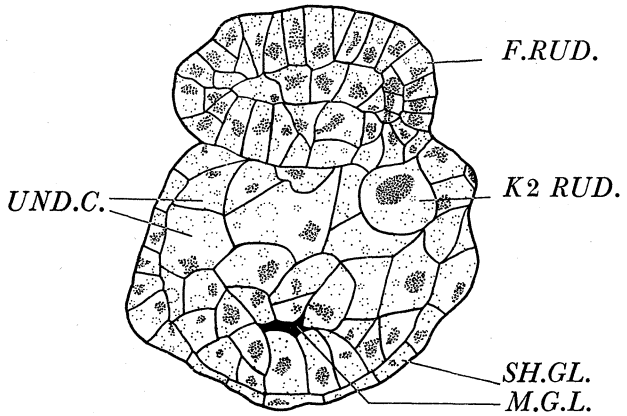

FIGURE 9. Horizontal section through postgastrula.

Internally, differentiation of the visceral organs begins shortly after the appearance of the oral invagination. The foregut runs back from the mouth in the median plane of the embryo, but is for some time blind, having no open connexion with the midgut. The midgut takes the form of a hollow sac bounded by a wall made up of very large, heavily yolked cells (figure 9, *M.G.L*). The hindgut rudiment develops as a solid rod of cells on the embryo's right side. Close to the anterior extremity of the developing hindgut is a single very large cell with a huge nucleus; this is the rudiment of the larval kidney (figure 9, *K2 RUD.*). The remainder of the visceral sac remains undifferentiated at first (figure 9), but soon becomes aggregated into two approximately spherical masses lying symmetrically against the antero-lateral walls of the midgut; these are the two midgut diverticula (figures 8*E* and 11*A–E*, *L.M.*, *R.M.*).

With the onset of differentiation of the visceral organs, spaces between and around them result (figures 8*D*, *E* and 11*A* to *E*). Prior to this the mantle (=shell gland) lined the shell over the whole of its inside surface, and also closely invested the visceral mass, which, before differentiation into its component organs, closely filled the shell. At the same time, the mantle becomes less securely attached to the inside of the shell in posterior regions, and eventually leaves contact with it in some areas; a space between the mantle and the shell comes into being. This space will be designated the shell cavity; it is not at this stage in communication with the exterior (figure 11*A*, *SH.CAV.*).

At first the mantle is, over its whole extent, very opaque due to the heavily yolked nature of its cells. With the completion of the first-formed part of the embryonic shell

(that part which encloses the visceral sac) this yolk is exhausted and the mantle in these regions becomes almost transparent. As development proceeds, restriction of yolk to those regions of the mantle which are still engaged in shell secretion, i.e. the mantle fold, becomes even more marked, and the mantle fold in life has the appearance of a sharply defined band of yolky cells, extending round the inside of the shell mouth (figure 11*D*, *E*, *M.F.*).

The first organs of the embryonic nervous system to appear are the paired otocysts (figures 10, 11*B*, *OT.*), in the cavities of which are deposited the spherical otoliths. The sacs become internally ciliated and the otoliths can be observed to rotate. The otocysts are described as originating by invagination in many gastropods but this is not the case

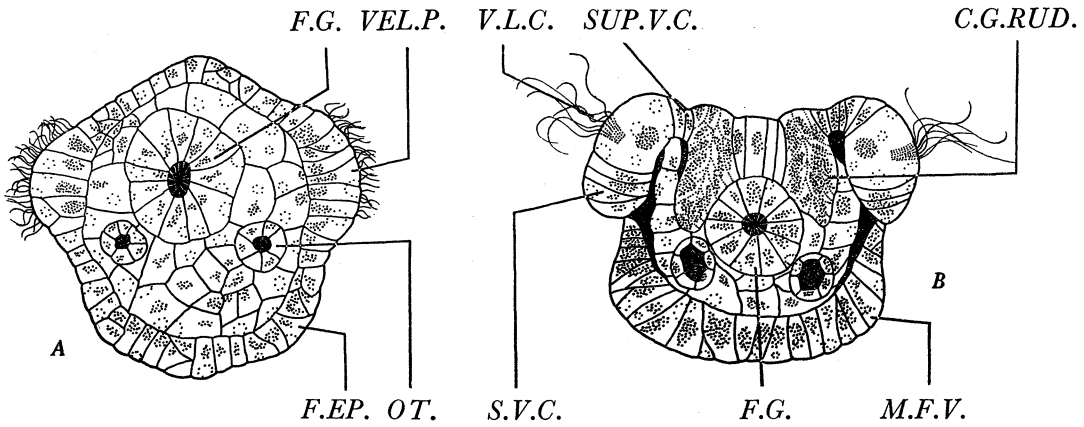

FIGURE 10. Transverse sections through the cephalopedal mass of: *A*. Postgastrula. *B*. Early veliger (section passing just posterior to the foot).

in *Adalaria*. The rudiments of the cerebral ganglia next appear; thickenings, one on either side, appear in the ectoderm of the area within the velar lobes (figure 10*B*, *C.G.RUD.*). Cells are cut off inwards and migrate back to form discrete ganglion rudiments, one on either side of the foregut. Migration of nervous elements across the top of the foregut soon establishes a cerebral commissure (figure 12, *C.C.*). The originally paired intravelar thickenings become linked and a shallow, trough-like invagination, deepest at its lateral extremities, becomes clear, increasing the surface for proliferation of nervous elements. The intravelar trough and invaginations persist for some days. The pedal ganglia develop shortly after the cerebrals, and are cut off from cells of the pedal ectoderm. There is no restricted site of proliferation; elements which form the pedal ganglia are proliferated widely, and pass up the foot to assemble in two masses, one on either side, close to the respective otocysts. A commissure linking these ganglia is present almost from the onset of their production.

Differentiation of the velum continues (figure 11*A* to *E*), the main velar row of cilia becoming stronger. Another tract of cilia differentiates on the velar lobes, below the main locomotor row. It soon becomes clear that this tract lies on a ridge, the subvelar ridge or subvelum, and leads round the velar lobes on either side, down to merge with the ventral border of the mouth (figure 11*C*, *S.V.*).

The foot becomes finely ciliated over its whole external surface, except for the posterior face, on which an operculum is secreted (figures 11*E*, 12, *OP.*). Especially long cilia appear

at the tip of the foot, which becomes papillate (figure 11*C*). The foot at this stage corresponds to the rudiment of the metapodium of later stages. The pedal epidermis, except in that region on which the operculum is secreted, differentiates into a single layer of columnar cells with enlarged nuclei. The body of the foot is filled with undifferentiated, heavily yolked cells. The epidermis shortly becomes exhausted of its yolk, except for a median band

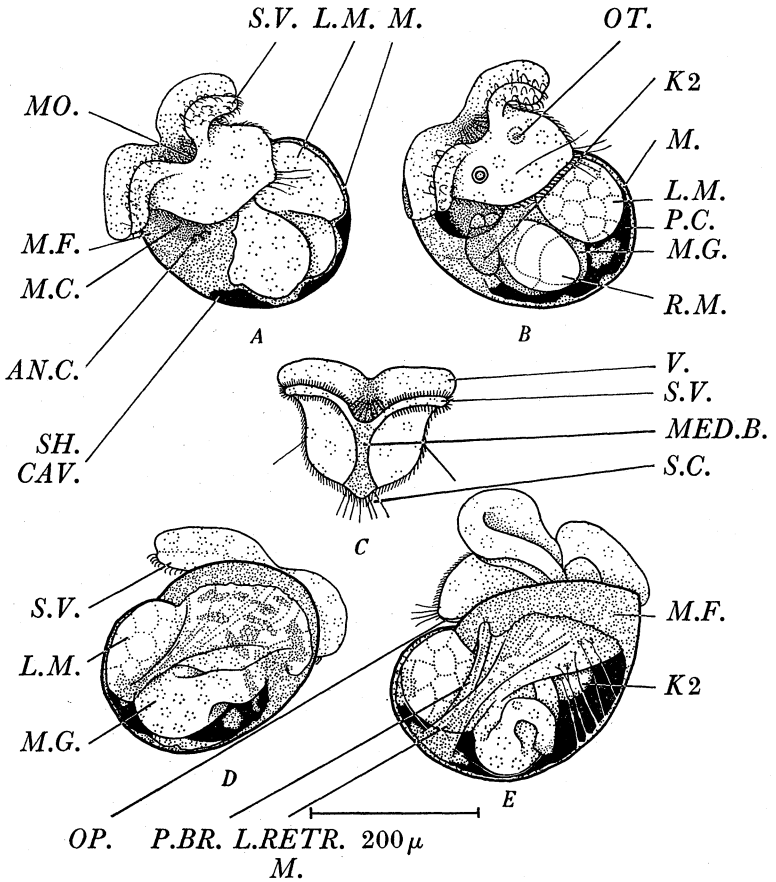

FIGURE 11. Further veliger development (drawings from life). *A*. Embryo at 16 to 17 days; right latero-ventral aspect. *B*. Embryo at 19 to 20 days; right latero-ventral aspect. *C*. Cephalopedal ciliation; ventral aspect. *D*. Embryo at 19 to 20 days; left latero-dorsal aspect. *E*. Embryo at 21 to 22 days; left latero-dorsal aspect. (Velar locomotor cilia not illustrated).

of yolky cells leading from the ventral border of the mouth to the papillate tip of the foot (figure 11*C*, *MED.B.*). In other species of dorid nudibranchs (Thompson 1957) this median band bears cilia which produce a current playing a part in the feeding mechanism of the free larva. No such function is performed by this band in *Adalaria*, for it is eliminated by the development of the propodium a considerable time before hatching.

Typically, a pair of multicellular metapodial mucus glands (figure 12, *MET.GL.*) develop. These have separate ducts to the exterior, opening on either side of the median yolky band, about half way down its length. Much variation in the relative size of these glands exists, but in the majority of cases the right member remains very small, while the left hypertrophies. The glands are usually flask-shaped, and, originating close to the otocysts, pass down the foot to their external openings. Their contents appear reticulate in sections, and hold mucus stains tenaciously. They are not visible in life. Various epidermal cells,

particularly on the left side of the foot, at its base, enlarge and become vacuolated. These are the lateral pedal glands; they are unicellular and many of them take up mucus stains.

As development proceeds, and the yolk, which earlier rendered the embryo opaque, is used up, internal detail becomes increasingly easier to discern in live material.

Secretion of the shell continues and this has the effect of carrying the mantle fold up away from the body of the embryo in dorsal and lateral regions; a shallow mantle cavity comes into being (figure 11 *A*, *B*, *M.C.*). This cavity is deepest on the right side, and in it lie the anal cells, now partially hidden from view.

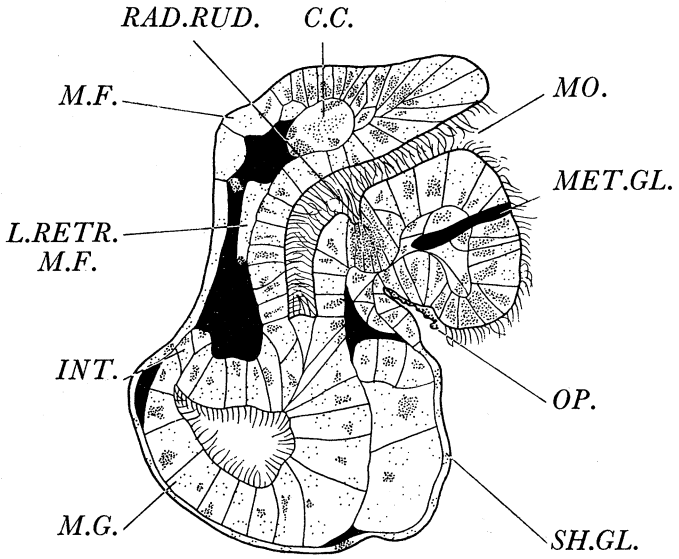

FIGURE 12 Sagittal section of embryo at 20 days.

The foregut comes to penetrate the wall of the midgut and their lumina communicate. Both foregut and midgut become internally ciliated. A lumen appears in the hindgut, which also acquires internal ciliation; the metachronal waves of these hindgut cilia travel towards the midgut. The paired midgut diverticula acquire lumina (more marked in the case of the left diverticulum) and they become connected with the lumen of the midgut (figure 13). From the first, the left diverticulum is larger than the right (figures 11 *A*, *B* and 13); this disparity becomes more and more marked as development proceeds. The cells composing the wall of the left diverticulum divide many times so that it comes to be made up of a large number of relatively small cells; this does not happen in the case of the right diverticulum, which remains composed of a few relatively large cells (figures 11 *A*, *B* and 13). The hindgut, at first blind at both extremities, acquires an open connexion with the midgut, mid-dorsally; in addition, it comes to penetrate the mantle fold on the right side of the embryo at the anus.

The radula sac rudiment appears first as a thickening, of cells with enlarged nuclei, in the ventral and ventro-lateral walls of the foregut, a short way behind the mouth opening (figure 12, *RAD.RUD.*). This rudiment subsequently grows down and back, forming a blind, tubular, posteriorly directed invagination of the ventral wall of the foregut.

The larval kidney (figure 9, *K2 RUD.*) comes to lie partially in the mantle fold, in a mid-lateral position on the right side. It at first consists of a single cell, but this divides twice, and so the organ comes to consist of a trio of flask-shaped cells, closely apposed and

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with a common pore to the exterior. Commencement of excretory activity by the larval kidney is marked by the appearance of a vesicle close to its pore to the exterior. This vesicle in life is colourless, as is the larval kidney, but in sections it can be seen that blackish, reticulate material accumulates in it throughout embryonic life (figures 15, 18, *ST.V.*). Shortly after commencement of activity, the larval kidney becomes free of the cytoplasmic yolk granules which at that time characterise all other cells of the embryo with the exception of the elements of the nervous system and of parts of the mantle.

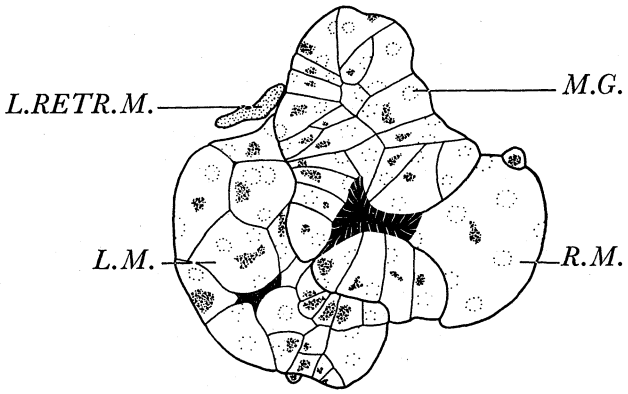

FIGURE 13. Transverse section through embryo at 20 days; passing through the most anterior part of the visceral mass.

The larval retractor muscle (figures 11 *D*, *E* and 13, *L.RETR.M.*) is made up of about ten long, spindle-shaped cells, which are initially heavily yolked. It becomes attached to the shell posteriorly, on the embryo's left side, and its anterior prolongations reach to all parts of the body. Its component muscle cells soon become capable of contraction, but, for reasons to be discussed later (pp. 21–2), are unable to effect any retraction of the embryo. Other muscular elements differentiate in the cephalopedal mass; in particular, muscle fibres running from the velar lobes into the sides of the foot, and circular fibres in the velum itself, are evident in life.

As the shell approximates to its maximum size, the mantle fold, hitherto apparently undifferentiated, undergoes striking histological changes, associated with the impending completion of shell secretion. The most anterior face of the mantle fold becomes differentiated into two layers: an outer, more anterior layer of columnar cells, and an inner layer of rounded cells (figures 15, 18, and 19). Both layers of cells have enlarged, deeply staining nuclei, and their cytoplasm becomes free of yolk granules. Progress of this histological change is more rapid in dorsal regions and on the right side, than on the left side; it never extends down to affect the mantle fold ventrally. It affects only, in fact, those regions of the mantle which line the mantle cavity.

C. *Formation of the optic ganglia and eyes* (22 to 24 days at 9 to 10 °C)

The onset of the development of the optic ganglia marks a turning point in the embryology of *Adalaria*. Hitherto, all ontogenetical differentiation has appeared to be directed at the production of a larval stage similar in all but very minor respects to that of other dorid nudibranchs (Thompson 1957). With the development of eyes, and with the occurrence

of the various other changes initiated contemporaneously, it becomes clear that a much more complex result is the goal.

Between 22 and 24 days after oviposition, the optic ganglia make their appearance, and within a further 1 to 2 days, optic pigment becomes visible in live embryos (figure 14, *L.OP.G.*). The elements which come to form the optic ganglia are cut off at deep invagina-

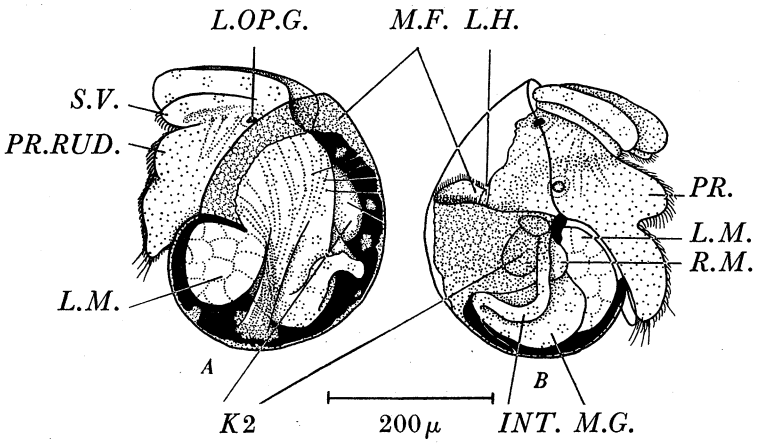

FIGURE 14. Later embryonic stages (drawings from life). *A*. Embryo at 23 to 25 days; left lateral aspect. *B*. Embryo at 30 days; right lateral aspect. (Velar locomotor cilia not illustrated.)

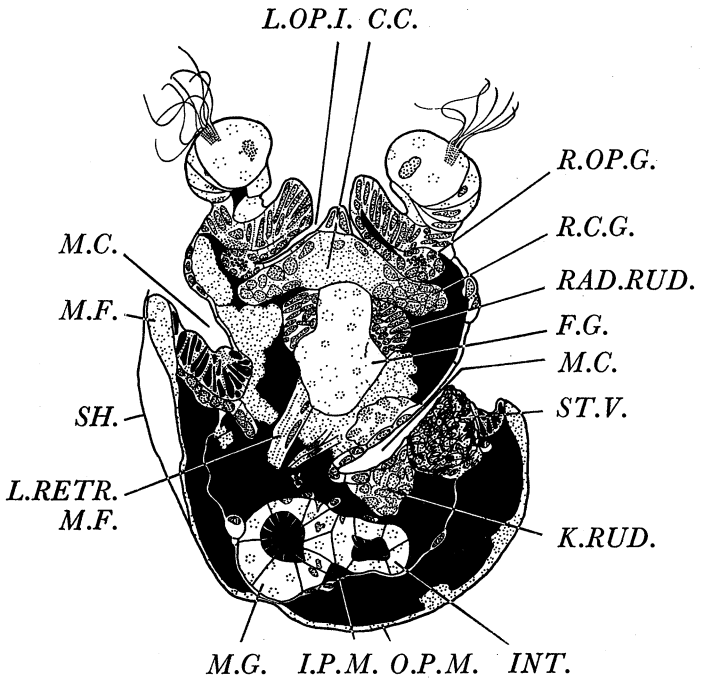

FIGURE 15. Horizontal section through embryo at 30 days; passing just above the level of the mouth.

tions which appear in paired regions of the intravelar ectoderm, dorsal to the regions at which the cerebral ganglia arose earlier (figure 15). Cells are cut off, migrate inwards, and form discrete ganglia dorsal to the cerebral ganglia. Development of the optic ganglia continues up to and after escape from the egg.

The optic pigment appears in the latero-dorsal face of each optic ganglion and takes the form of a shallow cup (figure 16*A*) in the concavity of which a small, spherical, hyaline

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lens is differentiated. The pigment is made up of a large number of blackish-amber granules. Soon after the appearance of eye pigment in live embryos, they become sensitive to sudden light changes, and usually respond by either partial or total retraction into the shell.

At the same time, the rudiments of the pleural ganglia make their appearance, being proliferated from paired ectodermal thickenings in the pedal epidermis, one on either side, just below the mouth (figure 16*B*, *R.PL.G.*). The elements produced migrate inwards and come to form two densely staining masses of cells dorso-lateral to their respective pedal ganglia.

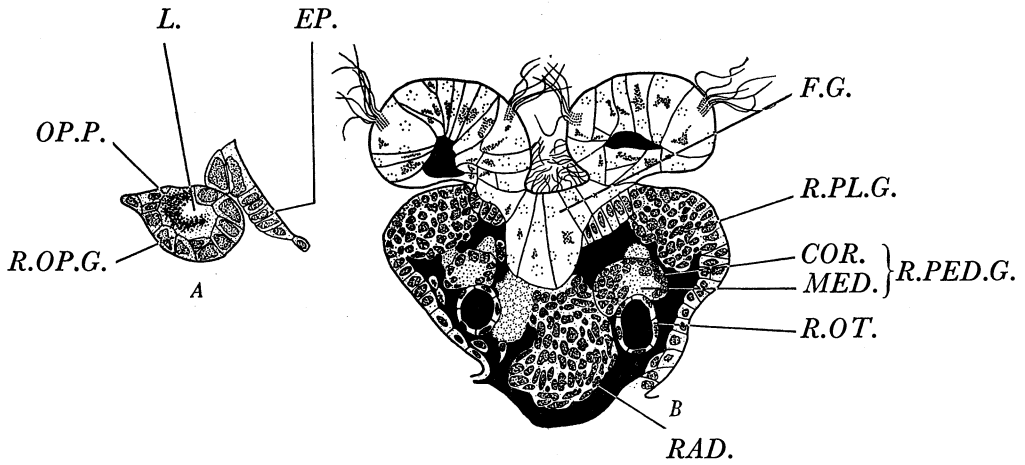

FIGURE 16. Horizontal sections of embryo at 30 days; same series as figure 15. *A*. Optic ganglion as it appears in section more dorsal than plane of figure 15 (not to same scale). *B*. section just ventral to the mouth.

The cerebral ganglia first, and then the pedals, become histologically differentiated into two distinct layers (figures 15, 16*B*, 18 and 19). The outer layer is densely nucleated, consisting of the cell bodies of the nerve fibres; the inner layer contains no nuclei, only nerve fibres. The outer layer will be termed the ganglionic cortex, the inner the ganglionic medulla. The cerebral and pedal commissures come to consist mainly of fibres. Connectives, again composed chiefly of nerve fibres, grow out from the cerebral ganglia, and, passing immediately in front of the otocysts, enter the pedals (figure 18, *R.C.P.C.*). The paired buccal ganglia, which lie one on either side of the foregut, probably arise as posterior migrations from the cerebral ganglia; they become linked by a narrow commissure running between the foregut and the radula sac diverticulum, and are soon differentiated into cortex and medulla. From the beginning the buccal ganglia are asymmetrically disposed; this will be dealt with more fully later (p. 30).

Contemporary with the beginning of the formation of the optic ganglia, the foot begins to undergo striking further development (figures 14, 17). Rapid multiplication of cells of the metapodial epidermis, near to the openings of the metapodial mucus glands, brings into existence the rudiment of the propodium. A marked effect of the beginning of this proliferation is that the median band of yolky epidermal cells (p. 16) becomes interrupted at this zone of mitotic activity, the extent of the interruption increasing with the growth of the propodium. Another consequence is that the external openings of the metapodial

glands come to be relatively further and further down the sole of the foot as the propodium develops. The propodium is from the first more strongly ciliated than the metapodium.

Accompanying the appearance of the rudiment of the propodium, the paired propodial mucus glands begin to develop. They arise by enlargement, division and differentiation of a few cells which lay between the otocysts. They aggregate into two masses, each of which

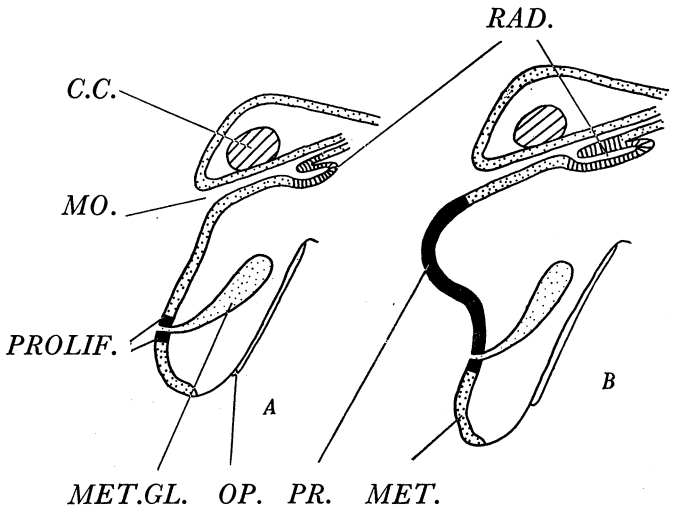

FIGURE 17. Diagrammatic sagittal sections to show mode of origin of the propodium. *A*. Early veliger. *B*. Late veliger.

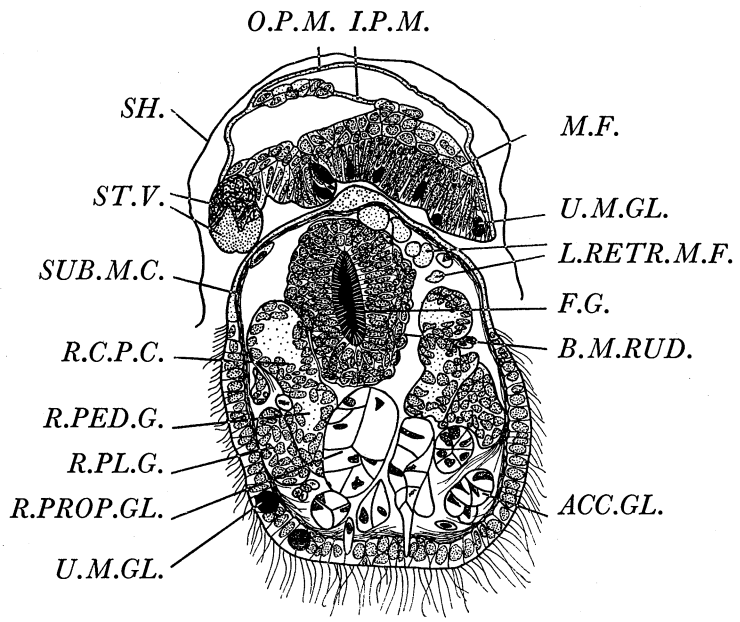

FIGURE 18. Transverse section of embryo at hatching; passing through the cephalopedal mass just behind the buccal pump rudiment.

becomes flask shaped, and acquires a separate duct to the exterior, opening just below the mouth (figures 18, 19, *PROP. GL.*).

From 2 to 3 days after the appearance of pigment in the eyes, secretion of the shell is completed and the mantle fold withdraws from the mouth of the shell (figures 14, 18 and 19). The space between the shell and the mantle, the shell cavity (p. 14), is now freely in

communication with the exterior. Withdrawal or detachment of the mantle fold from the shell mouth begins usually in dorsal regions and gradually spreads to the sides; the mantle fold remains attached to the shell ventrally. Retraction of the cephalopedal mass into the shell can now take place. This was earlier impossible, because of the restraining effect of the shell mouth—mantle fold connexion (that is to say, because of the restricted capacity of mantle cavity). Retraction in *Adalaria*, as in other dorids investigated (Thompson 1957), is not into the mantle cavity but into the shell cavity directly. Precocious severing of the connexion between the mantle fold and the shell mouth can be induced 1 or 2 days earlier than it would normally occur, by subjecting embryos to a sudden shock (mechanical agitation, or addition of a semi-toxic reagent, e.g. dilute alcohol).

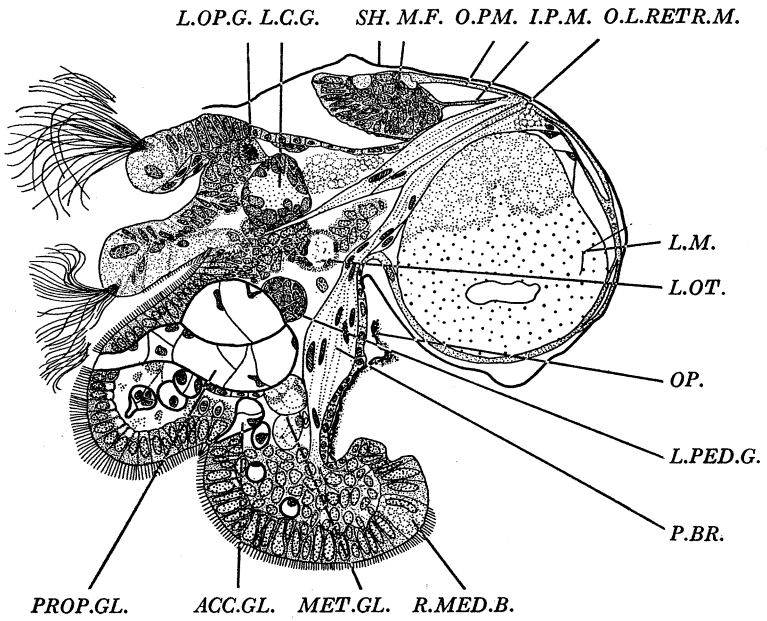

FIGURE 19. Longitudinal section through embryo shortly before hatching; passing to the left of the median plane.

In the radula sac, which has enlarged greatly and become consolidated by aggregation of mesoderm cells around it, the radular teeth develop. In those regions of the sac where teeth are being formed, the lumen is obliterated, and the teeth themselves appear to be laid down between interfaces of adjacent cells of the wall. The first teeth are not produced at the posterior extremity of the radula sac, but in a region about half way along its length.

A small number of mesoderm cells become aggregated close to the dorso-mesial face of the larval kidney. Initially, they are undifferentiated cells with large reticulate nuclei. There is no lumen enclosed within the aggregation, which is the rudiment of the adult kidney (figures 15, 23 and 25, *K.RUD.*).

D. *The structure of the embryo at hatching* (36 to 39 days at 9 to 10 °C)

(i) *The velum*

The paired velar lobes are disposed approximately in the shape of a figure 8 (figures 29, 30). Each lobe consists of a single row of large discoidal cells bearing the long, composite locomotor cilia, and of supravelar and subvelar supporting cells (figure 10, *SUP.V.C.*,

S.V.C.). The subvelar cells are arranged in several rows, are elongated with long nuclei, and form two ridges, running round the velar lobes below the main velar cell row, and meeting in the ventral borders of the mouth. The subvelar cells are strongly ciliated (figures 14, 30). In life the subvelar cilia beat continuously, whereas the main velar cilia can be stopped and started. Subepidermal spaces are present in the velar lobes, through which run fibres of the muscle systems of the embryo, and nerve fibres from the cerebral ganglia to the main velar cells. On fixation, the large composite cilia of the velum become frayed out into their components, and can be seen in sections to originate deep within the cytoplasm of the cells (figures 10, 15, 16). Very little yolk is present in the velar cells at hatching.

These observations are in general agreement with the more detailed work of Carter (1926, 1928), who examined the velar cells of various larval nudibranchs.

(ii) *The foot*

At liberation the foot is large and well developed. The original foot rudiment of the earlier veliger stages now forms only the metapodium of the larval foot. The propodium, which developed (figure 17) by ectodermal proliferation, is large and strongly ciliated.

An operculum is present on the posterior face of the metapodium; its form is described elsewhere (pp. 35–36, figure 31 *C*). The pedal epidermis is of single cell thickness; the epidermal cells have no cytoplasmic yolk granules and the median yolky band has almost disappeared (figure 19, *R.MED.B.*). The external surface of the foot (apart from that region next the operculum) is ciliated and various stiff longer cilia are present, particularly on the tip of the metapodium (figures 14, 29, and 30). Within the foot four types of gland can be recognized.

(*a*) The propodial mucus glands (figures 18, 19, *PROP.GL.*) are a pair of multicellular, flask-shaped glands. Their bulk lies between and below the otocysts and each has a separate duct, perforating the epidermis a short way below the mouth. The glands are made up of cells which, in sectioned material, stain uniformly dark green with alcian blue. Their nuclei are peripherally placed within the cells.

(*b*) The accessory pedal mucus glands (figures 18, 19, *ACC.GL.*) are abundant, small, flask-shaped multicellular glands. They are subepidermal, and open by separate ducts to the exterior, all over the sole of the foot. Histologically, they do not differ from the propodial glands.

(*c*) The metapodial mucus glands (figures 12, 19, *MET.GL.*) were originally a pair, but usually at hatching only the left member has persisted. In rare cases multiplication of this left member may occur. When, as is occasionally the case, both members are present at hatching, they lie close to the otocysts and extend down the foot to open to the exterior a short way in front of the tip of the metapodium. They are multicellular, each cell being vacuolated; these vacuoles appear in sections to be filled with material which stains pale green with alcian blue.

(*d*) The lateral pedal glands are unicellular, epidermal glands, and are most abundant and largest on the left side of the foot, near its base, but are present to a lesser extent in the corresponding position on the right side, and, sparsely, scattered over the sole of the foot (figure 18, *U.M.GL.*). In sections, some have contents which stain with alcian blue.

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Factors of the cephalopedal muscle complex (p. 29) can be seen in the foot, and its shape is very variable in life.

(iii) *The alimentary canal* (figures 34*B*, 35*A*)

The canal is throughout bounded by a wall of single cell thickness.

(*a*) *The mouth and foregut.* Above the mouth opening are the two velar lobes and below it is the strongly ciliated propodium. The subvelar ridges of the velum merge with the ventral border of the mouth; the effective beat of the subvelar cilia is towards the mouth. The borders of the mouth and the internal surface of the foregut are strongly ciliated.

Little or no yolk remains in the cytoplasm of the cells of the foregut; these cells have become smaller and the foregut lumen is accordingly more restricted.

From the mouth, the foregut travels back, rising steeply at first, then arching down to enter the midgut. The point of entry of the foregut into the midgut, which was earlier in the median plane of the embryo, at hatching has become displaced slightly to the right side. Viewed in optical section from above, the foregut lies in the median plane of the embryo, veering over slightly to the right just before entering the midgut.

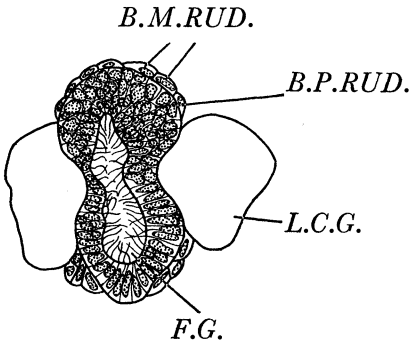

FIGURE 20. Transverse section through foregut of embryo at hatching; passing through the rudiment of the buccal pump.

Two foregut diverticula are present, one dorsal and the other ventral. The dorsal one is as yet rudimentary, and is no more than a thickening in the roof of the foregut, just behind the mouth; its cells have enlarged and become columnar. This is the rudiment of the buccal pump (figures 20, 36*A*, *B.P.*). The ventral diverticulum is blind, elongated and posteriorly directed; it is the radula sac. Mesodermal elements aggregated around it are the rudiments of the musculature of the buccal mass. Up to six pairs of teeth may be present in the radula sac at hatching; these teeth correspond to the large, hooked lateral teeth of the radula. A thickening in the floor of the most anterior part of the radula sac is the rudiment of the odontophore pad, on which the radular ribbon will later be supported during its use.

(*b*) *The midgut.* Throughout later embryonic life the intracellular reserves of the two midgut diverticula were progressively exhausted. However, this exhaustion did not affect the two organs in the same way. As the cells of the right diverticulum gave up their yolk, the overall size of the organ decreased, so that at hatching it is reduced to a cluster of cells lying on the right antero-lateral face of the midgut. It may be overlooked in life because it is partially hidden by the hindgut (figure 14*B*, *R.M.*). At liberation the cells of the right diverticulum are almost devoid of yolk.

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In the case of the left diverticulum, exhaustion of the yolk stores affected first those cells nearest the midgut (figure 21), and later spread over the whole organ (figure 22). As the cells lost their yolk, their nuclei became enlarged, giving a dark appearance in sections to these regions. This darkness was increased because those regions of the cytoplasm close to the nuclei tend to take up the nuclear stain also. In sections of early stages in the exhaustion of the yolk of these cells, ramifying islands of clear matter which holds mucus stains can be observed to extend around the yolk granules in the cytoplasm. In completely exhausted cells this substance, possibly enzymatic, fills that end of the cell away from the nucleus (figures 22, 33, *MUC.*). The nuclei are peripherally arranged.

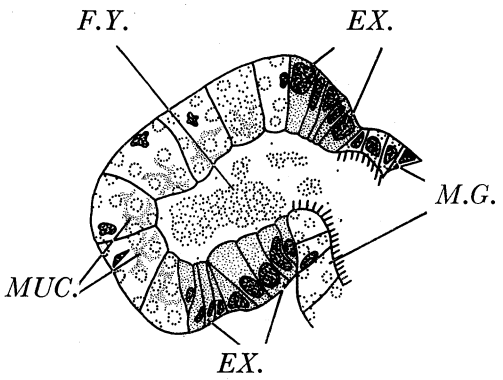

FIGURE 21. Horizontal section through left midgut diverticulum of embryo 7 days from hatching.

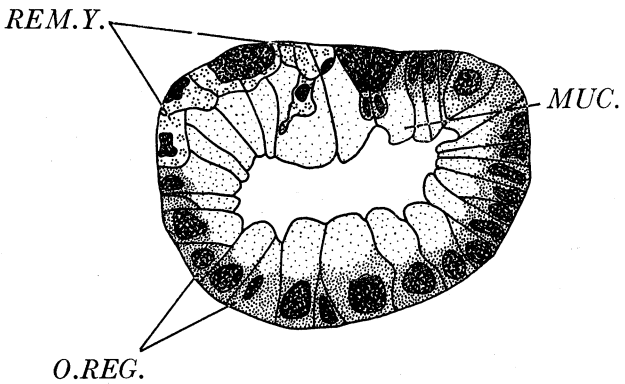

FIGURE 22. Horizontal section through left midgut diverticulum of embryo at hatching.

Neither of the midgut diverticula is internally ciliated. The midgut itself is strongly ciliated internally; these cilia are arranged on systems of raised ridges and can cause a rapid rotation of particles contained within the organ. During later embryonic life, it is always possible to observe yolk granules free in the lumen of the midgut (figure 21, *F.Y.*). The cells composing its wall have grown smaller and their cytoplasmic yolk stores are almost exhausted at liberation.

At hatching an increase in the overall size of the left midgut diverticulum has begun, and the organ can be seen to be extending across beneath the midgut (figures 29, 34 and 35). The most obvious consequence of this ventral extension of the diverticulum is that it brings about a slight rotation of the midgut (figures 34, 35). All parts of the midgut which lay dorsal to the hypothetical geometrical centre of the organ are displaced towards the

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left, and all parts which lay below this centre are displaced towards the right. Accordingly, the point of entry of the foregut into the midgut, formerly in the mid-line of the embryo, becomes displaced towards the right side; the point of communication between the hindgut and the midgut becomes displaced to the left of the median plane of the embryo. This rotation becomes more pronounced during pelagic life (p. 38).

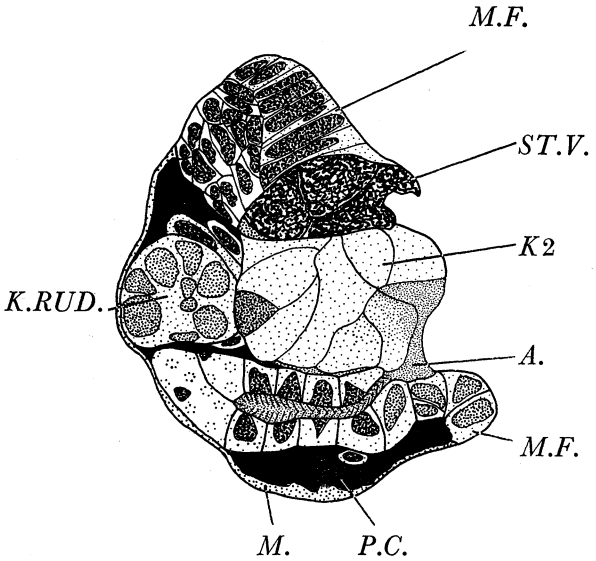

FIGURE 23. Horizontal section of a hatching larva, passing through the lateral extremity on the right side.

(*c*) *The hindgut.* This is a hollow tube, of uniform diameter and without any internal folding, running from the midgut, dorsally (but slightly to the left of the median plane of the embryo), to the anus, situated in a latero-ventral position in the mantle fold on the animal's right side. It is ciliated internally, and in life metachronal waves travelling towards the midgut are evident. Close to the terminal region of the hindgut, embedded in the mantle fold, are several clear vesicles. The anal opening is ventral to the external opening of the larval kidney (figure 23, *A.*).

(iv) *The larval kidney*

This is a flask-shaped organ made up of three large cells. Each cell contains a large vacuole whose contents stain uniformly greyish blue with haematoxylin. The larval kidney is partially embedded in the mantle fold, and is situated in a mid-lateral position on the embryo's right side, immediately above the anal opening (figures 14, 23 and 29, *K2*). Dorsal to the renal opening, and also embedded in the mantle fold, is a large vesicle which contains material that, in stained sections, appears irregularly reticulate and blackish (figures 15, 18 and 23, *ST.V.*). It is probable that this vesicle stores excretory products converted to an insoluble form during embryonic life.

(v) *The adult kidney*

The definitive kidney is in rudimentary form at hatching; it is a spherical aggregation of mesoderm cells lying above and behind the larval kidney (figures 15, 23 and 25, *K.RUD.*). No lumen is enclosed within this rudiment, but in more advanced embryos the cells may be centripetally vacuolated. The organ has no communication with the exterior.

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A small, undifferentiated aggregation of mesoderm cells close to the definitive kidney rudiment (and connected with it by a short solid rod of cells) is the rudiment of the adult heart.

(vi) *The mantle and the body cavities* (figure 24)

(*a*) *The mantle fold* is simply a flared collar standing out from the body behind the cephalopedal mass (figures 11, 14, 15 and 19). The outer margin of the collar is free, except in ventral regions where it remains in intimate contact with the mouth of the shell (figure 19). A restricted mantle cavity is subtended between the mantle fold and the dorsal body wall. This is in wide communication with the exterior, and is most capacious on the embryo's right side.

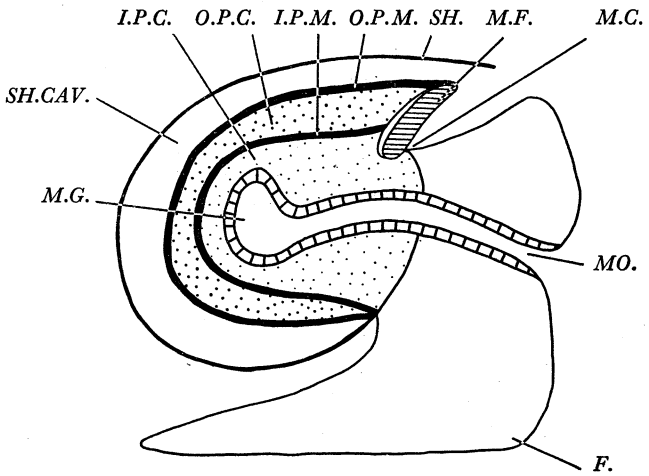

FIGURE 24. Diagrammatic sagittal longitudinal section, to show the relations of the body cavities.

The mantle fold is asymmetrical in that it has greater anterior extent on the left side, yet is thickest on the right side (figures 15, 25). This is correlated with the direction of the incipient coiling of the shell (p. 35). The histological differentiation undergone by the mantle fold some time before hatching has been dealt with (p. 18 and figures 15, 18 and 19).

The fold is ciliated externally and the outer columnar layer of cells is interspersed with unicellular mucus glands (figure 18, *U.M.GL.*). Contractile elements, part of the cephalopedal muscle complex (p. 29), differentiate in the inner layer of the mantle fold. Their contraction can vary the capacity of the mantle cavity; in sections of poorly fixed material, it can result in the appearance of irregular folds in the mantle.

(*b*) *Membranes enclosing the visceral organs* (figures 15, 18, 19, 24 and 25). Extending back from the outer margin of the mantle fold all round the embryo is a thin membrane which encloses the visceral organs in a perivisceral sac. This outer perivisceral membrane is the remnant of the shell gland investment which earlier closely overlay the visceral organs and secreted the earliest embryonic shell. After this task had been completed, and after the visceral organs had differentiated so as to leave spaces between them, the outer perivisceral membrane (=shell gland or mantle) virtually ceased to be intimately connected with either the viscera or the shell, except in a few regions; it came to delimit a fluid-filled sac in which the visceral organs lay, i.e. the perivisceral cavity.

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The inner perivisceral membrane lies within the perivisceral sac and effectively subdivides the perivisceral cavity into two parts, between which no direct communication was evident. Dorsally this inner membrane lies free in the perivisceral cavity, but in ventral regions it lies in close contact with the ventral faces of the midgut and diverticula, the hindgut, the larval kidney and the adult kidney. The inner membrane contains contractile elements, and is linked by them to the various organs it encloses, also to the trunk of the larval retractor muscle. The contraction of certain of these elements can constrict the midgut and the left midgut diverticulum, thus probably aiding interchange of particles

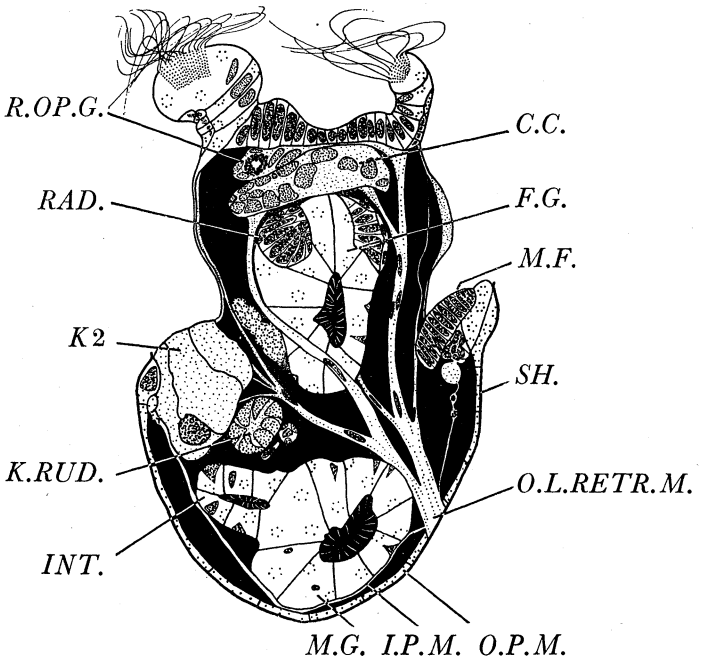

FIGURE 25. Oblique horizontal section through embryo shortly before hatching.

between the cavities of these two organs. A similar phenomenon has been described for *Ostrea edulis* by Millar (1955). The origin of the inner perivisceral membrane is not fully understood, but it seems probable that it arose by a split in the mantle at the time when the mantle was becoming less securely attached to the shell and viscera.

It is very probable that the inner perivisceral cavity corresponds to the 'coelom' of larval *Aplysia* (Saunders & Poole 1910).

(*c*) *Other body cavities*. Subepidermal spaces of blastocoelic origin occur in the cephalopedal mass (figures 10, 15, 18, 19 and 25).

(vii) *The 'larval heart'* (figure 14*B*, *L.H.*)

This is a vesicle which pulsates irregularly, placed high up on the embryo's right side. It made its appearance a few days before hatching. Its activity accelerates the flow of fluid into and out of the mantle cavity, which is induced by the mantle cilia. This flow brings about the dispersal of excretory products during larval life; they are discharged into the mantle cavity just below the larval heart. Muscular elements of the inner perivisceral membrane are connected with this 'heart'.

(viii) *The muscle systems*

(*a*) *The larval retractor muscle* (figure 26) has its origin on the inner surface of the shell, postero-laterally, to the left of the median plane of the embryo. From this origin, the muscle trunk runs forwards, passing between the dorsal extremities of the midgut and of the left midgut diverticulum, before dividing into its component branches (figures 18, 19, 25 and 26). The muscle is composed of a large number of elongated cells, the original number of component cells having become greatly increased. The pedal branch alone now consists of more than twelve cells. The nuclei of the muscle cells are small and elongated along the long axes of the cells. No nuclei occur in the main trunk of the muscle.

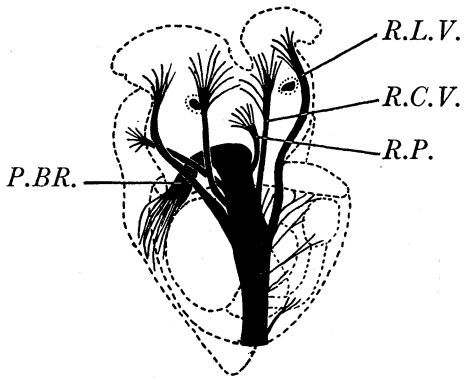

Figure 26. Reconstruction of the factors of the larval retractor muscle in a hatching embryo.

Apart from minute branches to the mantle and viscera, there are large branches to the central velar region (a pair), to the lateral velar regions (a pair), to the propodium (a pair), and a single very thick branch to the metapodium (figures 19, 25 and 26). This metapodial branch has insertions all over the inner surface of the operculum (figure 31*C*), and has many smaller branches to all parts of the meso- and metapodium.

The supposed 'twist' through 180° to which Pelseneer (1911) attributed considerable significance, is, in *Adalaria* (and in a variety of other nudibranchs examined) an illusion, best explained as a product of the slight divergence of course between the pedal branch of the retractor muscle, and the main trunk (figure 11*E*, 14*A*). The pedal branch, from the origin of the muscle on the shell, travels directly towards the foot; its fibres thus travel obliquely across the long axes of the fibres of the main trunk. This divergence gives rise to what might appear to be a twist in the muscle.

(*b*) *The cephalopedal muscle complex* (figure 18, *SUB.M.C.*) consists of a system of muscular elements lying beneath the epidermal layers of the foot and velum, forming a muscular sheath, the activities of which, in life, are partly responsible for the great mobility and plasticity of the cephalopedal mass. Elements of this system come to penetrate the mantle fold during late embryonic life.

(ix) *The shell* (figure 31*A*, *B*)

A full description of the shell is given on p. 35. It measures, in its maximum dimension in lateral aspect, between 0·28 and 0·30 mm. No growth of the shell takes place after the embryonic stage at which the mantle fold withdrew from the shell mouth (p. 21).

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(x) *The nervous system* (figure 27)

At hatching the nervous system consists of paired cerebral, optic, buccal, pedal and pleural ganglia, linked by a complex of commissures and connectives. Of the five pairs of ganglia, only the pleurals and optics are still incomplete. All the others are free of the epidermis and have become differentiated into cortex and medulla (see p. 20). Commissures are present linking the component members of all the pairs of ganglia except the pleurals and optics.

The cerebral ganglia lie one on either side of the foregut. They are linked together by a broad cerebral commissure which passes immediately in front of the buccal pump (figures 25, 27 and 36, *C.C.*).

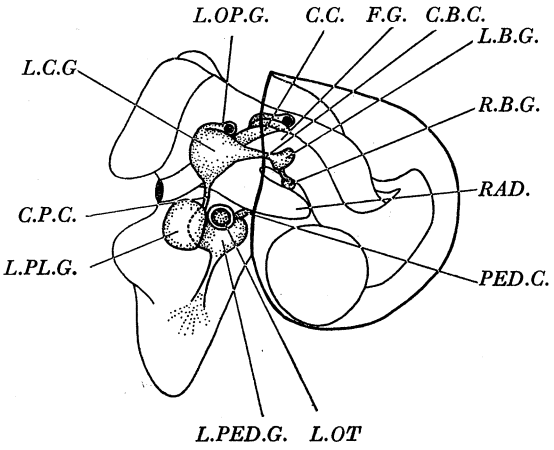

FIGURE 27. Reconstruction of the nervous system of a hatching embryo (left lateral aspect—diagrammatic).

The optic ganglia (figures 15, 16, 19, 25, 27 and 29, *OP.G.*) are a pair, one on either side, lying against the antero-dorsal faces of the respective cerebral ganglia. Each ganglion has a bulb-like expansion which contains granules of the blackish amber optic pigment, arranged in the form of a cup. In the concavity of each cup, which is directed upwards and outwards, is a small, clear, hyaline lens. The optic ganglia are broadly connected to their respective cerebral ganglia by short cerebro-optic connectives.

The pedal ganglia (figures 16, 18, 19 and 27, *PED.G.*) are a pair of kidney-shaped aggregations lying in the base of the foot, with the otocysts fitting closely into the 'notches'. They are linked by a narrow pedal commissure.

The pleural ganglia are a pair, lying one on either side, close to the dorso-lateral faces of the pedal ganglia (figures 18, 27, *PL.G.*).

The buccal ganglia are a pair, lying one on either side of the foregut a short way posterior to the cerebral ganglia. They are linked by a buccal commissure which passes between the foregut and the radula sac diverticulum. The buccal ganglia are asymmetrically disposed, that on the left side being tilted upwards around the foregut, and that on the right downwards around the radula sac (figure 27).

Cerebro-buccal connectives are present and continue behind the buccal ganglia, that on the right side passing over the oesophagus to the dorsal regions of the visceral mass and that on the left passing downwards to the ventral parts of the visceral mass. A thick pair of cerebro-pedal connectives are present, passing in front of the otocysts.

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The otocysts are a pair of internally ciliated sacs, bounded by a wall of single cell thickness. In life, they each contain a single large calcareous otolith, which slowly rotates under the influence of the continuous beat of the cilia. The otocysts lie against the anterior faces of their respective pedal ganglia, and the medulla of the ganglion extends up to the interface between the ganglion and the otocyst.

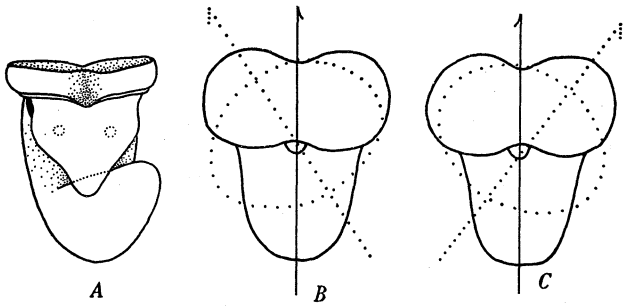

FIGURE 28. Diagrams illustrating asymmetries of the embryo. *A*. Generalized ventral view of embryo. *B*. Illustrating the relationship between the dorso-ventral axes of the cephalopedal mass (solid line) and of the first-formed part of the shell (interrupted line). *C*. Illustrating the relationship between the dorso-ventral axes of the cephalopedal mass (solid line) and of the visceral mass (interrupted line).

(xi) *Asymmetry of the embryo*

A study of live and sectioned veliger stages shows that, as well as the obvious asymmetry of the veliger indicated by the location of the intestine and larval kidney on the right side and the predominance of the larval retractor muscle on the left, more subtle asymmetries of the embryonic body occur.

These are basically two in number, each rendering the other more difficult to analyze.

(*a*) An incipient *sinistrality* in the relations of the dorso-ventral axes of the cephalopedal mass, on the one hand, and the shell, on the other. This is most clearly evident in live extended embryos (figure 28 *A*).

If the dorso-ventral axis of the cephalopedal mass be represented by a vertical line, then if it be imagined that the embryo is viewed from the anterior, a line representing the dorso-ventral axis of the first-formed part of the shell (that part which encloses the visceral mass) will appear to be displaced to the left (figure 28 *B*).

Because the dorso-ventral axes of the cephalopedal mass and of the last-formed part of the shell are coincident, the shell itself is an apparently sinistral one.

(*b*) An incipient *dextrality* in the relations of the dorso-ventral axes of the cephalopedal mass, on the one hand, and the visceral mass, on the other. This can be seen clearly in serially sectioned embryos.

If the dorso-ventral axis of the cephalopedal mass be represented by a vertical line, then if it be imagined that the embryo is viewed from the anterior, a line representing the dorso-ventral axis of the visceral sac (leaving the shell entirely out of consideration) will appear to be displaced to the right (figure 28 *C*).

This displacement shows very clearly when the foregut and radula sac are followed through successively more posterior transverse sections. These two organs are the only ones common to both cephalopedal and visceral masses. In the cephalopedal mass they are

both median and symmetrically placed, the foregut being immediately dorsal to the radula sac. As they pass back towards the visceral mass, they begin to conform to its dorso-ventral axis; in more posterior sections the radula sac appears to move out towards the right side of the cephalopedal mass, whereas it is simply beginning to take up its new position, still median, but this time median with regard to the axes of the visceral mass.

Although it has been possible to attempt the foregoing analysis of the asymmetries of the embryo, it has been found far more difficult to interpret them in terms of function and of relationship. The dextral displacement of the dorso-ventral axis of the visceral mass with regard to that axis of the cephalopedal mass can perhaps be attributed simply to relationship, for the vast majority of gastropods show such a displacement (which, if continued after embryonic life, will result in a dextrally coiled visceral mass). The sinistrality in relations between the cephalopedal mass and the shell is, however, extremely difficult to correlate with relationship (apart from the fact that it is characteristic of all opisthobranch veligers), and, indeed, has proved impossible to associate with any adaptation to larval life. Its structural aspects are elaborated on page 35.

(xii) *Liberation from the egg*

This occurs from 36 to 39 days after oviposition, at 9 to 10 °C.

All the embryos in any ribbon do not escape simultaneously, but the last healthy larva to hatch generally escapes between 1 and 3 days after the first. Once an embryo has escaped from the primary case, it is usually able to progress through the secondary membrane and the ribbon jelly quite easily. The jelly becomes more flaccid some days before liberation begins; similarly, the primary membrane becomes very pliable and even the pressure of a cilium of the embryonic velum is sufficient to distort its shape.

The mechanism by which escape from the primary case is effected is not fully understood; possibly it is aided by an enzyme, as Berrill (1931) suggested for *Acera*; certainly it cannot be traced to action of either the radula or the operculum.

6. The pelagic stages

The veligers, after escaping from the egg mass, swim strongly upwards. In culture this almost invariably results in their becoming trapped in the surface film of the water, for the larval shell has a peculiarly water-repellent quality. The larvae become lodged in the surface with the back of the shell projecting out of the water; the velum and foot remain beneath the surface, and so the ciliary apparatus of the larva is unaffected. In order to allow further development to take place, it was necessary to sink such marooned larvae by gently squirting water from a pipette on to the surface.

A. *Pelagic locomotion*

Locomotion is effected by the beat of the long velar cilia, which imparts a forward motion to the larva. The velum is well developed in *Adalaria* and the locomotor cilia are long in proportion (figure 30). The larva can swim in a straight line, without the spiralling so often characteristic of animals which swim by ciliary means.

Swimming activity is interrupted at intervals, the larva half retracts into the shell and sinks slowly. If, at any time while swimming, the larva encounters an obstacle, it creeps

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over it in the usual gastropod fashion, propelled by the cilia of the foot. During creeping, the velar lobes are brought together and held partially within the shell. If the obstacle is not suitable for metamorphosis of the larva, the velar lobes are protruded and the larva swims away.

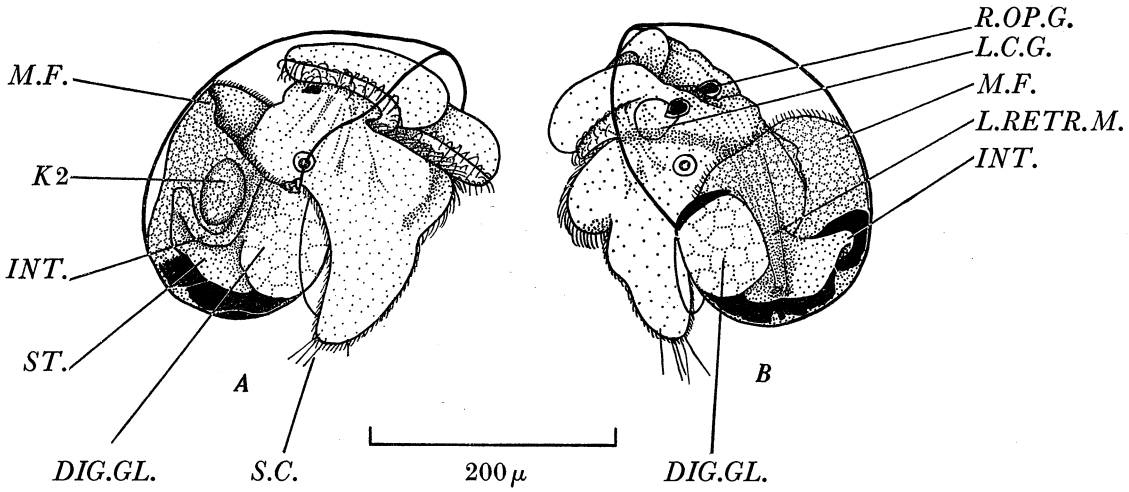

FIGURE 29. Early shelled larvae of *Adalaria proxima* (drawings from life). *A*. Right lateral aspect. *B*. Left latero-dorsal aspect. (The velar locomotor cilia are not illustrated.)

B. *Feeding*

Feeding occurs during swimming, for the beat of the long velar cilia serves a dual purpose in both imparting a forward motion to the larva and in bringing a constantly renewed supply of sea water within the reach of the ciliary apparatus. This apparatus was investigated using a technique devised by Drinnan (1954). Larvae were cemented by their shells to the tips of finely drawn glass rods, by means of an adhesive, Bostik 125. They were then examined in sea water in a Perspex vessel, constructed in such a manner that the glass rods are supported, yet can be rotated if necessary.

This technique was devised originally for examination of larvae of *Ostrea edulis*, which were dried on filter-paper before fixing them to the rods; with larvae of *Adalaria*, however, it was found more efficient to affix them while they are trapped in the surface film of the culture vessel. This modification greatly decreased the mortality incurred in transference to the inspection vessel.

Micro-organisms employed to demonstrate the feeding currents of *Adalaria* included *Chlorella stigmatophora* Butcher and *Isochrysis galbana* Parke.

The feeding currents are simple and are represented in figure 30. Particles in the water which the beat of the velar locomotor cilia causes to flow past the larva are swiftly conducted towards and into the mouth by the cilia on the subvelar ridges. Feeding is of relatively small importance to the larva of *Adalaria*, which can undergo normal further development either in sterile or artificially enriched sea water.

The borders of the mouth itself are strongly ciliated and direct a current into the foregut, the strong ciliation of the latter rapidly forcing any particles brought within its influence into the midgut. Once inside the midgut, particles are rotated rapidly by a raised band of cilia on the inner surface of its wall. Seemingly at random, particles pass into the lumen

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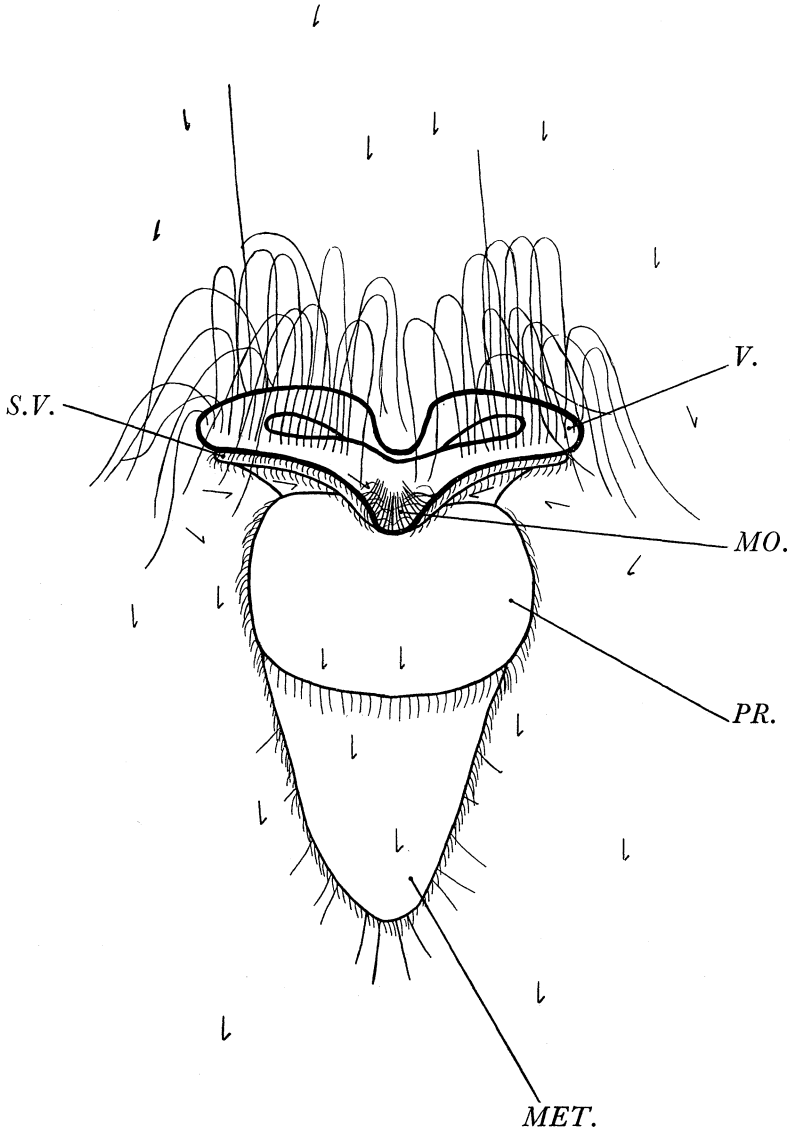

FIGURE 30. The cephalopedal ciliary apparatus of the larva of *Adalaria proxima* (ventral aspect).

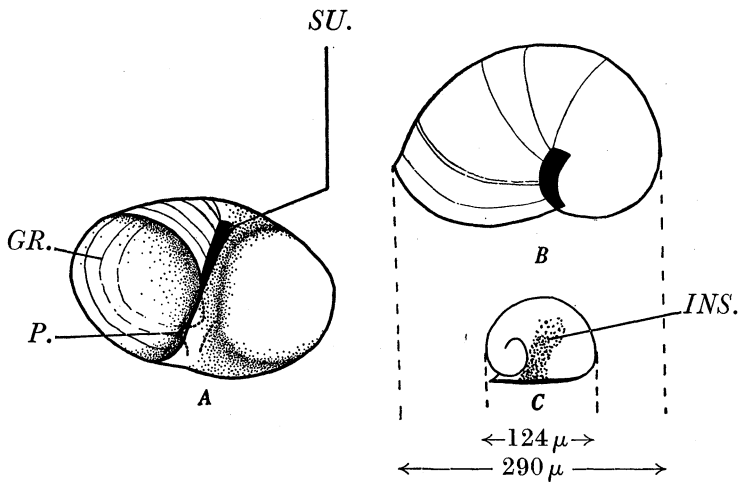

FIGURE 31. The larval shell and operculum of *Adalaria proxima*. *A*. Ventral view of the shell. *B*. Left lateral aspect of the shell. *C*. Operculum viewed from the inside.

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of the left diverticulum and are there ultimately ingested by cells of its wall. Digestion is intracellular, but it is not known whether it is assisted by extracellular digestion.

Contraction of muscular elements associated with the inner perivisceral membrane (p. 28) can cause temporary constriction of the midgut and diverticulum, and this no doubt aids the effective mixing of particles within their lumina.

C. *The larval shell and operculum* (figure 31)

The shell of *Adalaria* belongs to the type B of Vestergaard & Thorson (1938) and Thorson (1946). It is larger than that of any other dorid nudibranch (Thompson 1957) and measures 0·28 to 0·30 mm in its longest dimension in lateral aspect. If orientated in the usual way, it is plain that the shell is an incipiently sinistral one; this accords with the more anterior extent of the mantle fold on the left side in embryonic stages. No growth of the shell can occur during pelagic life.

The shell bears no characteristic sculpturing, but various scattered aggregations of minute pits may be present. Growth lines may or may not be visible.

(i) *Analysis of the sinistrality of the shell*

Dorid shell sinistrality is not in accord with the organization of the larval body itself. That the dorids are truly dextral organisms is shown by the following observations:

(*a*) The cleavage of the egg follows the course typical of that of dextral gastropods (p. 10 and figure 5 *A* to *G*; figure 50 *B* to *N*, plate 1).

(*b*) An incipiently dextral twist between the cephalopedal mass and the visceral mass was evident from a close examination of the relations between the dorso-ventral axes of those two divisions of the embryonic body (p. 31).

(*c*) The anus, larval kidney and deepest extent of the mantle cavity are all placed on the right side of the larval body, an arrangement identical with that in most dextral gastropods but no sinistral forms.

(*d*) The spiral of the operculum (figure 31 *C*), when viewed from the outside, follows, from its nucleus, a counter-clockwise course. (The reverse is, of course, the case if the operculum is viewed from the inside). This is typical of gastropods whose organization is dextral.

With these considerations in mind, it is clear that the larval shell of the dorid nudibranchs is truly a *hyperstrophic* one, the superficial sinistrality masking a fundamentally dextral organization of the larval body. Observations of a similar nature on various species of nudibranchs (Thompson 1957) tend to confirm the conclusion that the hyperstrophic larval shell is the rule in nudibranchs.

(ii) *Retraction of the larval body into the shell*

Retraction may be total or only partial, depending on the strength and nature of the stimulus. Partial retraction occurs after mild stimuli, such as a sudden change in light intensity; it involves the retraction of only the velar lobes and the upper part of the propodium. The attitude of partial retraction is adopted during the frequent pauses in swimming activity, during which the larva sinks passively. It is also the attitude adopted when the larva creeps over an obstacle.

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Complete retraction occurs after a violent mechanical or chemical stimulus, and involves the withdrawal of the whole cephalopedal mass into the shell cavity, followed by the closure of the shell aperture by the operculum, which lodges some way inside the shell mouth. After a short period, during which the velar locomotor cilia remain quiescent, the larval retractor muscle relaxes and the operculum is protruded slightly. The tip of the metapodium comes to project slightly into the medium, with the long stiff sensory cilia on its tip and sides (figure 30) outside the shell. This attitude may be maintained indefinitely.

During retraction the long velar cilia are first stopped, then brought together above the velar lobes; then the velar lobes themselves are brought together by contraction of elements of the cephalopedal muscle complex; next the velum is withdrawn into the shell cavity, then the propodium and then the metapodium as the foot arches up and back into the mouth of the shell. Retraction of the cephalopedal mass is brought about solely by the contraction of the larval retractor muscle, but contraction of muscular elements in the inner perivisceral membrane (p. 28) first pulls the visceral organs far back into the hump of the shell, to make room, as it were, for the head and foot.

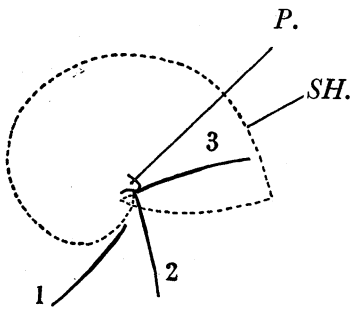

FIGURE 32. Successive positions occupied by the operculum during retraction of the larva into the shell.

Both retraction and extension of the larva are smooth, orderly processes; the former is accomplished chiefly by contraction of the larval retractor muscle, with some participation by muscular elements of the cephalopedal muscle complex and of the inner perivisceral membrane; the mechanism of extension is not fully understood, but obviously must involve integrated contraction of muscle elements to produce pressure changes within the various body cavities.

(iii) *The operculum during retraction* (figure 32)

When the larva is fully extended, the operculum is held well clear of the shell aperture; it is not hinged to the mouth of the shell in any way. During retraction (figure 32) it is brought round and back towards the shell mouth until its inner margin touches the peg on the right side of the shell, just inside the aperture (figures 31 *A*, 32, *P.*). This peg acts as a fulcrum and the outer edge of the operculum then travels through an arc which is completed when it is inside the shell mouth.

The operculum itself (figure 31 *C*) if viewed from the inside is approximately semicircular and convex. Pits where fibres of the larval retractor muscle were inserted can be seen in detached opercula, and they lie, as would be expected, chiefly on the left side.

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D. *Analysis of the larval phase*

The pelagic larval phase is divided into two distinct stages, the first occupying 1 or 2 days after hatching, the second of up to 14 days duration. During the first stage, the larvae tend to swim upwards; this behaviour is reversed at the start of the second stage.

It is convenient to name the first stage the *obligatory phase* of pelagic life, for, until the internal changes which take place during it are completed, the larva is incapable of metamorphosis. At the end of the obligatory phase these changes are complete, the upward-swimming behaviour is reversed and the *searching phase* begins. If at any time during the searching phase the larva alights on a favourable substratum, metamorphosis will occur. Searching may be continued for up to a fortnight before the larva dies. Attempts to alter the relative durations of the two phases by enrichment of culture media were unsuccessful.

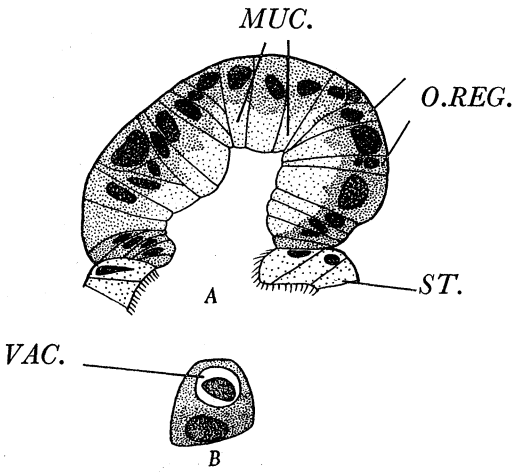

FIGURE 33. Section through the left midgut diverticulum (digestive gland) of early shelled larvae. *A*. Starved larva. *B*. Cell of diverticulum of a larva which was fed on plant micro-organisms.

(i) *The obligatory phase*

During the 1 or 2 days occupied by this phase, various changes in internal organization occur. These changes are as follows:

(*a*) *The mantle* (figures 37*A*, 38*A* and 39). The mantle fold becomes reflected back and, in a proportion of larvae approximately 2 days after hatching, has fused with the dorsal and lateral regions of the outer perivisceral membrane. The columnar layer of the mantle fold, which originally lined the embryonic mantle cavity, now forms the outermost layer of the new dorsal integument. Reflexion of the fold is caused mainly by differential growth processes, but probably is aided by contraction of muscular elements in the inner perivisceral membrane.

This reflexion of the mantle fold brings the anal and larval kidney apertures out still further to the side, and these openings are now directed laterally, instead of anteriorly, as was earlier the case.

(*b*) *The alimentary canal* (figures 33, 34 and 35). Histological changes: the left midgut diverticulum, which, until liberation, acted as a storage organ, during pelagic life functions, as it does in post-larval stages, as a digestive gland. The midgut itself, which in pelagic

feeding stages acts as a sorting chamber (in so far as any true sorting does in fact occur) will henceforth be referred to as the stomach. Sections of the digestive gland show food particles enclosed within cytoplasmic vacuoles. In life, these ingested organisms often give the wall of the digestive gland a green tinge.

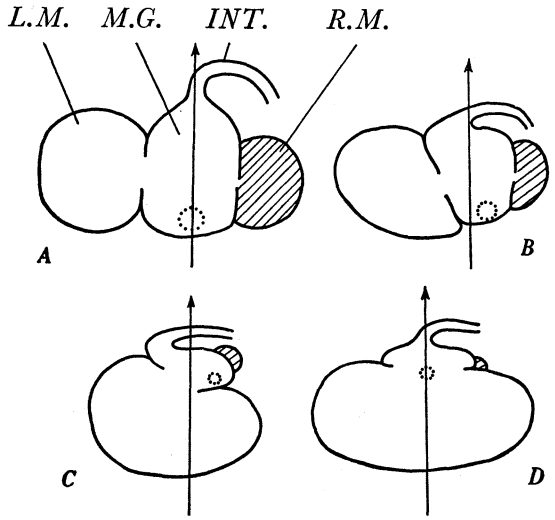

FIGURE 34. Diagrammatic series showing the disposition of the alimentary canal if viewed from the posterior of the animal. *A*. Before hatching. *B*. At hatching. *C*. In the early pelagic stages. *D*. After metamorphosis. The arrows represent the hypothetical dorso-ventral axis of the visceral mass. The dots indicate the position of the communication between the foregut and the midgut.

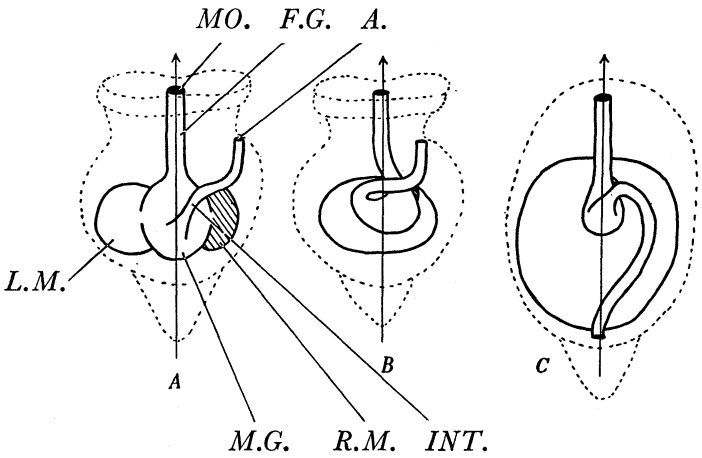

FIGURE 35. Diagrammatic series showing the disposition of the alimentary canal if viewed from above the animal. *A*. Before hatching. *B*. In the early pelagic stages. *C*. After metamorphosis. The arrows represent the antero-posterior axis of the animal.

Growth changes: the enlargement and extension beneath the stomach of the digestive gland, which was evident at hatching, continues, and the resultant rotation of the stomach becomes still more pronounced. This process is completed towards the close of the obligatory planktonic phase and at that time the digestive gland has come to lie across the visceral sac beneath the stomach; it has greater lateral extent on the right side than either the stomach or the right midgut diverticulum.

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It must be emphasized that this stomach rotation is to be regarded as the result of a growth process which is quite separate from any aspect of gastropod torsion. It involves the rotation of *some* of the organs *within the shell*; the relations between the shell and retractor muscle, on the one hand, and the cephalopedal mass, on the other, are not altered.

At the end of the obligatory phase, this stomach rotation is partially reversed, the extent of this reversal varying in different individuals. Differential growth of regions of the

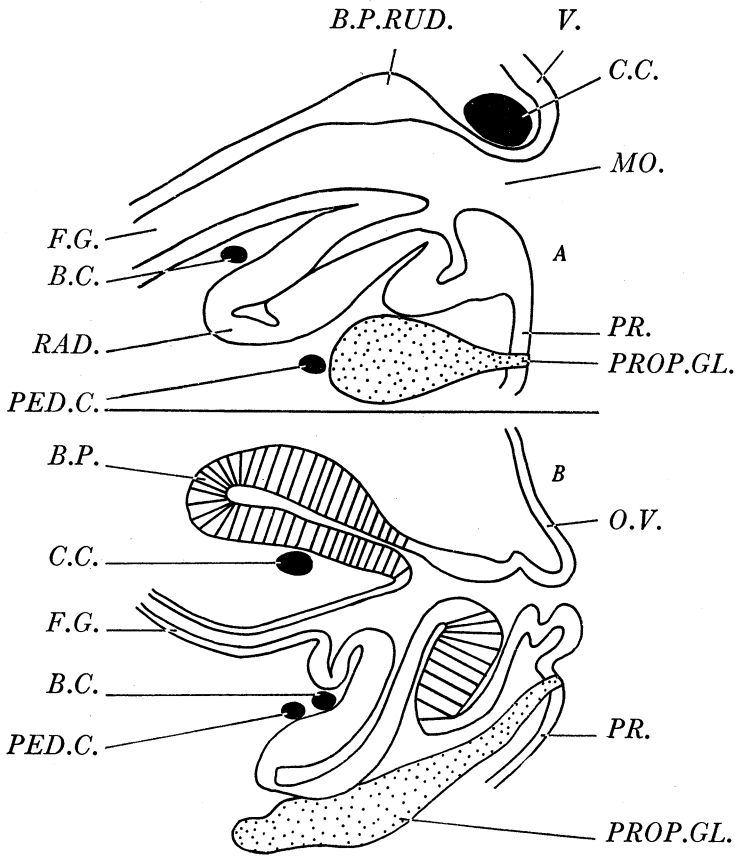

FIGURE 36. The buccal mass of *Adalaria proxima* in diagrammatic sagittal longitudinal section. *A*. In the shelled larva. *B*. In the post-metamorphal stages.

stomach brings about a return to the earlier condition with the foregut-stomach and hindgut-stomach connexions once again approximating to their original median positions. Hence, it is convenient to regard the initial stomach rotation as a temporary mechanical consequence of the enlargement and ventral extension of the digestive gland, for which a correction, by differential growth of parts of the stomach wall, is soon made.

At the onset of the searching phase, the opening of the stomach into the digestive gland has so widened that in some individuals their lumina are completely confluent (figure 33 *A*).

(*c*) *The larval musculature*. Throughout the obligatory planktonic phase, it is the larval retractor muscle (aided in a co-ordinatory capacity by the cephalopedal muscle complex and by contractile elements associated with the inner perivisceral membrane) which is chiefly responsible for retraction of the larval body into the shell. As the mantle becomes reflected, however, muscular elements differentiate which run from it down into the foot on both sides. On the left side, these merge into the pedal branch of the larval retractor

muscle. On the right they are more strongly developed; it was found impossible to decide whether this muscle (figure 39, *R.F.RETR.M.*), to be referred to as the right foot retractor muscle, is homologous with the columellar muscle of dextral, spirally coiled gastropods, delayed in development as so often occurs, or whether it is a separate development, merely a mirror-image of the few fibres on the left side, which follow the same course, and develop at the same time.

(*d*) *The nervous system.* Proliferation of the pleural and optic ganglia continues, but is still not complete at the close of the obligatory planktonic phase. Concentration of the existing ganglia begins, their connectives and commissures shortening and thickening.

(*e*) *The excretory system.* The larval kidney in life is colourless, as also is its storage vesicle. Contrary to expectation, the storage vesicle does not void its contents.

Throughout the obligatory phase, the larval body becomes less securely attached to the shell. Several connexions between the visceral mass and the shell are severed and when the larva reaches the stage when it is first competent to metamorphose, it is held to the shell only at the origin of the larval retractor muscle and by the mantle fold at the ventral mouth of the shell.

(ii) *The searching phase*

Searching behaviour commences at the end of the obligatory phase, and will be continued for up to a fortnight if a favourable substratum is not encountered. If, however, searching for a week has not resulted in the location of such a substratum, then a slowly increasing proportion of larvae becomes abnormal, and incapable of progressive further development.

During the searching phase, various further changes take place which are in the nature of being anticipatory of metamorphosis. These anticipatory changes are as follows.

The reflected mantle fold begins to spread forwards and backwards over the upper surface of the larva. Development of the pleural and optic ganglia may be completed. Paired thickenings then appear in the intravelar area which are the rudiments of the rhinophoreal tentacles. Much later, the shell may be cast, while the operculum and velum are retained, and the resulting shell-less forms (which would almost certainly be short-lived in nature) may live for several days in culture. Even later, the operculum may be cast and the velar lobes reduced in size. Although these last-mentioned forms are obviously atypical, a (decreasing) proportion of them remains capable of healthy progressive development if allowed access to a suitable substrate.

The searching phase may be best summarized by the following division into two somewhat artificial parts.

(*a*) Throughout the first week after the close of the obligatory phase, the larvae are viable (i.e. capable of normal progressive metamorphosis), and, if at any time during this period the correct substrate (see below) becomes available, they will settle and metamorphose.

(*b*) During the second week an increasing proportion of larvae which have been denied access to the correct substrate will cast the shell and operculum and even reduce the velum. The resulting shell-less forms, although a proportion remains viable in culture conditions, probably play no part in the economy of the species in nature.

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E. *Settlement of the larva*

(i) *Conditions under which settlement will occur*

Larvae were reared in aerated sea water in litre or half-litre glass beakers at 9 to 10 °C. The only substrate on which settlement and metamorphosis of larvae of *Adalaria proxima* will occur is a live colony of the polyzoan *Electra pilosa*. Larvae did not distinguish between the varieties *typica* and *dentata* of this species, although adult *Adalaria* were never observed on *E. pilosa typica* in nature.

Settlement will not occur on the following species of Polyzoa, unless *E. pilosa* is also present.

Membranipora membranacea	*Flustrella hispida*
Alcyonidium polyoum	*Flustra foliacea*
Schizoporella unicornis	*Hippothoa hyalina*

Experiments showed that, for a substrate to be acceptable to larvae of *Adalaria proxima*, it must have both of the following characteristics:

(i) The 'smell' of live *Electra pilosa*. (Dead colonies of the polyzoan will not induce settlement.)

(ii) The texture of *E. pilosa*.

Of these, the first seems to be the more important, for a small proportion of larvae will metamorphose in the presence of that feature of the polyzoan alone (for instance, on plankton silk which separates searching larvae of *Adalaria* from a live colony of *E. pilosa*).

(ii) *Behaviour of larvae which encounter any physical obstacle during the obligatory planktonic phase*

During the obligatory phase the behaviour mechanism of the larvae compels them to swim upwards. This tendency is maintained until the end of the phase, in 1 to 2 days after hatching. Hence, they will rarely encounter any substantial obstacle, either in natural or culture conditions. If, however, an obstacle is experimentally placed in their path, they will, on touching it, creep over its surface, by means of the cilia of the foot. If the obstacle is not *E. pilosa* they soon swim away and resume their upward course. If it is a colony of this polyzoan, the negative geotaxis is overcome and the larva creeps over it for a day or more before metamorphosing.

Hence, the duration of the obligatory phase is determined chiefly by the behaviour of the larva; by almost ensuring that no substrata will be encountered during the first 1 to 2 days after liberation, this negative geotaxis delays metamorphosis long enough for the larvae, in nature, to be dispersed over a wide area before searching commences.

(iii) *Behaviour of larvae which encounter any physical obstacle during the searching phase of pelagic life*

When, on the descent from the surface layers of the medium which characterizes the beginning of the searching phase, the larva encounters any obstacle, the velar lobes and the upper part of the propodium are retracted and the animal creeps actively over the surface of the substratum. If the substratum is not a live colony of *E. pilosa*, the velar lobes

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are soon extruded and the larva swims away for a while before again descending. If, however, the substratum is *E. pilosa*, creeping continues and the animal never again uses the velar locomotor cilia.

The length of time elapsing between the location of the correct substratum by larvae of *A. proxima* and the completion of metamorphosis varies, and depends on, amongst others, the following factors.

(i) On whether or not the larva has reached the end of the obligatory phase. If not, then it will take the length of time by which it falls short of that, in addition to the usual time taken for metamorphosis.

(ii) On how long the larva has been searching. The longer, the greater the anticipatory changes that will already have occurred, and the shorter the time before metamorphosis is complete.

(iii) On the turbulence of the medium. This effects only the most obvious of the changes involved in metamorphosis, namely, the casting of the shell and operculum. This can be accelerated by gently squirting water from a pipette on to the metamorphosing larva.

In view of these factors, it will be appreciated that no precise estimate of the time required for metamorphosis to occur is possible. Generally, however, it is possible to state that larvae which locate a suitable substrate during the early part of the searching phase usually cast the shell and operculum within 1 day.

7. Metamorphosis

Metamorphosis involves numerous structural alterations proceeding contemporaneously; the nine most obvious features are shown in figure 37. These changes result in the conversion of the veliger form, adapted to a planktotrophic swimming life, to the dorid form, adapted to browsing, littoral life.

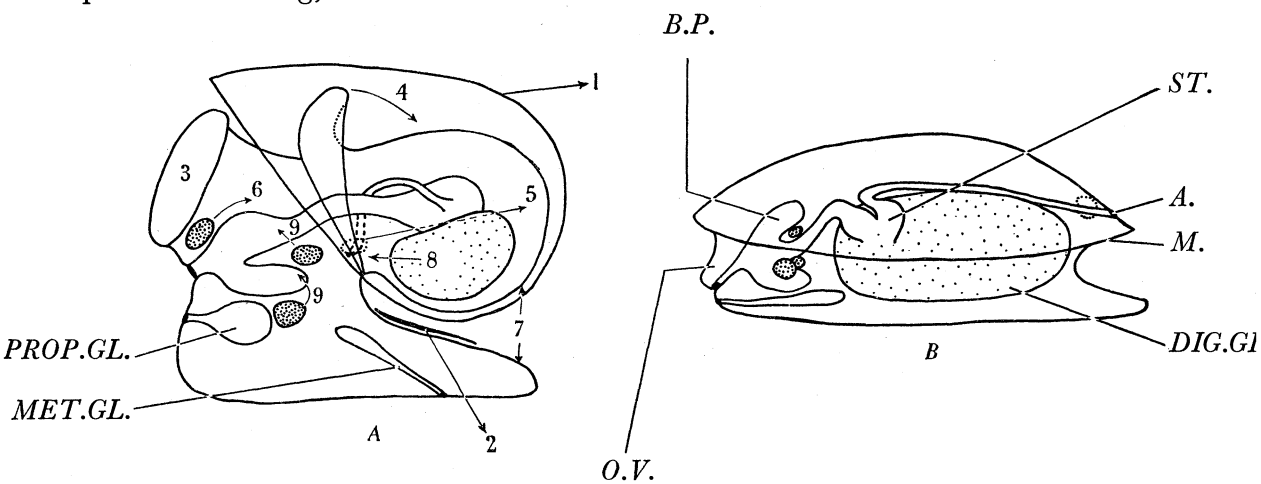

Figure 37. Changes involved in metamorphosis (diagrammatic). *A*. Pelagic larva; lateral aspect. (1) Casting of shell. (2) Casting of operculum. (3) Transformation of velar lobes into oral veil. (4) Inversion and spread of mantle fold. (5) Detorsion and reversal of visceral flexure. (6) Movement of cerebral commissure to a position posterior to the buccal pump. (7) General flattening of the body; broadening of the region of communication between the foot and the visceral mass. (8) Great enlargement of the digestive gland. (9) Concentration of ganglia. *B*. Post-metamorphal stage; lateral aspect.

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A. *Changes in external form* (figures 40, 41)

With the loss of the shell, the post-larva assumes a more flattened shape; accordingly, the region of communication between the foot and the visceral mass widens greatly and the digestive gland comes to lie partly within the foot.

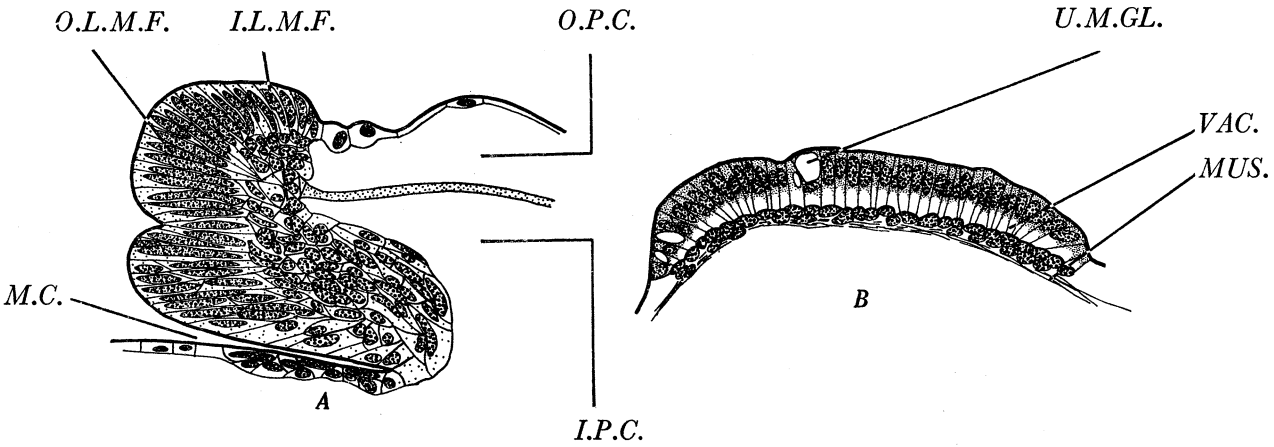

FIGURE 38. The mantle fold; longitudinal sections. *A*. Searching larva. *B*. Newly metamorphosed post-larva.

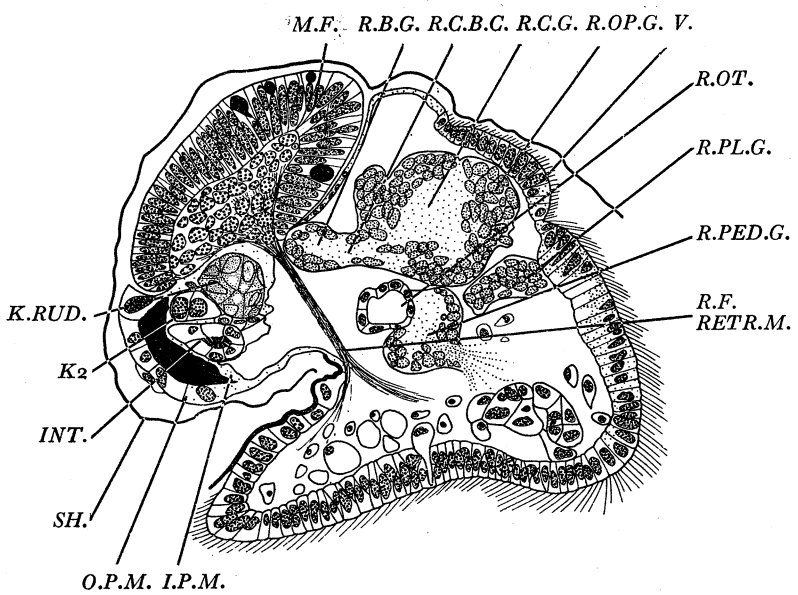

FIGURE 39. Longitudinal section, to the right of the median plane, through a shelled larva in the early stages of metamorphosis.

B. *The mantle* (figures 38, 39, 40, 41)

The mantle fold, which became reflected back during early pelagic life, fuses with the outer perivisceral membrane behind the fold. By rapid division of the cells of the columnar layer of the mantle fold, it spreads forwards and backwards over the dorsal surface of the post-larva; the perivisceral cavity becomes more or less obliterated, and the inner and outer perivisceral membranes indistinguishable. The 'larval heart' disappears.

Backward extension of the inverted mantle fold is more rapid on the right side of the post-larva than on the left. In consequence, the anus, larval kidney, adult kidney and heart rudiment are all carried along the right side with the extending mantle.

Anterior extension of the mantle is more rapid at the sides than in the middle. It extends over the region under which the eyes lie, and then gradually encircles and isolates the paired intravelar prominences which are the rudiments of the rhinophoreal tentacles (figure 41 *A*, *B*).

C. *The velum* (figures 39, 40 and 41)

The velar locomotor cilia are resorbed and the velar area reduced to a slightly thickened layer of epidermal cells. It ceases to be divided into two marked lobes, and becomes flattened, giving rise to the oral veil of post-larval stages.

D. *The nervous system*

The existing system of ganglia becomes still more concentrated, by further shortening and thickening of the commissures and connectives. The dorso-ventral flattening of the body displaces the cerebral commissure from its original position, anterior to the rudiment of the buccal pump, backwards, so that in post-metamorphal stages it crosses the foregut behind the pump (figures 36, 37). Development of the optic and pleural ganglia is completed. The intravelar epidermis becomes thickened at the sites of proliferation of the optic ganglia, these thickenings forming the rudiments of the rhinophores. These rudiments are soon encircled and isolated by the mantle as it spreads forwards (figures 40, 41).

E. *The muscle systems*

The larval retractor muscle becomes reduced and is indistinguishable in post-larval stages. The right and left foot retractors cease to be discrete, and either disappear altogether or play an insignificant part in the adult muscle complex, which is derived from the cephalopedal muscle complex of the larva.

F. *The alimentary canal* (figures 34 to 37, 40 and 41)

Cells are proliferated inwards from the wall of the hitherto sac-like digestive gland; these aggregate into ramifying cell-bridges subdividing the lumen. This process continues until the organ has no longer a discrete lumen, but is made up of a complex of anastomosing cell bridges.

The position of the anus moves from the larval site (latero-ventral on the right side) (figure 37 *A*) to the post-larval and adult one, subterminal and median (figure 37 *B*). This movement of the anal complex is, of course, one which reverses torsion as that process affects the adult gastropod, but it is important to note that it results also in a loss of gastropod visceral flexure.

The movement of the anal complex back along the animal's right side is divided into two stages.

(*a*) An initial, relatively rapid change brought about by the inversion of the mantle fold. This inversion affects the terminal region of the hindgut which lies embedded in the fold, and results in the anus becoming directed first laterally, and then posteriorly, instead of, as was earlier the case, anteriorly.

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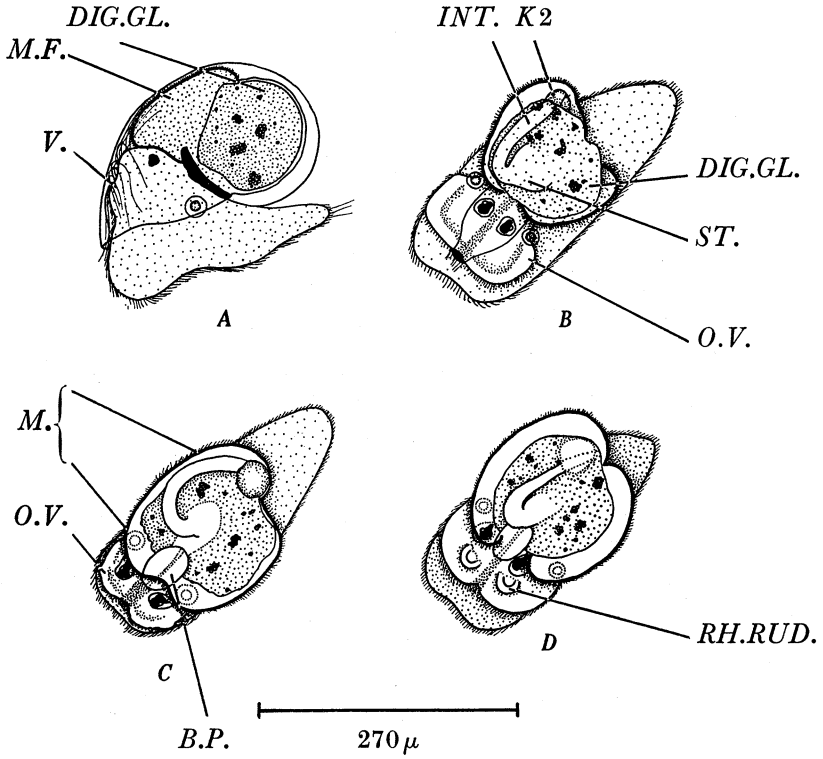

FIGURE 40. Metamorphosis (drawings from life). *A*. Shelled stage, lateral aspect. *B* to *D*. Shell-less post-larval stages, in dorsal aspect.

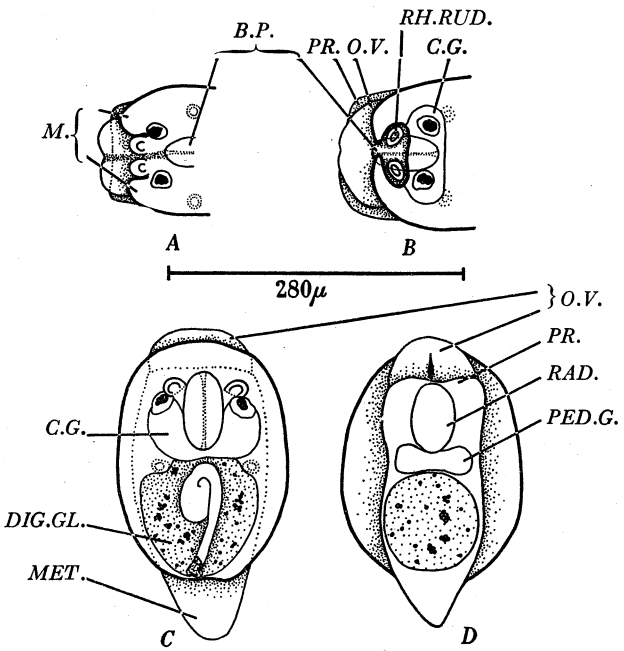

FIGURE 41. Metamorphosis (drawings from life). *A* and *B*. Head end only, showing enclosure of the rhinophore rudiments by the extending mantle; dorsal aspect. *C*. Benthic stage at completion of the metamorphal changes; dorsal aspect. *D*. Benthic stage at completion of metamorphal changes; ventral aspect.

(*b*) A subsequent, relatively slower change, brought about by the faster posterior extension of the mantle on the right side during metamorphosis. This extension carries the anal complex (anus, larval kidney and its storage vesicle, adult kidney and heart rudiment) round to the adult position at the posterior extremity of the visceral mass. The meeting and fusion of the posteriorly extending sides of the mantle renders the anus slightly subterminal (figures 40, 41).

The point of connexion between the hindgut and the stomach remains unaltered and so the characteristic dorid hindgut loop is brought into existence. On leaving the stomach the hindgut extends anteriorly for a short distance, before looping back past the right side of the stomach to the anus (figures 37, 40 and 41 *C*).

The stomach becomes divided into two chambers by a folding in its wall. The more anterior lobe receives the foregut and the posterior lobe gives off the hindgut (figure 37 *B*).

The digestive gland enlarges greatly, extending forwards as far as the otocysts and pedal ganglia (figures 37, 40 and 41). The majority of its bulk, after metamorphosis, lies within the foot. It comes to surround the stomach on all sides, leaving only the dorsal face free. The enlargement of the digestive gland carries the stomach forwards towards the mouth. Much of the foregut's former length is now unnecessary and it becomes arched steeply upwards, before descending to enter the most anterior lobe of the stomach (figure 37 *B*).

The buccal mass musculature differentiates, and, when metamorphosis is complete, the radula is capable of being protruded through the mouth. Until this time, however, it is probable that the diet is augmented by ciliary intake of encrusting diatoms, protozoa, etc., for the gut is brown in life, and particles may be observed circulating in the stomach.

8. Subsequent development

On completion of metamorphosis, the juveniles begin to feed on the polyzoan on which they had settled. Post-metamorphal growth is rapid (figure 42). The young dorids move about little as long as the food is abundant; they become active only when the polyzoan epifauna around them is depleted.

Various internal changes take place which are in continuation of processes initiated during metamorphosis.

The adult kidney enlarges and replaces the larval kidney which, with its storage vesicle, becomes reduced in size and disappears soon after metamorphosis. The definitive kidney becomes elongated and lies alongside the intestine (figure 43). A lumen soon appears between the cells composing the kidney. The heart rudiment becomes flattened and lies above the intestine and kidney; it differentiates into the pericardium with the auricle and ventricle contained within. The subepidermal spaces of the larval head and foot form the haemocoel. The solid rod of cells which earlier connected the rudiments of the kidney and the heart, acquires a lumen and forms the reno-pericardial tube (figure 43, *RPD.*). A thickened dilation at the posterior end of this tube, where it enters the posterior extremity of the pericardium on the right side, is the reno-pericardial syrinx (figure 43, *RPS.*) or pyriform vesicle (Hancock 1865). The renal organ is prolonged posteriorly into a narrow tube which terminates in the renal pore, on the right side of the anal opening (figure 43 *R.*).

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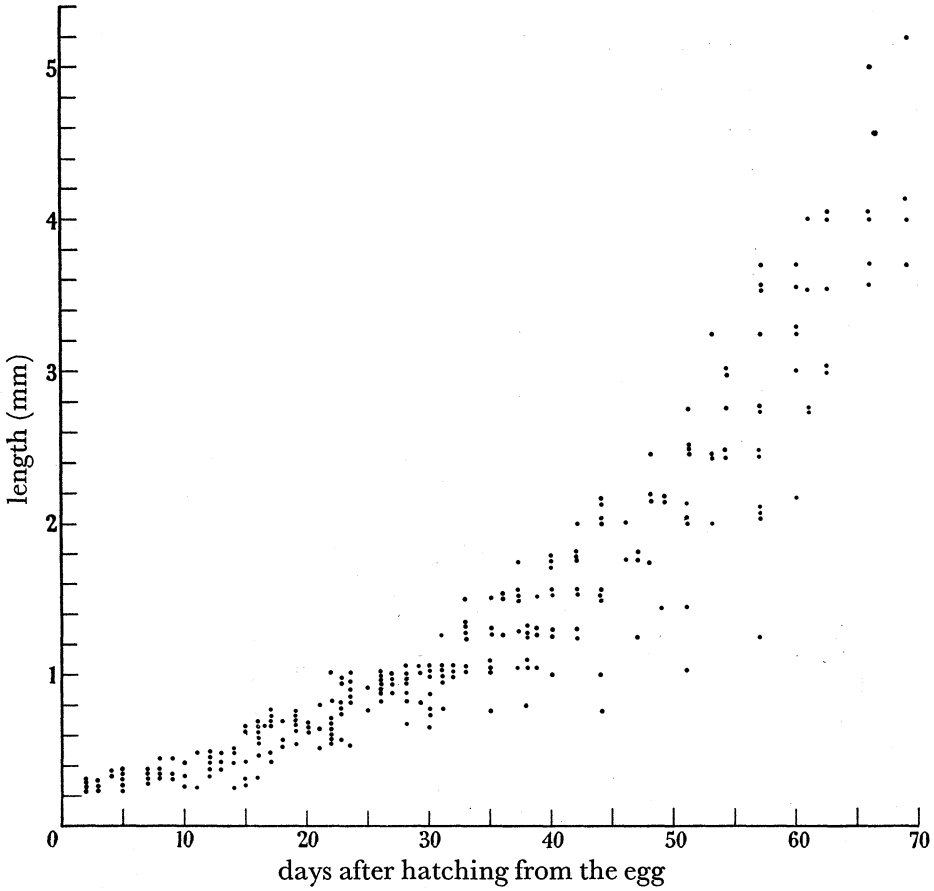

FIGURE 42. Growth rate of post-metamorphal stages during the first ten weeks of benthic life (at 9 to 10 °C).

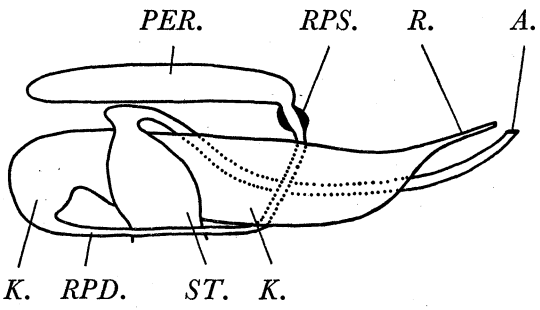

FIGURE 43. Diagrammatic reconstruction of the renal system of benthic stages; left lateral aspect.

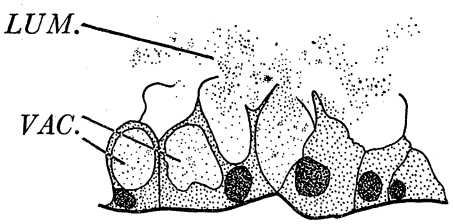

FIGURE 44. Section through the wall of the renal organ of a juvenile *Adalaria proxima*.

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The kidney increases in size throughout life, and in later stages becomes subdivided. Its wall consists of vacuolated cells and presents a characteristic appearance in sections (figure 44). It appears that excretory products accumulate in the vacuoles and are discharged into the renal cavity by rupture of the cells.

The mantle spreads over the dorsal surface of the young dorid and eventually comes to project out as a skirt all round the animal. The mantle epidermis is histologically simple in young post-larval stages (figures 38*B*, 45*A*), but in later life becomes rich in large epidermal glands. These glands are of two types (figure 45*B*). Glands of type 1 are found chiefly on the tubercles which characterize the dorid's back in later stages; they are multicellular

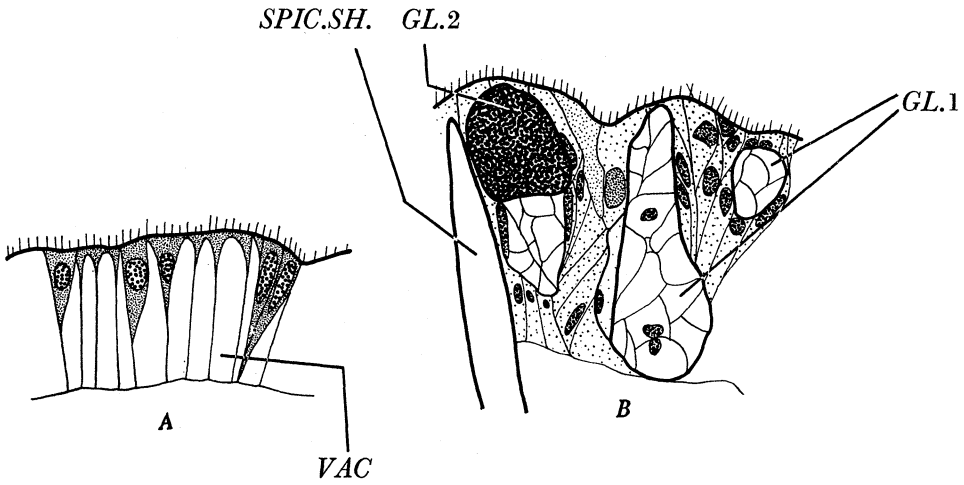

FIGURE 45. Sections through the mantle of benthic stages. *A*. of a juvenile, length 0·5 mm. *B*. of adult.

and their contents take up only mucus stains. Glands of type 2 predominate on the mantle between the tubercles, but are also abundant in the epidermis of the oral veil; so densely do their contents stain with haematoxylin that it is impossible to state whether they are unicellular or multicellular.

Calcareous spicules are laid down in the subepidermal layers of the mantle. The first-formed spicules are all orientated with their long axes transverse to the antero-posterior axis of the animal (figure 46*A*). Subsequently, longitudinally orientated spicules appear in the mantle and along the sides of the foot (figure 46*B*). Then radially arranged spicules are secreted in the mantle skirt (figure 46*C*). Finally, the mantle becomes tuberculated (figure 46*D*) and vertical spicules are laid down within the tubercles (figure 46*E*). With the appearance of spicules, internal detail becomes increasingly more difficult to discern in live material.

Concentration of the ganglia continues. The pleural and optic ganglia fuse with the cerebrals. The cerebro-pedal connectives shorten and thicken, and the pedal commissure, which earlier was below, now comes to lie above the radula sac (figures 36*B*, 47). When this ontogenetical euthyneury is complete, the asymmetry of the visceral nerves (p. 30) is no longer discernable.

The rhinophores enlarge and become lamellated. Depressions in the mantle around the bases of the rhinophores form deep pits into which they can be retracted (figure 46*D*,

E). Spicules develop in the subepidermal layer of each rhinophore. Stout nerves connect the rhinophores with the respective cerebral ganglia.

When the dorid reaches a length of between 1 and 2 mm, the anal branchiae begin to develop, first as thickenings, and then as outpushings of the mantle around the anus. The first branchiae formed are in an arc anterior to the anus, later ones completing the circle around it. The branchiae are simply pinnate.

The body musculature is derived from the subepidermal complex of the larva; none of the other larval muscle systems could be traced through to the adult form. The adult

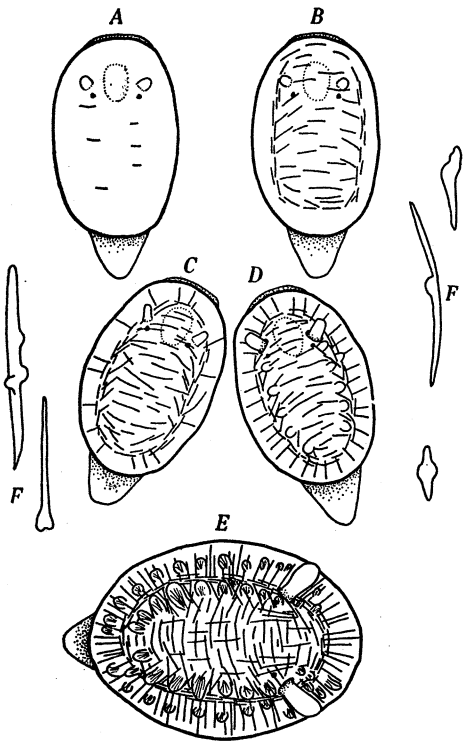

FIGURE 46. Post-metamorphal development (drawings from life; not to same scale). *A*. Juvenile, length 0·31 mm; dorsal aspect. *B*. Juvenile, length 0·37 mm; dorsal aspect. *C*. Juvenile, length 0·47 mm; dorsal aspect. *D*. Juvenile, length 0·54 mm; dorsal aspect. *E*. Juvenile, length 2·0 mm. dorsal aspect. *F*. Spicules, much enlarged.

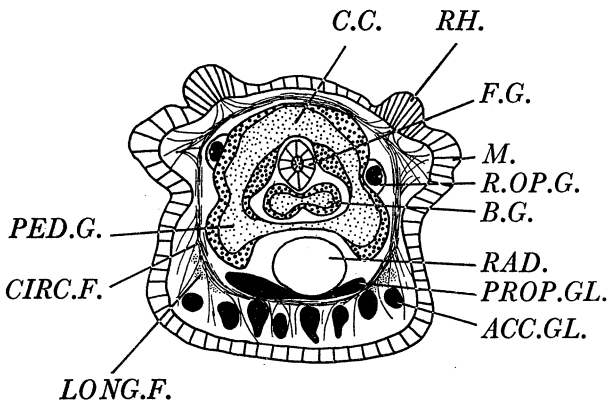

FIGURE 47. Transverse section through the head region of a juvenile *Adalaria proxima*, 0·5 mm in length (semi-diagrammatic).

system consists of a complex of circular and longitudinal muscle bands with branches to all parts of the mantle, foot, and oral veil (figure 47, *CIRC.F.*, *LONG.F.*), which are responsible also for the retraction of the anal branchiae and the rhinophores. Muscle fibres differentiate around the intestine and the renal duct.

The buccal mass (figure 36*B*) enlarges greatly, and paired tubular multicellular salivary glands develop. The buccal pump becomes pedunculate and its walls greatly thickened and muscular.

The histological structure of the digestive gland in the adult was not investigated in any detail; it resembles the descriptions and illustrations of Forrest (1953) for *Archidoris*. The adult digestive gland is derived almost entirely from the left midgut diverticulum of the embryonic and larval stages; the right diverticulum forms only a very small region of the gland, close to the point where the oesophagus enters. In sections of early post-larvae this region is recognizable, for it consists of cells having a greater affinity for eosin. In later stages both histologically and anatomically it is indetectable.

The internal surface of the intestine becomes increased by folding; faeces, which in early post-metamorphal stages are minutely particulate, in later stages are cemented together and expelled as elongated pellets.

Such investigations as were made into the development of the sexual organs are described on p. 7.

9. Repetition of the cycle

A population consisting of individuals from three laboratory-reared spawn masses was maintained in slowly running sea water during the period up to and including their breeding season in the following year (i.e. from June 1956 to April 1957). At approximately fortnightly intervals from September 1956, onwards, they were counted, measured, and provided with fresh food. The amount of food which they were given was always in excess of their needs.

Figure 48 shows the mean lengths of the population throughout the period, the maximum and minimum length measurements recorded on each date, the fortnightly mortality and the amount of spawn produced. Figure 49 shows the temperature fluctuations during the period.

The spawning season extended from mid-February to early April, during which time 150 egg masses were deposited. The number of individuals taking part in spawning was fifty-nine, so that an average of slightly less than three egg masses was produced per individual. No estimates were made of the number of eggs produced, but it was noted that the masses were generally much smaller than the average for the species. Of the spawn ribbons, 117 were attached to the glass walls or floor of the vessel, and 33 to fronds of *Fucus serratus*. Death of the breeding generation followed closely on the cessation of spawning.

A high proportion of the egg masses produced contained eggs which failed to develop; this was to be expected (Thompson 1957) in view of the temperature at which the adults had been cultured. Nevertheless, it was possible in a number of cases to obtain settlement and metamorphosis of larvae from some of the egg masses, so as to extend the investigation into the second generation.

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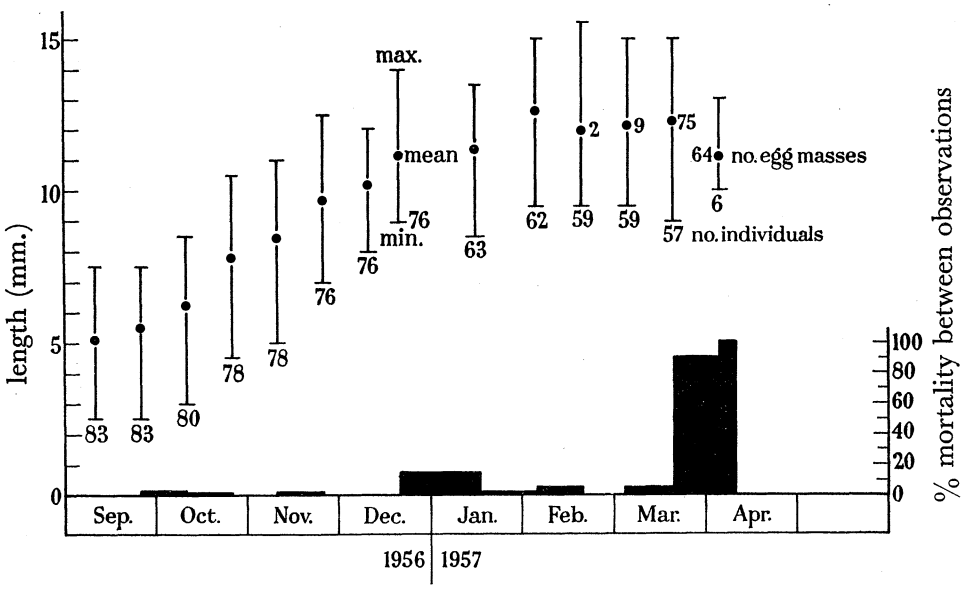

FIGURE 48. Periodic observations on a population of *Adalaria proxima* reared in the laboratory.

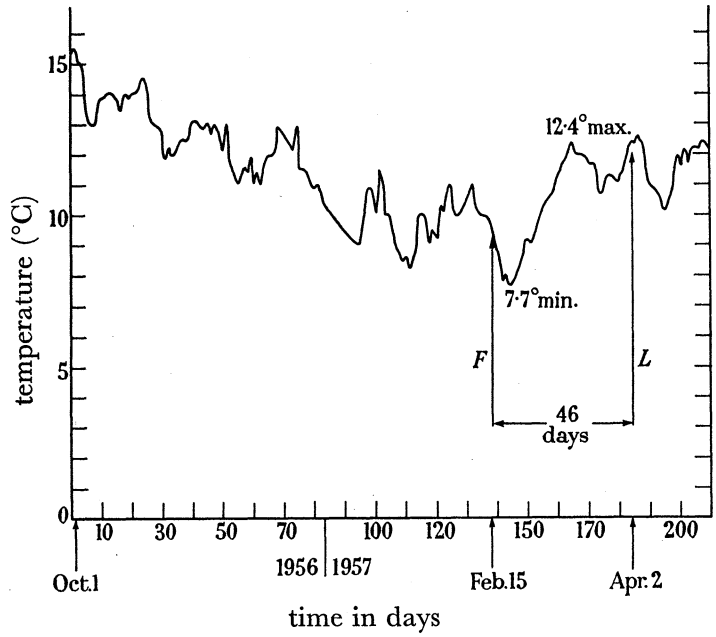

FIGURE 49. Water temperature variation during the course of the experiment detailed in figure 48. F = first spawn; L = last spawn.

Mortality at different stages of the life cycle

Estimates were made of the mortality among progeny from a single spawn ribbon (produced in the laboratory) which, on oviposition, contained 1320 eggs.

Uncleaved egg—free larva	Mortality, 10·76%
Free larva—post-metamorphal stages	Mortality, 84·51%
First 6 weeks of benthic life	Mortality, 11·39%

(The percentage given represents that proportion of the animals which died during the specified stages.)

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Mortality during the remainder of the cycle (in progeny from different spawn masses) is expressed in figure 48. It is clear that by far the most critical stages in the life cycle are those surrounding settlement and metamorphosis, even in the relatively sheltered environment offered by laboratory culture.

10. Discussion

Ecological considerations

The life cycle of *Adalaria proxima* is a simple one. The settling larvae search until they either find a colony of *Electra pilosa* (an encrusting polyzoan) or die. The population is thus established in the regions of greatest abundance of this polyzoan, namely (at least as far as the Menai Straits is concerned) in the *Fucus serratus* zone of the shore. The dorids probably maintain this littoral position during the remainder of the cycle by their very sluggishness when the food is in excess of their needs. *Electra pilosa* breeds through the greater part of the year, and so the food supply is constantly replenished.

The dorids grow to maturity by the following spring, and, provided the temperature of the sea has risen to above approximately 6 °C, begin reproductive activity in mid-February. Feeding intensity diminishes before and during the breeding season (Thompson 1957). In 1956, following a severely cold winter, the sea temperature did not rise above 6 °C until late March, and the onset of reproduction was delayed until that time. The death of the breeding generation occurs soon after the consumption of all the food reserves stored in the digestive gland; its place is taken by the new generation of juvenile dorids.

Adalaria proxima is a boreo-arctic species, the recorded distribution being (Lemche 1941): East Greenland, Massachusetts, Iceland, Faroes, White Sea, Murman Coast, West Coast of Norway, Kattegat, Baltic, North Sea, and British coasts. It has been stated to be present in the Mediterranean (Pruvot-Fol 1954), but this record is without firm foundation (Pruvot-Fol 1955). On British coasts it is recorded as far south as the Bristol Channel (Purchon 1947), and there is a single record from the English Channel (Garstang 1895).

The reproduction of *A. proxima* is related to this northern distribution in the usual ways: its life history is a simple one, and there is a single short annual breeding season; the breeding period extends over a relatively cool temperature range; the eggs are larger than in more southern species and a relatively small number of them are produced; the developmental period is long and the embryos hatch at a relatively advanced stage (Thompson 1957); the minimal length of the larval phase is short and a tendency towards independence of the phytoplankton is evident.

The larval phase is almost certainly retained in *A. proxima* simply as a dispersal mechanism, and the selection of a suitable substrate for settlement is governed by an elaborate and highly specific sensory mechanism. It is by means of this mechanism that the species establishes itself at the beginning of each period of benthic life in the region of the sea bottom which is most suited to post-metamorphal development.

Embryological considerations

As would be expected in the forms which are the most remote from the original ancestral gastropod, the embryological processes of the dorid nudibranchs (if, as seems certain, *Adalaria* is representative in all important respects) differ widely from what most modern authorities agree to have been the primitive pattern.

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Asymmetry of organization, which is presumed to have entered the history of the gastropods with the advent of torsion (although this theory may have to undergo some revision in the light of recent discoveries), is evident very early in the embryology of *Adalaria*. Torsion in *Adalaria* is no longer detectable as a mechanical process; all the organs which are involved in ontogenetical torsion are, from their first appearance, placed in their post-torsional positions. The embryonic nervous system shows only traces of the ancestral streptoneury. Torsion in this species is accomplished during the later stages of cleavage, and even the anal cells at their first appearance are in the post-torsional position.

Torsion as it affects the visceral organs in the opisthobranch embryo never approaches the full 180° twisting found in living Diotocardia; the full advantages of torsion to the gastropod larva are achieved if the mantle cavity is brought to a position behind the head, but the accompanying change in position of the anus, while being unnecessary for the achievement of the extra protection which torsion makes possible for the larva, is in fact positively an inconvenience, in some respects, to the adult gastropod. (This point, which is a central one in modern theories of gastropod torsion, is developed particularly fully by Yonge (1947).) Hence, torsion, as it involves the position of the anus, has been halted in the embryonic opisthobranch at a position approximately appropriate to the adult state. Pelseneer's assertion (1911) that in a wide variety of nudibranchs (including some dorids) the positions of the anus and mantle cavity move during embryonic life through 180°, is denied. This author also states that the position of the anus in the nudibranch larva is mid-dorsal, behind the head; this is not the case in any of the dorids investigated (Thompson 1957), nor in any other species of nudibranch whose larvae were examined in order to check this point (including *Idulia coronata*, *Limapontia depressa*, *Eubranchus exiguus*, *Facelina drummondi* and *Cratena aurantia*).

In the dorid nudibranch, the probable original purpose of torsion, namely, to bring the larval mantle cavity to a dorsal position behind the head, so rendering it possible to accommodate the head therein during retraction, is redundant; the unimportance of the shell to any but the veliger stages renders possible an early withdrawal of the mantle fold from the shell mouth, which enables the head and foot to be retracted directly into the cavity of the shell. Torsion still takes place, however; the subsequent fate of the mantle fold, which forms the complete dorsal integument of the post-larval benthic stages, renders its presence behind the head necessary for reasons other than those which were important to the ancestral veliger. So closely was torsion interwoven with the pattern of the evolution of the dorids, that it still takes place in the embryo but for different reasons, and in a different manner, from the original ones.

It is possible to distinguish five ways in which torsion is brought about, drawing examples from the various gastropods whose development has been studied.

(1) 180° torsion achieved by muscle contraction alone. (Found in no living forms, except possibly in *Acmaea* (Boutan 1899) but it is possible that this was the original mechanism.)

(2) 180° torsion achieved in two stages, an initial movement through 90° brought about by muscle contraction, followed by a slower movement through the remaining 90° by differential growth processes, e.g. *Haliotis*, *Patella*, *Calliostoma*, etc. (Crofts 1937, 1955; Smith 1935).

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(3) 180° torsion achieved by differential growth processes alone, e.g. *Paludina* (Drummond 1902) and *Pomatias* (Creek 1951).

(4) Torsion achieved by differential growth processes, the change in position of the anus being halted at a site approximately appropriate to the adult state, e.g. *Aplysia* (Saunders & Poole 1910), most Monotocardia?

(5) Torsion no longer recognizable as a movement of the pallial complex, the organs being, from their first appearance in the embryo, in the post-torsional positions, e.g. *Adalaria*.

The antero-posterior axes (i.e. the axes of greatest symmetry and the axes indicating the direction in which locomotion is effected) of the larval and adult stages of *Adalaria* are coincident. It is on the basis of this identity of orientation that the axes of the embryonic and larval body have been named throughout this work.

Torsion, then, in the veliger of *Adalaria*, although it is not visible as a mechanical process, would involve a movement of the pallial complex from a ventral to a dorsal position. This point is emphasized here only because many authors have stated that developmental torsion in gastropods involves a movement of the pallial complex from a *posterior* to an *anterior* position; this is the result when translated into the adult gastropod, because of the post-metamorphal posterior extension of the foot and the consequent shifting upwards of the visceral mass, but it is surely confusing to so name the axes of the veliger that it appears to swim along the production of its own dorso-ventral axis.

Metamorphosis in *Adalaria* involves no violent change in orientation, merely a general flattening of the body consequent on the loss of the shell. The distinction between head, foot and visceral mass becomes indistinct, and the dorid form is assumed. Detorsion is combined with reversal of the more fundamental gastropod process, visceral flexure, as the anal complex moves back along the right side to the adult postero-dorsal position. It is remarkable that just as torsion in Diotocardia is brought about in two stages, one relatively rapid, one relatively slow, so, in *Adalaria*, is detorsion (see p. 44).

The dorid nudibranchs, on the basis of anatomical studies of the modern opisthobranchs, are believed to have arisen from shelled forms, and to represent the culmination of the tendency towards the enclosure of the shell by the mantle manifest in present day tectibranchs. In *Adalaria* the outer layer of the adult dorsal integument (cloak or mantle) *is derived from the layer which lined the embryonic and larval mantle cavity*. If the dorids had evolved from shelled ancestors by progressive enclosure of the shell by the mantle, it is precisely this layer which would be outermost when the folds closed over the shell dorsally. Yet in the development of dorids, no vestige of this enclosure remains, the shell being catastrophically discarded at metamorphosis. Ontogeny in this case not only fails to recapitulate phylogeny, it contradicts it, achieving the same end by strikingly different means.

That the dorsal integument of *Adalaria* is derived wholly from the embryonic mantle fold is beyond doubt. The views of Garstang (1890), of Fischer (1880–7), and of Herdman & Clubb (1892), that the dorsal integument of the dorid is the product of the fusion of pleuropodia or epipodia, are mistaken. The pleural innervation of the dorid dorsum demonstrated by Herdman & Clubb (1892) is clearly a reliable guide to its homology, as was suggested by Gilchrist (1895). In that case, it seems certain that the cerata of the eolid nudibranchs are not homologous with the dorsal papillae (sometimes called cerata)

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of certain dorids (e.g. *Polycera*); the latter are products of the mantle in exactly the same way as are the tubercles on the back of *Adalaria*. By the same criterion, the dorsal processes of the dendronotids and tritoniids are probably also products of the mantle. The pedal innervation and presumably epipodial origin of the eolid ceras points to a fundamental cleavage early in the history of the nudibranchs.

In conclusion, it is interesting to speculate on the fact that the dorid nudibranchs are probably the most highly evolved group of living Gastropoda, that is to say, they have passed through the greatest number of fundamental structural changes from what we must presume to have been the original form. The prosobranchiate condition is ancestral to the opisthobranchiate, and the dorid nudibranchs are among the most advanced opisthobranchs. Yet, paradoxically, the complex evolutionary steps which have led to the dorid have resulted in a secondary return, in many respects, to the original condition. In the adult dorid, only unimportant traces remain of the three most important steps in the structural evolution of the gastropods, visceral flexure, torsion, and bilateral asymmetry.

11. Summary

1. An account, based on observations on a population in the Menai Straits, is given of the natural history of *Adalaria proxima* (Alder & Hancock), an annual species of dorid nudibranch.

2. Small populations of *A. proxima* were reared through the complete life cycle in the laboratory; estimates of spawn productivity, mortality and pattern of activity of the species under these conditions were made. The most critical stages in the life cycle were shown to surround settlement and metamorphosis.

3. Descriptions, in tabular form, are given of the spawn of some other species of dorids. The eggs of *A. proxima* are larger, fewer of them are produced than in any of the other species, and the developmental period is longer. The larvae of *A. proxima* are less dependent on the phytoplankton than the larvae of other species of dorids, and the feeding mechanism is less complex in important respects.

4. The pelagic larval phase of *A. proxima* consists of two distinct stages, during the first of which the larvae swim upwards, this behaviour being reversed at the commencement of the second stage. Selection of a suitable substrate for settlement and metamorphosis is governed by an elaborate and highly specific sensory mechanism, by means of which the species establishes itself, at the beginning of each period of benthic life, in the region of the sea bottom most suited to post-metamorphal growth and development.

5. The development of *A. proxima* through all stages of its life cycle is described. Torsion is brought about during the later stages of cleavage and is not recognizable as a mechanical process. The relevant organs occupy their post-torsional positions from their earliest appearance in the embryo. Only traces of the ancestral streptoneury are evident. Torsion in dorids no longer occurs for the same reason, nor in the same manner, as in the ancestral gastropod; the dorid larval mantle cavity does not serve to accommodate the head during retraction into the shell.

During metamorphosis into the benthic form, detorsion and reversal of visceral flexure occur; the post-metamorphal stages are bilaterally symmetrical in all but unimportant respects. The dorsal integument of the adult is derived from the embryonic mantle fold.

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List of abbreviations used in the figures

anus
GL. accessory gland
. anal cells

buccal commissure
buccal ganglion
blastopore
RUD. rudiments of buccal mass
musculature
buccal pump
RUD. rudiment of buccal pump

. cerebro-buccal connective
cerebral commissure
cerebral ganglion
RUD. rudiment of cerebral ganglion
F. circular muscle fibres
ganglionic cortex
. cerebro-pedal connective

GL. digestive gland (left midgut diverticulum)

ectomeres
. endomeres
epidermis
. embryonic shell
exhausted cells

oot
. foot epidermis
foregut
D. foot rudiment
free yolk

gland of type 1
gland of type 2
genital opening
growth lines

F. inner layer of mantle fold
insertions of fibres of pedal branch of larval retractor muscle
intestine
. inner perivisceral cavity
. inner perivisceral membrane

dult kidney
larval kidney
UD. rudiment of larval kidney
D. rudiment of adult kidney

L.C.G. left cerebral ganglion
L.H. 'larval heart'
L.M. left midgut diverticulum
LONG.F. longitudinal muscle fibres
L.OP. lips of operculum
L.OP.G. left optic ganglion
L.OP.I. left optic invagination
L.OT. left otocyst
L.PED.G. left pedal ganglion
L.PL.G. left pleural ganglion
L.RETR.M. larval retractor muscle
L.RETR.M.F. factor of larval retractor muscle
LUM. lumen of kidney

M. mantle
MAC. macromere
M.C. mantle cavity
MED. ganglionic medulla
MED.B. median band of yolky cells
MET. metapodium
MET.GL. metapodial gland
M.F. mantle fold
M.F.V. ventral part of mantle fold
M.G. midgut
M.G.L. lumen of midgut
MO. mouth
MUC. islands of mucus-staining matter
MUS. muscle fibres of the cephalo-pedal muscle complex

O. ovum
O.L.RETR.M. origin of larval retractor muscle
O.L.M.F. outer layer of mantle fold
OP. operculum
O.P.C. outer perivisceral cavity
O.P.M. outer perivisceral membrane
OP.P. optic pigment
O.REG. outer regions taking up nuclear stains
OT. otocyst
O.V. oral veil

P. peg
P.BR. pedal branch of retractor muscle
P.C. perivisceral cavity
PED.C. pedal commissure
PED.G. pedal ganglion
PER. pericardium
PR. propodium
PROP.GL. propodi
PR.RUD. rudiment

*Q*1 first quartet of
*Q*2 second quartet

R. renal duct
RAD. radula sac
RAD.RUD. rudime
R.B.G. right buccal
R.C.B.C. right cerel
R.C.G. right cerebr
R.C.P.C. right cereb
R.C.V. right central
REM.Y. remaining
R.F.RETR.M. righ
RH. rhinophore
RH.RUD. rudiment
R.L.V. right lateral
R.M. right midgut
R.MED.B. remains yolk-la
R.OP.G. right optic
R.OT. right otocyst
R.P. right propodia
RPD. reno-pericard
R.PED.G. right ped
R.PL.G. right pleur
R.PROP.GL. right p
RPS. reno-pericardi

S.C. sensory cilia
SEC.M. secondary
SH. shell
SH.CAV. shell cavit
SH.GL. shell gland
SH.GL.I. shell glan
SPIC.SH. spicule sh
ST. stomach (midg
ST.V. storage vesicl
SU. suture line
SUB.M.C. subepide
SUP.V.C. supravela
S.V. subvelum
S.V.C. subvelar cells

U.M.GL. unicellula
UND.C. undifferent

V. velum
VAC. vacuole
VEL.P. velar promi

ial gland
t of propodium

micromeres
of micromeres

ent of radula sac
l ganglion
bro-buccal connective
al ganglion
bro-pedal connective
l velar branch
yolk reserves
t foot retractor muscle

t of rhinophore
l velar branch
diverticulum
s of median band of
aden cells
c ganglion
t
al branch
dial duct
dal ganglion
ral ganglion
propodial gland
lial syrinx

egg membrane

ty
(mantle)
d invagination
neath
gut)
le

ermal muscle complex
ar cells

ls

ar mucus gland
tiated, yolk-laden cells

inence

UD. rudiment of larval kidney
'D. rudiment of adult kidney

ens
. left buccal ganglion

PED.G. pedal ganglion
PER. pericardium
PR. propodium
PR.M. primary egg membrane
PROLIF. site of proliferation

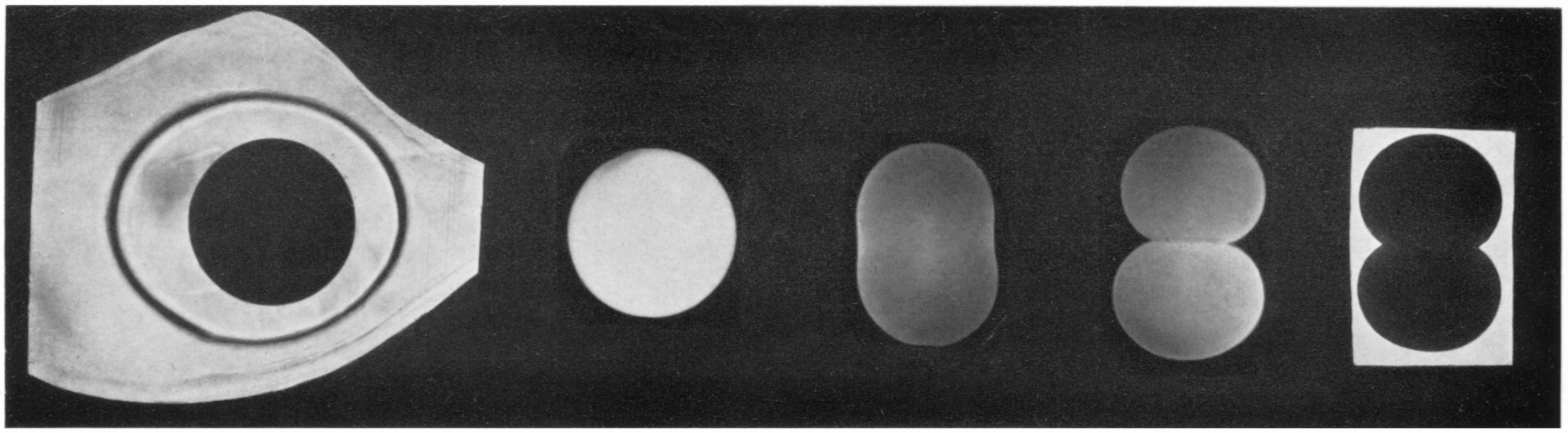
V. velum
VAC. vacuole
VEL.P. velar promi
V.L.C. velar locomc
V.P.F. ventral polar

PHILOS
TRANS

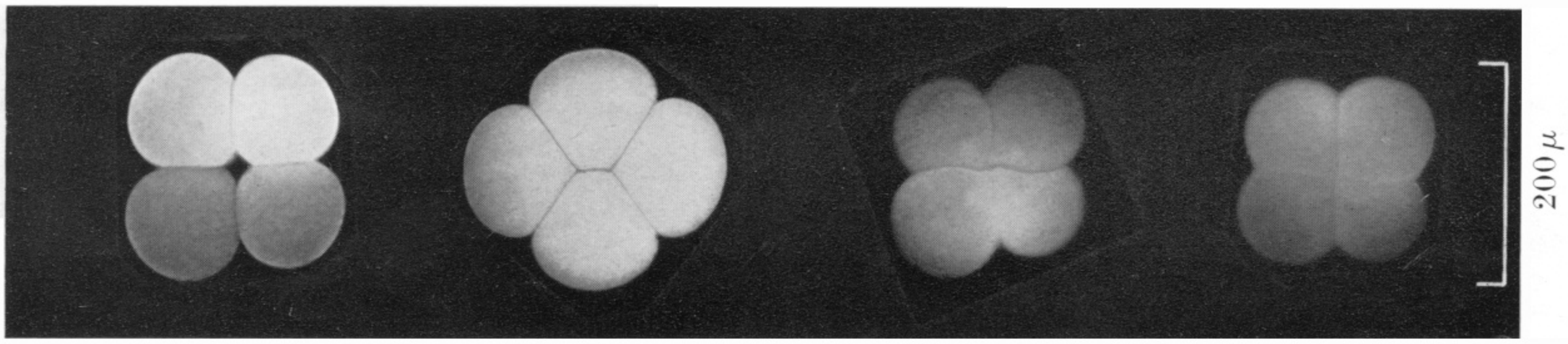
inence

otor cilia

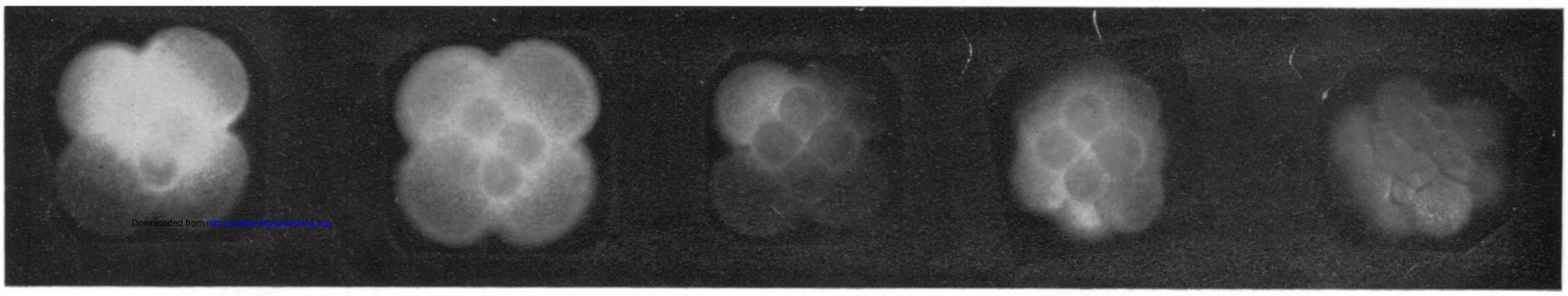
r furrow



A *B* *C* *D* *E*



F *G* *H* *I*



J *K* *L* *M* *N*

FIGURE 50. Microphotographs of cleavage stages of *Adalaria proxima*. All are of live embryos.

A. Uncleaved egg with its envelopes; direct lighting.
B. Localized translucency prior to extrusion of first polar body; dark ground illumination.
C. First cleavage beginning; dark ground illumination.
D. Early two-cell stage; dark ground illumination.
E. Slightly later stage; direct lighting.
F. Early four-cell stage; dark ground illumination. From animal pole.
G. Later four-cell stage; dark ground illumination. From vegetative pole.
H. The same, from animal pole; dark ground illumination.
I. Beginning of third cleavage division; nuclear spindles visible; dark ground illumination. From animal pole.
J. Transitory six-cell stage; dark ground illumination. From animal pole.
K. Transitory seven-cell stage; dark ground illumination. From animal pole.
L. Embryo after completion of third cleavage; dark ground illumination. From animal pole.
M. Embryo after production of second quartet of micromeres; dark ground illumination. From animal pole.
N. Embryo after division of first quartet (sixteen-cell stage); dark ground illumination. From animal pole.

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Q2 second quartet of micromeres

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RAD. radula sac
RAD.RUD. rudiment of radula sac
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R.C.B.C. right cerebro-buccal connective
R.C.G. right cerebral ganglion
R.C.P.C. right cerebro-pedal connective
R.C.V. right central velar branch
REM.Y. remaining yolk reserves
R.F.RETR.M. right foot retractor muscle
RH. rhinophore
RH.RUD. rudiment of rhinophore
R.L.V. right lateral velar branch
R.M. right midgut diverticulum
R.MED.B. remains of median band of yolk-laden cells
R.OP.G. right optic ganglion
R.OT. right otocyst
R.P. right propodial branch
RPD. reno-pericardial duct
R.PED.G. right pedal ganglion
R.PL.G. right pleural ganglion
R.PROP.GL. right propodial gland
RPS. reno-pericardial syrinx

S.C. sensory cilia
SEC.M. secondary egg membrane
SH. shell
SH.CAV. shell cavity
SH.GL. shell gland (mantle)
SH.GL.I. shell gland invagination
SPIC.SH. spicule sheath
ST. stomach (midgut)
ST.V. storage vesicle
SU. suture line
SUB.M.C. subepidermal muscle complex
SUP.V.C. supravelar cells
S.V. subvelum
S.V.C. subvelar cells

U.M.GL. unicellular mucus gland
UND.C. undifferentiated, yolk-laden cells

V. velum
VAC. vacuole
VEL.P. velar prominence
V.L.C. velar locomotor cilia
V.P.F. ventral polar furrow

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