Studies in Tunicate Development. Part II. Abbreviation of Development in the Molgulidae

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VI. *Studies in Tunicate Development. Part* II.—*Abbreviation of Development in the Molgulidæ.*

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Contents.

1. Introduction.

The development of simple ascidians as a whole has been already described (Berrill, 1929), but its abbreviation in certain members of the family Molgulidæ merits separate treatment and forms the main subject of this paper. The influence of viviparity and yolk accumulation on the development and the general biology of these forms will also be discussed.

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There is now known in more or less detail the development of 24 species of the Molgulidæ, 12 of which are described here for the first time, while confirmatory descriptions are given in the case of five others.

The developmental histories of the species under discussion were determined at the following marine biological laboratories :—*Molgula ampulloides*, *M. simplex*, *M. complanata*, *M. citrina* and *Eugyra arenosa* were examined at Plymouth, 1929 ; *M. citrina* (*nana*), at Kristineberg, Sweden, 1926 ; *M. bleizi*, *M. oculata*, *M. occulta* (*roscovita*), and *M. citrina* (*echinosiphonica*) at Roscoff, Brittany, 1926 ; *M. arenata* and *M. manhattensis* at Woods Hole 1927 ; and *M. retortiformis*, *M. citrina* (*M. litoralis*), *M. canadensis* and *Eugyra pilularis* at St. Andrews, Bay of Fundy, 1927–28.

Grateful acknowledgements are made to Dr. A. G. Huntsman, Dr. Marcel Prenant, and to the late Herr Magnus Aurivillius, without whose assistance the necessary material could not have been obtained. The expenses involved in visiting Kristineberg and Roscoff were in part defrayed by a grant from the Royal Society, while the visits to St. Andrews and Woods Hole were made while holding a fellowship from the International Education Board and were further facilitated through the hospitality afforded by the Biological Board of Canada.

Developmental material was obtained without difficulty from viviparous species, *Molgula citrina*, *M. complanata*, *M. canadensis* and *M. bleizi*, but in the other cases artificial fertilisations were made. The method used was the same as that described in the first paper on this subject (Berrill, 1929).

The development of the viviparous species *Molgula cooperi* and *M. papillosa* was determined from preserved material very kindly supplied by Dr. Huntsman and Dr. W. G. Van Name, while thanks are due to Mr. A. M. Lucas for the loan of permanent preparations and sections of embryos and larvæ of *Molgula robusta* (cp. Lucas, 1927).

The development of *M. verrucifera* was determined from specimens lent by Mr. Barnhart, of La Jolla, California, through the courtesy of Dr. W. E. Ritter, while that of *M. platei* and of *M. kolaensis* from embryos lent by Dr. A. Ârnbâck-Christie-Linde, from the Swedish State Museum.

2. Historical.

The difference in development between various members of the family Molgulidæ has been the subject of considerable discussion since the year 1870, when Lacaze-Duthiers first observed that a tailed tadpole-like stage did not occur in the development of all the species he examined.

He described at Roscoff a species at first identified as *Molgula tubulosa*, but later recognised that he was dealing with a new species and one with a very peculiar development. In a paper published in 1874 he gave to it the name *Anurella roscovita*, though not before Kupffer had independently discovered the adult form in the North Sea and ascribed to it the name *Molgula occulta*. It has been suggested elsewhere (Berrill,

1928) that the *Molgula conchilega* of FORBES and ALDER may be synonymous, and if so would possess priority.

LACAZE-DUTHIERS noted that the development differed in many particulars from that of all other ascidians, the development of which was then known; instead of a tadpole-like larva with an otolith, hatching from the egg-membrane and possessing a considerable period of free-swimming life, no sign of a tail was developed, nor an otolith, and the embryo emerged from the egg-membrane through its rupture by a superficial translucent region. He further stated that the embryo after hatching developed arm-like processes and moved in an amœboid manner, that these arms later grew so that two had a common origin on one side, three on the other side of the body, that the internal part of the embryo developed into a small ascidian in no way distinguishable, except through the absence of an otolith from the region of the permanent ganglion, from an ascidian of the same age that had developed from a metamorphosed tadpole. The principal stages can be seen in fig. 8, but the reader is referred to the numerous drawings contained in the original monograph.

LACAZE-DUTHIERS, however, made a most significant observation in connection with this “ direct ” development, and that was that lying alongside the rudimentary stomach of the advanced larva was a mass of highly refractive cells and in the same position occupied by the phagocytised tail cells of the tadpole larva of the urodele forms. He not unnaturally suggested that the mass of cells in the two cases were homologous. Apart from these observations he noted that gastrulation occurred, and also made some statements concerning the nature of the early cleavage. The first division was said to be almost always unequal and the succeeding divisions to result in the formation of micromeres and megameres, as was known to be the case in the Molluscs with which the ascidians were then thought to have affinity.

At this time the early cleavage of the ascidian egg had not otherwise been investigated, and it will be shown that the observations of LACAZE-DUTHIERS in this connection were erroneous.

The discovery of this abbreviated type of development caused considerable discussion, especially as it was confirmed by the discovery of a similar development in *Molgula simplex** and *Molgula macrosiphonica* by KUPFFER. There were two possible interpretations, that the direct development had been secondarily evolved from a more primitive tadpole-like larva, in which case the mass of cells mentioned above would represent the last trace of the tail, or that the “ direct ” type was the more primitive, the mass of cells the forerunner of the tail, and that the tailed larva was evolved from this tailless form. The latter theory was strongly presented by KUPFFER, but the other has been accepted generally as being the more probable.

In 1877 LACAZE-DUTHIERS published an account of the Molgulidæ of the Roscoff region and described no less than five species as possessing the direct type of develop-

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* There is much uncertainty as to the identity of the species described by KUPFFER as *Molgula simplex*, see p. 290.

ment, viz., *Molgula occulta* (*roscovita*), *M. oculata*, *M. bleizi*, *M. solenota*, *M. simplex*; while *Molgula complanata*, *M. ampulloides*, and *M. echinosiphonica* (*citrina*) he described as possessing tailed larvæ.

The only later paper, apart from some that were purely speculative and which brought forward no additional facts, was that of DAMAS, 1902. In this he described the development of *Molgula bleizi* from sectioned material and his results may briefly be summarised here.

No observations were made on the type of cleavage, though the statements of LACAZE-DUTHIERS in this connection were questioned on theoretical grounds. Gastrulation is apparently typical and the blastopore closes through the posterior growth of its dorsal lip. The upper layer of the roof thus formed becomes neural plate and the lower chordal cells (see fig. 18); the neural tube is formed through the invagination of the plate; that is, all the observations so far made by DAMAS are in harmony with CONKLIN'S later account of the development in *Styela partita*. DAMAS states further that there is precocious differentiation of mesoderm on either side of the notochord cells and that the number of elements is very restricted, but it is doubtful whether the differentiation is any more precocious than that of tissues generally in the development of the more typical simple ascidians.

However, while no tail outgrowth occurs, the essential elements seem to be present in the form of chordal cells, with lateral mesoderm and dorsal neural cells, and according to DAMAS typical disaggregation occurs as in urodele forms, that of the muscle cells (active organ) preceding that of the chordal cells (passive organ). It was found impossible to follow in any detail the metamorphic changes in the nervous system.

DAMAS suggests that the mesodermal cells act as phagocytes and that caudal disaggregation is precocious in and typical of anural development, but since the time factor was ignored, it is difficult to see upon what grounds the latter part of this statement is based.

The later development, *i.e.*, of the peribranchial sacs, the pericardium and epicardium, etc., was shown to be much the same in both anural and urodele types. DAMAS summarises his and LACAZE-DUTHIERS' observations on the anural development as follows:—

1. The embryo retains a rounded form throughout development. It never produces a caudal elongation.
2. The tunic or test is formed very early and attains great thickness.
3. Two groups of ampullæ are formed, one on either side of the body. These are larval organs of a somewhat transient existence.
4. At the side of the digestive canal at an early stage of development there is to be found a mass of cells representing the caudal organs of the urodele larva.

GIARD (1872) had suggested that the type of development could be correlated with the type of habitat of the adult, those living unattached in sand having anural embryos, but, as DAMAS pointed out, this theory fell to the ground, for while all the species then known to be living free were thought to possess tailless development, among species that were attached to some solid substratum both tailless and tailed larvæ occurred.

The only other theory put forward was that of HARTMEYER (1911), who suggested that the anural type of development could be correlated with the presence of viviparity; this was based on the facts that the viviparous species, *Molgula bleizi*, had been stated to possess this type of development, and that statements made concerning the remaining species known to have anural development left the question open. It was known, however, that certain species of *Molgula* that were viviparous produced tailed larvæ, so that this theory has held but little weight.

Since these early descriptions no new evidence has been brought forward until very recently, but now the development is known for about twenty members of the Molgulidæ and they will be described in turn before further discussion is made. First, of those species the development of which was described by LACAZE-DUTHIERS the following have been re-examined:—*Molgula bleizi*, *M. echinosiphonica* (*citrina*), *M. roscovita* (*occulta*), *M. oculata*, *M. ampulloides*, *M. simplex*, and *M. lanceplaini* (*complanata*), leaving to be confirmed the development of *Molgula solenota*, and the *Molgula macrosiphonica* of KUPFFER. Additional species that have been examined are *Molgula retortiformis*, *M. arenata*, *M. canadensis*, *M. papillosa*, *M. cooperi*, *M. verrucifera*, *M. platei*, *M. kolaensis*, *Bostrichobranchus* (*Eugyra*) *pilularis* and *Eugyra arenosa*. The species *Molgula arenata* and *Bostrichobranchus pilularis*, while no publication was made, were independently examined by A. M. LUCAS in 1925, who has also described a further species, viz., *Molgula robusta* (1927). *Molgula manhattensis* and *Molgula citrina* (form *littoralis*) have been described as well in a paper by CASWELL GRAVE (1926).

In order to make more obvious certain significant points, all the figures of development, unless otherwise stated, are drawn to the same scale with the aid of a camera lucida. The scale is the same as that to which the figures are drawn in the first paper dealing with ascidian development (BERRILL, 1929).

Owing to the existing confusion and uncertainty concerning the identity of most species of *Molgula*, it has been thought necessary to give the source of material in full detail in each case and also the less doubtful synonyms. It may be mentioned that the species as herein described are in accordance with the systematic views of CHRISTIE-LINDE (1928), but are in marked contrast to those of HARTMEYER (1923). The various species are grouped in four sections, oviparous forms with urodele development, viviparous forms with urodele development, oviparous forms with anural development, and viviparous forms with anural development; while the subsequent discussion deals first with the origin and influence of viviparity and second with the significance of anural development.

3. (*a*) Species with Urodele Larvæ.

I. Oviparous.

1. *Molgula tubifera*, Örsted, 1844.

Syn. *Ascidia ampulloides*, Van Beneden, 1847.
Molgula ampulloides, Kupffer, 1875.
Caesira ampulloides, Hartmeyer, in Bronn's Tierreich.
Molgula manhattensis (part), Hartmeyer, 1923.
Molgula manhattensis var. *ampulloides*, Berrill, 1928.

Habitat and Distribution.—Occurs typically in shallow sheltered waters throughout the coasts of north-western Europe, from the White Sea to Southern Spain. Usually attached to algæ, submerged wood structures, etc. May occur in the inter-tidal zone.

Source of Material.—The individuals examined were obtained mostly from the Salcombe Estuary (water of salinity 34 per cent.), English Channel, in very shallow water; others from the sides of pontoons, Millbay Docks, Plymouth, summer of 1925 and spring of 1926.

Dimensions.—The average size of the individuals used for cultures was 20 mm. The species seems to become sexually mature at about 15 mm. and to reach a maximum size of 30 mm.

Development.—Development invariably takes place outside the parent body, while the eggs are small and are produced in relatively large numbers. The ovum proper (diameter 0·11 mm.) is separated from the chorion or egg-membrane by the perivitelline fluid in which float a limited number of inner follicle cells. Attached to the outer side of the chorion are the outer follicle cells, cells that are highly vacuolated and assist greatly in the flotation of the egg (fig. 1, FI).

At 18° C. the tadpole develops and hatches in about 10 hours, and the hatching process is that of digestion of the egg-membrane as described in Part I of this study (Berrill, 1929). This method is, however, by no means infallible, and in this species often as many as 50 per cent. individuals fail to hatch as tadpoles, and only do so at a later stage by means of rupture of the chorion by the primary ectodermal ampulla. When hatching normally, the active free-swimming period seems to vary from one hour to ten hours. Van Beneden (1846) gives the period of free-swimming life as 12 hours. Sometimes a whole culture may have settled and commenced metamorphosis within two hours of hatching, at other times the free-swimming period may be prolonged into days, and from the few experiments made it seems most probable that it is influenced in the same way as was shown for the larvæ of the Ascidiidæ (Berrill, 1929).

The primary ectodermal ampulla appears and grows as the tail becomes phagocytised, and eventually attains a length often exceeding that of the tail. When fully formed, several secondary ampullæ appear and a final condition is reached when two clusters are formed, two ampullæ arising from a common base on one side of the body and three from a common base on the opposite side, as may be seen in fig. 1, J. Waves of

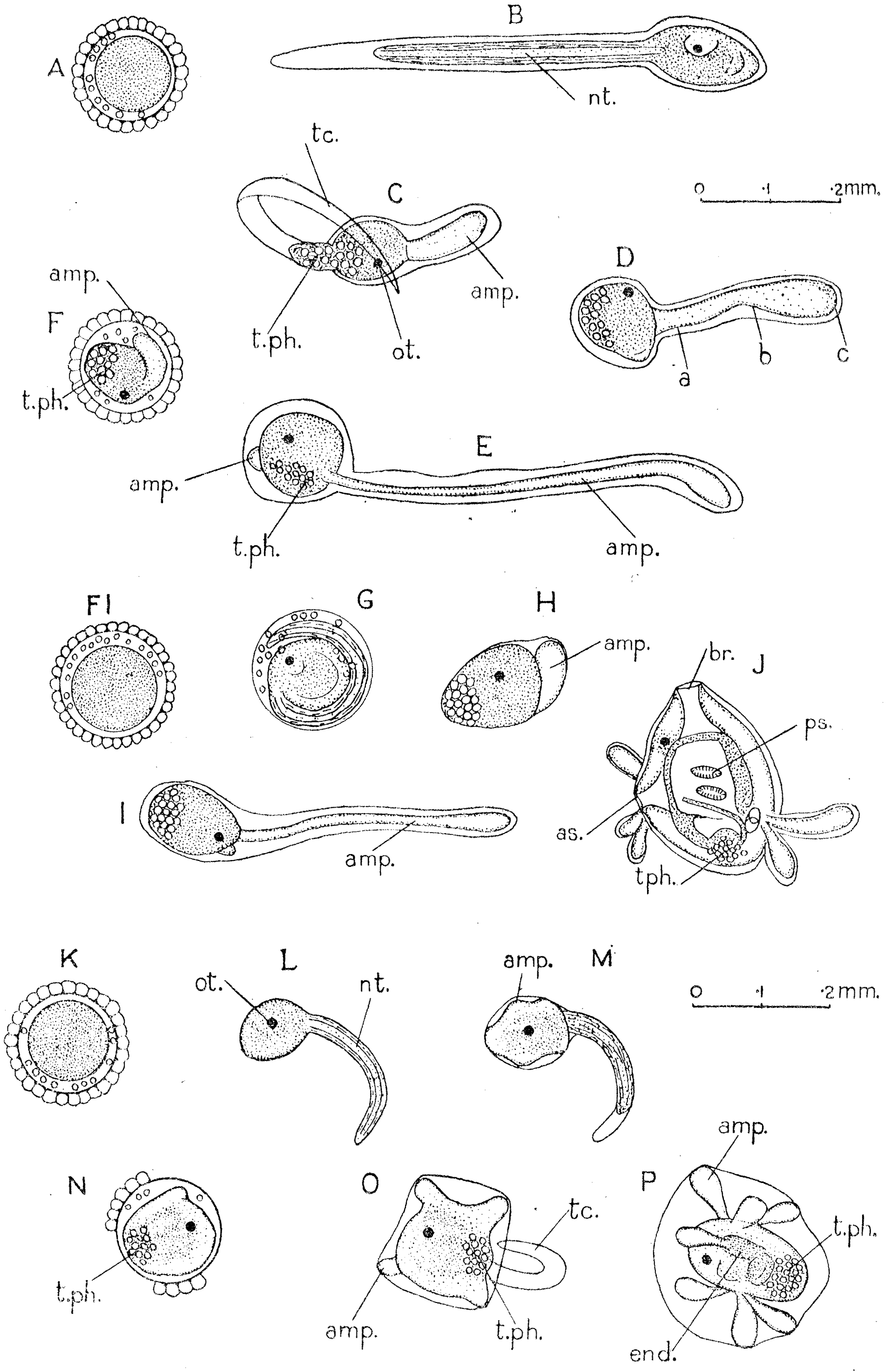

FIG. 1.—The development of oviparous urodele molgulids. A–F, *M. manhattensis*; FI–H, *M. simplex*; I–J, *M. tubifera*; K–P, *M. oculata*; showing eggs, tadpole larvæ, absorption of tail, and outgrowth of primary and secondary ampullæ. F and N represent larvæ that have absorbed their tails without hatching; that shown in F would break through eventually, that in N would not, according to the rate of ampullary outgrowth. FI represents egg of *M. tubifera* as well as that of *M. simplex*. *amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *end.*, endostyle; *nt.*, notochord; *ot.*, otolith; *tc.*, tail cuticle; *tph.*, tail phagocytes; *a–c*, extent of movement of wave of constriction *b*; *ps.*, protostigma.

constriction pass along the primary ampulla towards its distal end, about one per 100 seconds, and usually two can be seen at any given moment. The respiratory and other functions of these structures have already been discussed in detail in the above-mentioned paper. The single atrial invagination occurs approximately at the time of hatching of the tadpole, though the siphons, atrial and branchial, do not become functional until much later (see fig. 1, J).

It should also be noted that the single sense organ, the otolith, does not become dislocated or disappear during metamorphosis, but eventually is seen to lie close to the adult ganglion.

2. *Molgula manhattensis*, De Kay, 1843.

Syn. *Caesira manhattensis*, Van Name, 1912.
Molgula manhattensis (part), Hartmeyer, 1923.

Habitat and Distribution.—Occurs typically in shallow water, attached to Zostera weed, stones, wharves, etc., and ranges from Boston, Mass., to North Carolina. Is known to occur down to a depth of 24 fathoms.

Source of Material.—The individuals examined were obtained from Zostera at Woods Hole, Mass., in September, 1927.

Dimensions.—The specimens used for cultures were about 15 mm. diameter. The species apparently becomes sexually mature at about 12 mm. and attains a maximum size of about 25 mm. diameter.

Remarks.—This is a species very closely allied to *Molgula tubifera*, and in fact Hartmeyer included this last-named species and others within *Molgula manhattensis*.

Development.—The development is remarkedly similar to that of *M. tubifera*, in the nature and size of the tadpole, the outgrowth of a long primary ectodermal ampulla, and in the size of the egg and associated structures. This may be seen by comparing figs. 1, A–F and 1 FI, I, J.

Development occurs outside the parent body and its rate is close to that of *M. tubifera*, while as far as could be judged from two cultures, the free-swimming period of the tadpoles are much the same. The ability to hatch by means of rupture should the normal digestion method fail is well developed. The respiratory waves of constriction along the primary ampulla are readily seen, and were first recorded in this species by Willey (1900), who assigned to them this function.

3. *Molgula oculata*, Forbes, 1848.

Syn. *Anurella oculata*, Lacaze-Duthiers, 1877.
Caesira oculata, Hartmeyer, in Bronn's Tierreich.
Molgula oculata (part), Hartmeyer, 1923.

Habitat and Distribution.—Occurs living imbedded in open sand flats, away from the shore regions, throughout the English Channel and North Sea.

Source of Material.—The specimens were obtained, together with numerous individuals of *Molgula occulta,* from sand and shell-gravel in about 15 fathoms, three miles north from the mouth of the St. Pol river, Roscoff, Brittany, in September, 1926.

Dimensions.—The range in size of the individuals discovered, all of which were sexually mature, was from 20 mm. to 60 mm. greatest dimension.

Remarks.—This species was considered by HARTMEYER (1923) to be identical with *Molgula roscovita*, LAC.-DUTH., and *Molgula occulta*, KUPFFER, and, as already pointed out (BERRILL, 1928), this identification was facilitated by an erroneous account of the development of *M. oculata* by LACAZE-DUTHIERS (1877). He described the development of both *Molgula oculata* and *M. roscovita* as being of the direct type, though he distinguished between the respective adults. The differences between the adults of the two species have been stressed in the above-mentioned paper, and it was also shown that there is undoubtedly an association between the two forms, at least as regards the English Channel. Both at Roscoff and at Plymouth dredgings on sandy ground included both species.

Development.—LACAZE-DUTHIERS, however, described *Molgula oculata* as forming no tadpole-like larva, but cultures made at Roscoff during the summer of 1926 from large individuals (6 cms. diameter) that were indubitably of *Molgula oculata* invariably produced such tadpoles. Cultures were made on six occasions, and it was found that typical tadpoles were formed that hatched by the digestion method after about ten hours at 19° C. The only sense organ developed was the otocyst and this seems to retain its position throughout metamorphosis and become involved near the permanent ganglion, as in *Molgula tubifera* and *M. manhattensis*.

Unlike the last-mentioned species, the ectodermal ampullæ appear approximately altogether and no single one predominates. Associated with this relatively slow ampullary growth is the almost certain failure of an embryo to hatch should the primary method of membrane digestion break down. It should be noted that the final condition of the ampullæ, which, though short, exhibit waves of constriction, is a group of three on one side of the body, of two or three on the opposite side, and a single short anterior ampulla (fig. 1, K–P).

The egg with its follicle cells is almost identical in size and nature with those of *Molgula tubifera* and *M. manhattensis*. From examination of numerous adult individuals of *Molgula oculata* it is certain that the species is typically oviparous.

4. *Molgula simplex*, ALDER and HANCOCK, 1870.
    Syn. not *M. simplex*, KUPFFER, 1872.
        not *M. macrosiphonica*, KUPFFER, 1872.
        M. manhattensis (part), HARTMEYER, 1923.

Habitat and Distribution.—English Channel and North Sea. The species is typically attached and free from sand, mud, etc., although occasionally specimens have been dredged unattached, together with *M. oculata*, when both have been covered with adherent particles.

Source of Material.—Mature specimens were obtained in fair quantity from *Chaeto-*

pterus tubes from the Eddystone grounds at Plymouth, July, 1929, and occasional unattached individuals from adjoining sand patches.

Remarks.—This species seems to be very closely related to *Molgula tubifera,* sufficiently so for HARTMEYER to have included it under that name. In doing so, however, he confined his attention to the gut-loop, branchial folds, etc., and ignored the details of the branchial wall proper and the structure of the gonads. ALDER and HANCOCK give a description of *Molgula simplex* that differs from that of *M. tubifera* (*ampulloides*) in the following manner:—The stigmata are arranged in large and distinct spirals, one to each branchial pouch (those of *M. tubifera* being small, irregular and indistinct); the margins of the ovary, seen from the exterior, form a double series of ovarian tubules, and the testes forming two or three large irregular rounded nodules on the inner side of the ovary (in *M. tubifera* the testis is found at the periphery and outer surfaces of the ovary, see fig. 1, p. 18, ÂRNBÂCK, 1928).

The specimens found at Plymouth conform exactly to ALDER and HANCOCK'S description, and are easily distinguished from *M. tubifera* (*ampulloides*) which occurs in shallower water in the same district. Moreover, ALLEN, 1903, identified specimens obtained from Chætopterus tubes as *M. simplex,* as did HARTMEYER himself in 1909 during his visit to Plymouth.

ÂRNBÂCK-CHRISTIE-LINDE, 1928, p. 23, questions whether KUPFFER, 1872, in his account of *M. simplex* and *M. macrosiphonica* and their development was describing distinct forms. The importance of this is seen below.

Development.—Eggs from the individuals collected from Chætopterus tubes possessed red pigment, occasionally absent, which eventually became confined to the endoderm of the larvæ. Apart from the question of colour, the eggs are identical with those of *M. tubifera* and *M. manhattensis.*

A typical tadpole is formed, with its single sense organ or otolith. Hatching takes place through digestion of the egg membrane, there is a free-swimming period of several hours, and as the tail later becomes absorbed, a single anterior ampulla grows out. The latest stage obtained is shown in fig. 1, H, great difficulty having been encountered in obtaining artificial fertilisations. The adults could not be kept alive. The species was reared to a definite tadpole stage on three different occasions. Therefore KUPFFER could not have been dealing with this form when he described the development of *M. simplex* as direct. LACAZE-DUTHIER'S apparent confirmation (1877) of KUPFFER may be ignored, since he made no personal observations upon the type of development.

5. Another oviparous species is the form common at St. Andrews, Bay of Fundy, and identified as *Molgula papillosa,* VERRILL, by HUNTSMAN (1912). Unfortunately none of the numerous attempts to obtain natural or artificial fertilisation were successful, so that the type of development, direct or indirect, remains undetermined. ÂRNBÂCK (1928) includes this form under *Molgula manhattensis,* but it is very doubtful whether such procedure is justifiable.

II. *Viviparous.*

6. *Molgula citrina*, Alder and Hancock, 1848.

Syn. *Molgula littoralis*, Verrill, 1871.
Molgula nana, Kupffer, 1871.
Molgula echinosiphonica, Lacaze-Duthiers, 1877.
Caesira citrina, Van Name, 1912.
Caesira citrina, Hartmeyer, in Bronn's Tierreich.

Habitat and Distribution.—Occurs in shallow and deep waters, attached to algæ, other ascidians, etc., on both sides of the Atlantic, from the White Sea to Brittany, and from the St. Lawrence to Narragansett.

Source of Material.—Individuals were examined from various localities. Form "*nana*" was found on one or two occasions attached to algæ in about 5 fathoms in the Gullmarfjord, west coast of Sweden, and examined at Kristineberg. Form "*echinosiphonica*" was discovered at Roscoff in the locality described for it by Lacaze-Duthiers in 1877, in fairly large numbers, namely, attached together with *Molgula bleizi* to individuals of *Styelopsis grossularia* lining the roof of certain rock grottoes in the intertidal zone. Form "*littoralis*" was found and examined at St. Andrews, New Brunswick, in very large numbers. The individuals formed clusters attached to the submerged brushwood of old fish traps. Some specimens were also encountered at Woods Hole, together with *Molgula manhattensis.*

Dimensions.—Sexually mature at 5 mm., maximum size 18–20 mm.

Remarks.—Within this species both Hartmeyer (1923) and Ârnbâck (1928) include also *M. littoralis*, Verrill, *Molgula nana* Kupffer and *Molgula echinosiphonica*, Lac.-Duth.

Caswell Grave (1926), however, maintains that the European forms as typified by *M. nana* and *M. echinosiphonica* are specifically different from the American form of *Molgula citrina.* This question has already been discussed (Berrill, 1928), but the nature of the differences between the two types is shown here to a fuller extent.

Molgula nana was examined at Kristineberg and *Molgula echinosiphonica* at Roscoff during the summer of 1926, while *Molgula citrina* (*littoralis*) was examined at St. Andrews and Woods Hole 1927–8; in fig. 2 all the drawings, with the exception of B and C, were made from American forms. Figs. 2, B and C, are of *Molgula echinosiphonica* but represent equally *Molgula nana.*

Development.—Before discussing the variations that occur in the development of this species, the more typical development of the east-coast American type will be described. The ovum has approximately double the diameter of that of the above oviparous species, *i.e.*, about 0·21 mm. The perivitelline space is small and more or less packed with inner follicle cells. The outer follicle cells are markedly flattened out over the surface of the chorion.

Development invariably occurs within the atrial cavity of the parent and up to a thousand embryos, at all stages of development, may be found within one individual. A typical Molgulid tadpole is formed as is shown in fig. 2, C and D. At this stage the tail has grown to its full length and the vacuolated notochordal cells are on the verge of fusing to form a single turgid rod; there is an otocyst but no eye, and the atrial invagination is just commencing (not shown in the figure). Fig. 2, D, however, represents a tadpole that has been hatched prematurely and artificially. In the oviparous species, with their small eggs, the tadpole hatches by the digestion method at this stage. In *Molgula citrina* hatching is delayed, and apparently never occurs by means of digestion of the chorion by an enzyme. Usually the tadpoles hatch when they have attained the appearance shown in fig. 2, E. Here it is evident that great extension of the trunk region of the tadpole has occurred, and there is also visible the first outswelling of the lateral and posterior ectodermal ampullæ. The anterior extension also foreshadows

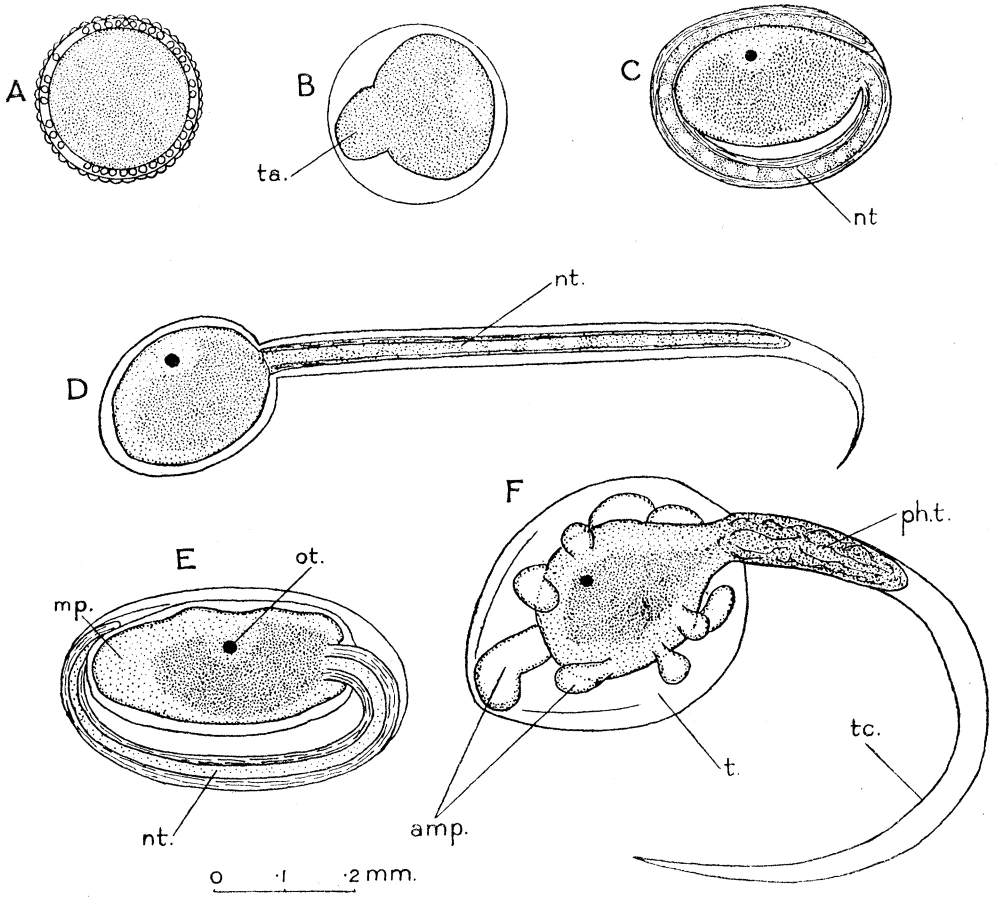

FIG. 2.—Development of *Molgula citrina*. A, egg; B., short-tailed embryo; C, well-advanced larva, notochord vacuolated; D, tadpole hatched prematurely at stage equivalent to normal hatching stage of *M. manhattensis*; E, tadpole on point of hatching, notochord one vacuole; F, tadpole metamorphosing (note precocious outgrowth of ampullæ, foreshadowed in E). *amp.*, ampulla; *mp.*, mental process; *nt.*, notochord; *pht.*, phagocytising tail; *ot.*, otolith; *t.*, test; *tc.*, tail cuticle.

the outgrowth of the primary ampulla. The immediate effect, however, of such extension is the extreme stretching of the chorion or egg-membrane and this, combined with the twitching movements of the tail, eventually causes the rupture of the membrane and the hatching of the tadpole. Then, after a brief period of free-swimming life, the larva settles, the tail becomes phagocytised, the anterior and lateral ampullæ become very prominent.

A completely metamorphosed individual is shown from two aspects in fig. 3, D and E. It will be seen that there is but a single atrial siphon, that the otolith is embedded near the permanent ganglion, the phagocytised tail forms a mass of highly refractive cells at the side of the stomach, the primary renal vesicle is well developed, and the ectodermal ampullæ form a cluster on either side of the body. This is a stage reached in about ten days from hatching, at 12° C.

Of the remaining drawings, fig. 3, A, represents a type encountered fairly commonly at St. Andrews. Fixation has occurred and metamorphosis proceeded to much the same extent as in the case D and E, but phagocytosis of the tail has not commenced. The tail, in fact, still exhibits twitching movements, though its tip shows signs of degeneration. The reader is referred to a similar condition produced by hyper-alkalinity in larvæ of the Ascidiidae, described in Part I (Berrill, 1929). Its significance in this instance will be discussed later when the development of the remaining species of *Molgula* has been described. It should be noted, however, that despite the absence of tail absorption the continued growth of the trunk region and its characteristic rotation are apparently normal.

Figs. 3, B and C, are of two stages of development of *Molgula echinosiphonica* and *M. nana*. That is, the tadpole usually fails to hatch as such and the tailed phase of development is passed through entirely within the egg-membrane, and the larva finally emerges through the continued swelling of the larval test and outgrowth of ectodermal ampullæ, thereby rupturing the membrane. It will be seen that the tadpoles themselves are identical with those of the American type; while the tail is still perfect in form, ectodermal ampullæ appear, one primary and anterior, and a group on either side of the body. In all there is an otocyst but no eye. Thus the only difference between the two types seems to be in the relative toughness of the egg-membrane. In the American type the larvæ succeed in rupturing the membrane before the tail commences to be phagocytised; in the European phagocytosis of the tail usually starts and is completed before membrane rupture occurs. But sections of the European type of a stage rather earlier than that shown in fig. 3, B, show a degree of differentiation identical with that figured by Caswell Grave (1926, figs. 5 and 6).

This author, however, has assumed that the difference in mode of hatching between the American and European forms is complete and constant, and suggests that it warrants a specific distinction. In reality, between 80 and 90 per cent. of the larvæ of *Molgula nana* and *M. echinosiphonica* hatch post-metamorphically and there are always a few that hatch out as tadpoles. While among the numerous individuals studied at

St. Andrews it was found that on an average 2–3 per cent. hatch post-metamorphically, so that the difference is but one of degree only. A discussion of the marked difference between the free-swimming tadpole of *Molgula citrina* and those of *Molgula tubifera*, *manhattensis*, and *oculata*, will be postponed until the remaining viviparous species have been described.

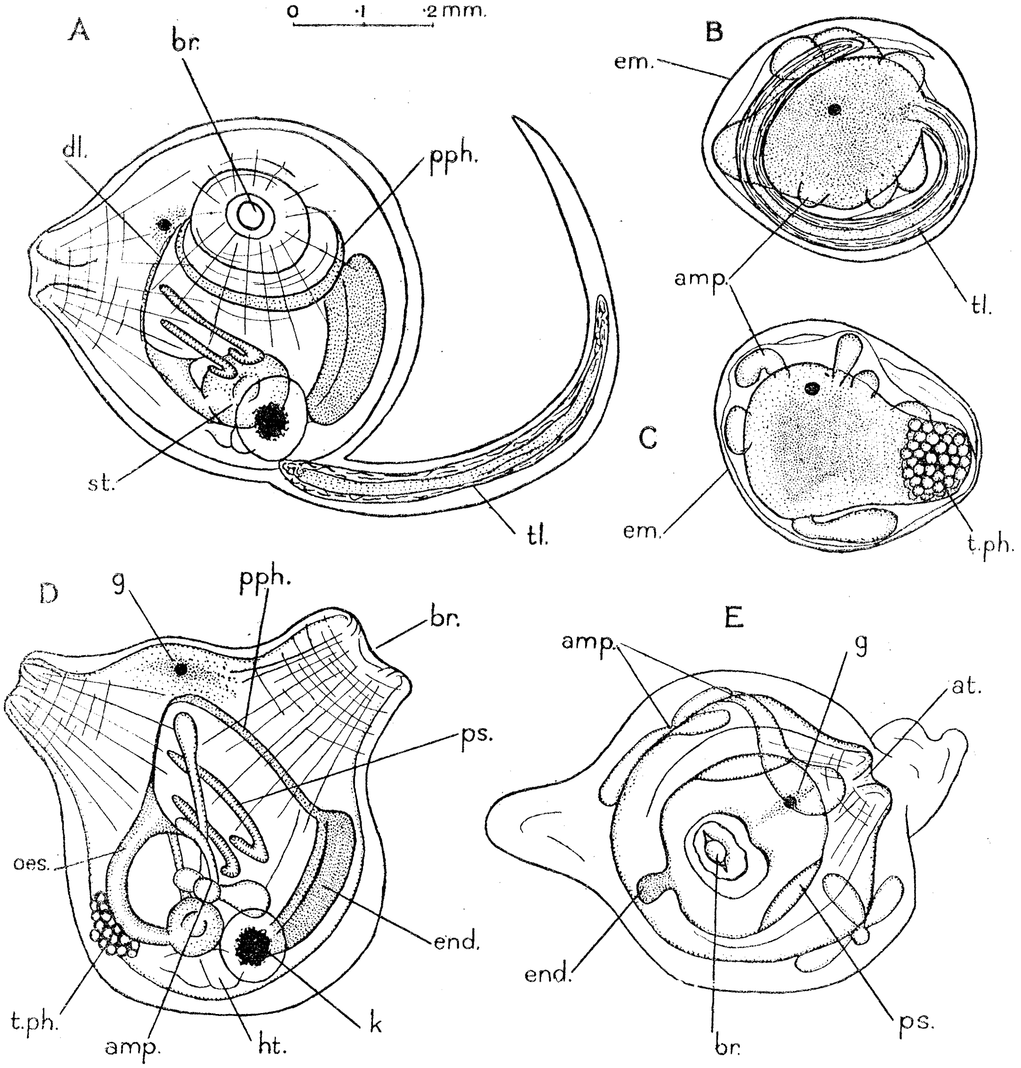

FIG. 3.—Development of *Molgula citrina* (continued). A, fully metamorphosed larva with tail still intact and twitching; B and C, individuals of form "*echinosiphonica*" metamorphosing before hatching (note precocious ampullary outgrowth); D, normal and fully metamorphosed individual. E, dorsal view of same. *amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *dl.*, dorsal lamina; *end.*, endostyle; *em.*, egg-membrane; *g.*, ganglion; *ht.*, heart; *k.*, renal vesicle; *oes.*, oesophagus; *ps.*, protostigmata; *pph.*, peripharyngeal band; *st.*, stomach; *tl.*, tail; *tph.*, tail phagocytes.

7. *Molgula complanata*, ALDER and HANCOCK, 1870.

Syn. *M. papillosa*, VERRILL, 1871.
Ctenicella lanceplaini and *C. morgatæ*, LACAZE-DUTHIERS, 1877.
Lithonephrya eugyranda, DAMAS, 1902.
Caesira papillosa, VAN NAME, 1912.
Caesira canadensis, HUNTSMAN, 1912.

Remarks.—This species, according to both HARTMEYER, 1923, and ÂRNBÂCK, 1928, includes all those forms falling within the genus *Ctenicella* of LACAZE-DUTHIERS, 1877. It is not doubted that they are very closely related together, but they are sufficiently divergent for at least three types to be distinguished, at least as regards development. The outstanding adult features common to all are the reversal in the orientation of the gonad and its ducts, the subdivision of the lobes of the atrial siphon, viviparity, and the production of tadpole larvæ. The reader is referred to the discussion given by ÂRNBÂCK (1928, p. 45) on the significance of many differences in adult structure.

Section (*a*).—1. *Molgula complanata*, ALDER and HANCOCK.
2. *Caesira papillosa*, VAN NAME.
3. *Molgula canadensis*, HUNTSMAN.

These three forms are grouped together since no differences have been discovered in their development, although it has not been followed to the same extent in all.

Source of Material.—1. Numerous small individuals of "*complanata*" found in small depressions and crevasses in stones throughout Plymouth Sound, and a few larger specimens (10 mm.) from Millbay Docks, July-September, 1929.

2. One preserved specimen of "*papillosa*," lent by Dr. W. G. VAN NAME, taken at U.S.F.C. Sta. 32 off Cape Ann in 90 fathoms, 1877.

3. Two specimens from the Swedish State Museum, lent by Dr. ÂRNBÂCK-CHRISTIE-LINDE, taken from West Spitzbergen, 1861, in about 20 fathoms, one from sand, one from rock (see ÂRNBÂCK, 1928, p. 44, habitat).

4. Living specimens of "*canadensis*" found growing with hydroids attached to scallop shells in about 5 fathoms at the mouth of l'Etang river, Bay of Fundy, August, 1927.

Dimensions.—The individuals found at Plymouth varied in greatest diameter from 3 to 10 mm., all being sexually mature. They averaged around 8 mm.

Development.—Fig. 4 represents the development of *Molgula complanata* as determined at Plymouth. The follicle cells are flattened out against the egg-membrane as in other viviparous forms, considerable embryonic growth occurs, and a tadpole hatches by means of rupturing the egg-membrane, has a very short free-swimming period and already shows ampullary outgrowth.

The anterior ampulla or mental process grows out to a tremendous extent and exhibits waves of constriction. After a day or so it is mostly resorbed, often being replaced by a second growing out from the same region, but usually the condition seen in fig. 4, F, is that found several days after fixation; that is, a forking or division occurs and in place of the earlier waves of constriction there is an alternation of dilatation and contraction of the distal extremities. This type of respiratory movement is therefore almost identical with the pulsating ampullæ of larval and adult Botryllids. The ampulla in *Molgula complanata* eventually shrinks and disappears as the heart becomes functional.

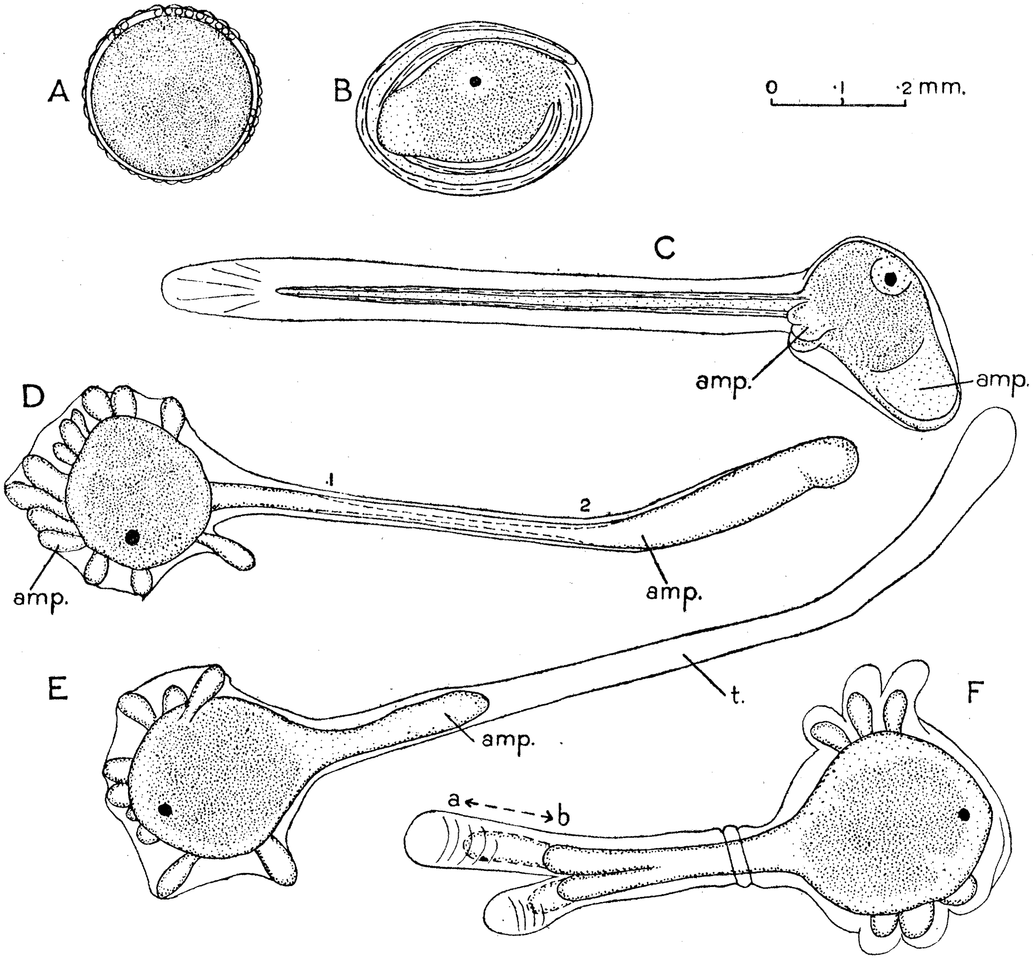

FIG. 4.—Development of *Molgula complanata*. A, egg; B, tadpole on point of hatching; C, tadpole, with slow metamorphosis showing commencement of rotation; D, individual with primary and secondary ampullæ, 1–2 being region exhibiting wave of constriction; E, 24 hours later showing resorption of primary ampulla; F, 36 hours later showing double primary ampulla exhibiting dilatation and contraction. D, E and F are from different individuals. *amp.*, ampulla; *t.*, test secreted by primary ampulla.

Section (*b*).—*Molgula lanceplaini.*

Two individuals attached to weed were found from Asia Shoal, Plymouth Sound, that fitted more closely than any other the description given by LACAZE-DUTHIERS for his *Ctenicella lanceplaini*. They had eggs the size figured for *M. complanata*, but produced tadpoles smaller in the trunk region but with a relatively longer tail.

Section (*c*).—*Molgula morgatæ.*

Syn. *Lithonephrya eugyranda*, GIARD, 1880, and DAMAS, 1902.

Recorded only from the north coast of Brittany.

Development.—Fig. 5, taken from LACAZE-DUTHIERS, shows the tadpole of this form. The scale is unknown, but it is seen that the tail is relatively short, about one and a half times the length of the trunk, also that the two posterior ampullæ are plainly visible before metamorphosis. This form is of especial interest in that it has been suggested that the tadpole represents an intermediate type between a normal tadpole and the tailless larvæ of some other molgulids (GARSTANG and GARSTANG, 1928, p. 38, and this paper, p. 337).

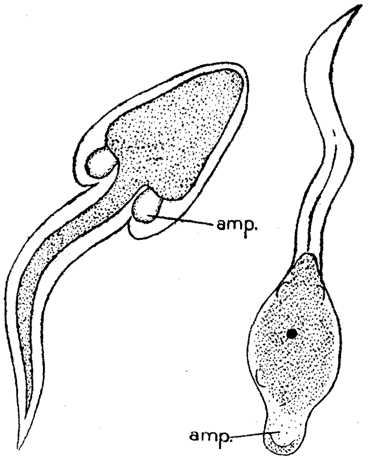

FIG. 5.—Tadpole and metamorphosing tadpole of *Molgula morgatæ* (after LACAZE-DUTHIERS). Scale unknown. *amp.*, ampulla.

8. *Molgula cooperi*, HUNTSMAN, 1911.

Syn. *Caesira cooperi*, HUNTSMAN, 1911.
? *Molgula regularis*, RITTER.

Habitat and Distribution.—Found only at Departure Bay, British Columbia, in 5 to 15 fathoms, in sand and gravel, firmly attached to larger objects. Individuals covered by a coating of fine sand.

Source of Material.—Preserved specimens from the original collection, lent by Dr. A. G. HUNTSMAN.

Dimensions.—Individuals sexually mature at 8 mm. greatest diameter, reaching a maximum of 15 mm.

Remarks.—The species is viviparous, as has been stated by HUNTSMAN (1911).

Development.—Fig. 6, G, H, I, shows the size and proportions of the eggs and larvæ, and the embryonic increase in size typical of viviparous forms is evident. The ectodermal ampullæ also are visible in tadpoles that have yet to hatch and metamorphose.

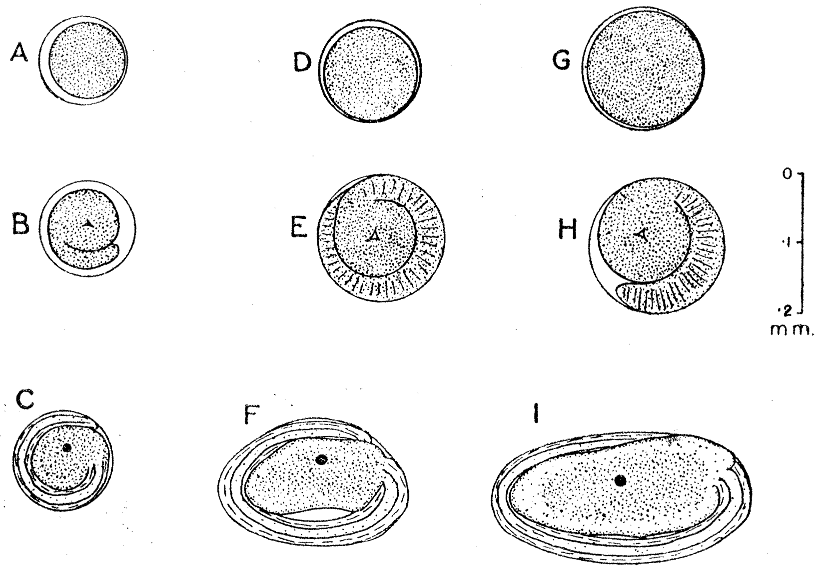

FIG. 6.—Development of *Molgula platei* (A, B, C), *M. verrucifera* (D, E, F), and *M. cooperi* (G, H, I). A, D, G, eggs (follicle cells omitted); B, E, H, developing embryos; C, F, I, tadpole larvæ on point of hatching.

9. *Molgula verrucifera*, RITTER and FORSYTH, 1917.

Habitat and Distribution.—Has been recorded only from La Jolla, California, from the littoral zone. Is usually attached to the exposed surfaces of rocks, and has a heavy coating of sand.

Source of Material.—Specimens from the Scripps Institute, Cal., lent through the courtesy of Dr. W. E. RITTER and Mr. BARNHART.

Dimensions.—8 to 10 mm. greatest dimension.

Remarks.—Development takes place within the atrial cavity of the parent (see RITTER and FORSYTH) and it can be seen from fig. 6, D, E, F, that there is relatively an enormous increase in size before hatching, and also that the ectodermal ampullæ appear before metamorphosis.

10. *Molgula platei*, HARTMEYER, 1912.

Habitat and Distribution.—Described by HARTMEYER, from Calbuco, Chile, and recorded by ÂRNBÂCK from a collection of *Molgula platei* and *Molgula manhattensis* made off the coast of New Jersey. Attached.

Source of Material.—Embryos extracted and lent by Dr. A. ÂRNBÂCK-CHRISTIE-LINDE, from specimens from the New Jersey collection.

Dimensions.—About 12 mm. greatest dimension. This seems to be the average adult size, but the range is imperfectly known.

Development.—*Molgula platei* possesses eggs at least as small as those of either *Molgula manhattensis* or *M. tubifera*, viz., 0·11 mm., and as small as are known to exist among ascidians, yet the eggs and developing embryos are retained within the atrial cavity of the parent.

Typical tadpole larvæ are formed (see fig. 6, A, B, C).

11. *Molgula heydemanni*, MICHAELSEN, 1915.

Habitat and Distribution.—Recorded in dredgings off the west coast of Africa. The species is attached by the ventral side, and has its surface encrusted with sand, etc.

Dimensions.—Maximum size but 7 mm., and sexually mature apparently at 2 or 3 mm. diameter.

Remarks.—According to MICHAELSEN, this species is the smallest solitary ascidian. It forms aggregates and reproduces viviparously.

Development.—MICHAELSEN states that numerous embryos and tailed larvæ were found in the atrial cavity. The fact that the adults are said to occur in small aggregates would seem to imply that the tadpoles escape in batches and in many cases, at least, fall to the bottom without an active swimming stage.

3. (*b*) SPECIES WITH ANURAL DEVELOPMENT.

I. *Oviparous.*

12. *Molgula retortiformis*, VERRILL, 1871.

Syn. *Molgula grönlandica,* TRAUSTEDT, 1880.

Habitat and Distribution.—Occurs in most arctic and sub-arctic waters, though is not truly circum-polar, and extends south with the Labrador Stream to Cape Cod. The species is typically attached to hard bottoms of various nature, usually in shallow water ranging down to not more than 120 fathoms.

Source of Material.—Individuals found in fair quantity occurring among clusters of *Molgula citrina, M. papillosa* (HUNTSMAN), etc., attached to submerged stakes and brushwood of fish-traps at St. Andrews, New Brunswick. Occasionally also from dredgings from the same district, together with *Boltenia ovifera* and *Tethyum pyriforme americanum.*

Dimensions.—An average size for this species seems to be about 40 mm., sexual maturity not being reached until it surpasses 20 mm., and possibly 25 mm. greatest dimension. The maximum size recorded is about 100 mm. height and 90 mm. breadth, so that this represents the largest northern Molgulid.

Remarks.—The species is quite definitely oviparous though the eggs are large; cultures were obtained by means of artificial fertilisation and had to be maintained at a temperature below 10° C. to be successful.

Development.—The general course of development is shown in fig. 7, and it is obvious that at no stage of development is there any sign of tadpole-like larva. The embryo remains round in form until it is ready to hatch and never is there any sign of a tail extension. After two to three days at 7° C. many embryos rupture the egg-membrane by the swelling of its outer translucent region as is shown in fig. 7, C, but in many cases the first two or three ectodermal ampullæ appear and the larval test becomes obvious before rupture occurs. This is shown in fig. 7, D and E, but whether the embryo hatches at stage C or E, the course taken, apart from the time of membrane rupture, is the same: that is, the body of the embryo first swells and then the ampullæ and larval test appear. There is never any sign of membrane digestion.

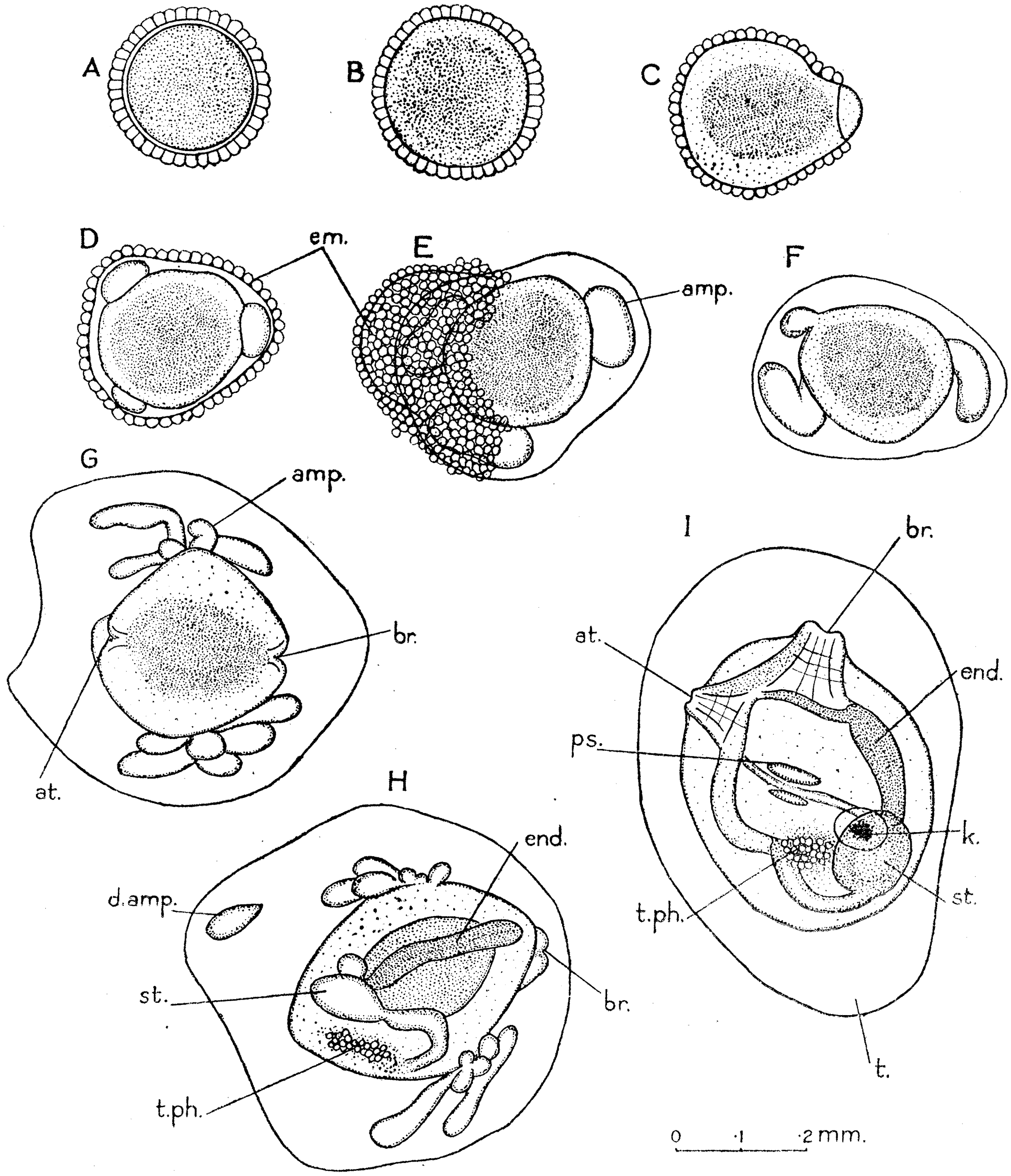

Fig. 7.—Development of *Molgula retortiformis*. A, egg; B, embryo shortly before hatching; C, embryo hatching at early stage; E, hatching at later stage, showing method of rupturing; F, embryo hatched; G, post-larval form showing siphons and lateral clusters of ampullæ; H, more advanced individual showing detached ampulla, alimentary canal, and cluster of cells of high refractive index at base of stomach; I, functional metamorphosed individual with siphons, protostigmata, renal vesicle, and cell cluster near stomach representing phagocytosed tail rudiment. *amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *d. amp.*, detached ampulla; *em.*, egg-membrane; *end.*, endostyle; *k.*, renal vesicle; *ps.*, protostigma; *st.*, stomach; *t.*, test; *tph.*, tail phagocytes.

The egg is both pigmented (purplish) and heavily yolked, so that the internal development is difficult to follow until a late stage when it has expanded and loosened out and the fundamental organs of the adult have been differentiated. The ampullæ eventually form a cluster of about six on either side of the body, and frequently the distal end of one becomes constricted off to lie well out in the relatively thick larval test. Fig. 7, G, represents the stage reached after 7–8 days at 7° C., while H and I that reached after 11–15 days from fertilisation. Apart from the complete lack of any tail formation and the absence of the otocyst, the development is remarkedly similar to that of *Molgula citrina*. The hatching mechanism is the same, the larval test swells and ectodermal ampullæ appear at approximately the time one would expect them to, had a tailed larva been produced; the ampullæ, at first few in number and exhibiting waves of constriction, finally form a cluster on either side of the body; a branchial and a single atrial siphon appear at about the same time, the differentiation of the alimentary tract and feeding mechanism and protostigmata is the same; while, lastly and unexpectedly, a mass of highly refractive cells appears at the side of the stomach in exactly the same position as that occupied by the refractive cells resulting from the phagocytosis of the tail in the case of *Molgula citrina* and other forms.

That is, neither a tail nor larval sense organs are formed, but otherwise the whole later course of development is identical with that which results from the metamorphosis of a tadpole.

It should also be noted that the follicle cells of the egg are numerous and highly vacuolated in spite of the fact that the ovum is relatively large; and that therefore, since *M. retortiformis* is oviparous, the shrinkage and stretching out of the follicle cells in other species is to be correlated with viviparity rather than increase in the size of the egg. More fully, increase in size of the egg causes the original limited number of outer follicle cells to become stretched and flattened over the surface of the chorion; in the case of viviparous species this was a matter of indifference and so they remained in this condition, but in a form such as *Molgula retortiformis*, where development proceeds in the water outside the parent body, the buoyancy of the egg would be affected and so the number would need to be increased to counteract the stretching and keep constant the specific gravity.

13. *Molgula occulta*, Kupffer, 1875.

Syn. ? *Ascidia conchilega*, O. F. Müller, 1776.
? *Molgula conchilega*, Alder and Hancock, 1907.
Anurella roscovita, Lacaze-Duthiers, 1877.
Molgula oculata (part), Hartmeyer, 1923.

Habitat and Distribution.—Is found widely distributed on the coasts of north-west Europe, living unattached embedded in sand, often in association with *Molgula oculata*, usually in extraordinary abundance.

Source of Material.—Individuals were obtained at Roscoff in large quantities in August and September, 1926, in the sand flats near the Roche de Loup, at low water of high spring tides. Others were obtained from dredgings, together with *Molgula oculata*, north of the St. Pol river in the same district. In July, 1926, large numbers of what is believed to be this species were dredged in 20 fathoms from muddy sand in the Koljö Fjord, near Kristineberg, but unfortunately attempts at natural and artificial fertilisation in this case were unsuccessful, owing probably to an insufficiently low temperature.

Dimensions.—Individuals apparently become sexually mature at about 18 mm. greatest dimension, attaining a maximum size of about 30 mm.

Remarks.—*Molgula occulta* was first discovered by LACAZE-DUTHIERS in 1870 at Roscoff, where it occurs in remarkable abundance free in the sand at extreme low-water tide level; but he did not give to it his name *Anurella roscovita* until 1877 when KUPFFER had independently discovered the same species in the North Sea and labelled it *M. occulta*. At Roscoff, Plymouth, and at Kristineberg dredgings that contained specimens of *Molgula occulta* invariably did so in very large numbers, and at Plymouth and Roscoff usually a few individuals of *Molgula oculata* would be found among them. Superficially these two species look much alike, but the adult differences have been described in some detail already (BERRILL, 1928); also a tentative explanation of LACAZE-DUTHIER'S error was made at the same time.

Development.—His account of the development of *Molgula occulta*, however, is both full and accurate. Fig. 8 shows the egg, hatching embryos, and later stages drawn from living material, but the reader is referred also to the numerous excellent figures of this development in LACAZE-DUTHIERS' account (1877). The species is quite definitely oviparous though the above author's description left the question open, and the egg has the same diameter as that of *Molgula tubifera*, *M. manhattensis*, and *M. oculata*, differing only in that the outer follicle cells are not so highly vacuolated.

At 19° C. the embryo hatches in the manner shown in fig. 8, B and C, after about 10 hours' development, *i.e.*, it hatches by means of membrane rupture as in *Molgula retortiformis*. The embryo swells and then the ectodermal ampullæ grow out. These never exceed five in number and all appear within an hour or two of hatching, as in fig. 8, D, and exhibit limited waves of constriction. This phenomenon caused LACAZE-DUTHIERS to describe the embryo as hatching through the rupture of the egg membrane and progressing by means of amœboid movement. Of the five outgrowths, two eventually arise from a common base on one side of the body and three on the other. The convergence of the ampullary bases in the case of every species so far examined seems to be due to the involvement of the body surface between their bases in their continued elongation, and conceivably such growth in length ceases when the bases fuse together.

The development as a whole is the same as that of *Molgula retortiformis*, differing only to the same extent from that of the tadpole-producing forms. That is, the ampullæ and larval test, the branchial and single atrial siphons, the differentiation of the digestive tract, and the appearance of the protostigmata are the same as if they had all resulted from a metamorphosing tadpole.

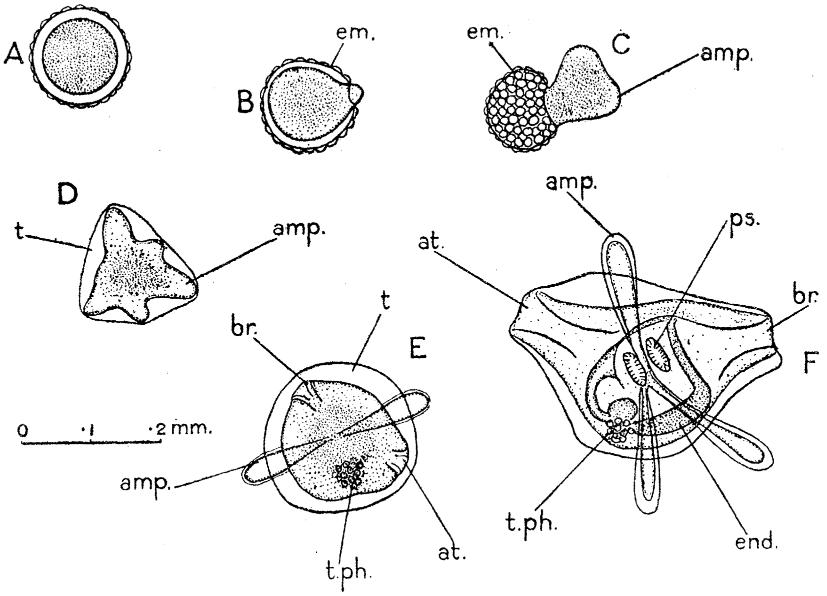

FIG. 8.—Development of *Molgula occulta* (*roscovita*). A, egg; B, C, embryos hatching; D, hatched larva showing five ampullæ (the " amœboid processes " of LACAZE-DUTHIERS); E, left side of more advanced individual showing two ampullæ, siphons and cluster of " tail " cells; F, completely metamorphosed form from right side showing three ampullæ, " tail " cells near stomach, and protostigmata. *amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *em.*, egg-membrane; *end.*, endostyle; *ps.*, protostigma; *t.*, test; *tph.*, tail phagocytes.

There is, however, no sign of tail development nor of an otocyst, nor does hatching occur by means of membrane digestion even though the egg is very similar to those that possess this method. It should also be noted that again a group of highly refractive cells appear in the same relative position as do those resulting from the phagocytosis of the tail in the urodele species, *i.e.*, alongside the stomach. *Molgula occulta* and *Molgula oculata* were cultured on several occasions almost simultaneously and the cultures maintained at the same temperature, so that the time factor in development becomes comparable. It was found that the tadpoles of *Molgula oculata* hatch (at 19° C.) at practically the same time as do the embryos of *Molgula occulta* and that the ectodermal ampullæ first appear in both forms after 12–14 hours. An account of the early cleavage and internal development of this species will be left till later.

14. *Molgula arenata*, STIMPSON, 1852.

Syn. *Caesira arenata*, VAN NAME, 1912.

Habitat and Distribution.—Has been recorded only from Cape Cod to Long Island Sound, in which region it occurs living unattached embedded in sand.

Source of Material.—About a dozen individuals were dredged in sand in 5 to 10 fathoms, off Falmouth Heights, Vineyard Sound, near Woods Hole, in September, 1927. Permanent preparations of whole larvæ and sections thereof, made in 1925, lent by Dr. A. M. LUCAS.

Dimensions.—Maximum size, 20 mm., sexually mature at 15 mm. or less.

Remarks.—This form is characteristically laterally compressed, and is oviparous.

Development.—Artificial fertilisations were made and cultures reared at 19° C. for 45 hours, the course of development through which period being shown in fig. 9. The size of the egg and nature of the follicle cells are practically identical with that of *Molgula occulta* (see fig. 8, A). Fig. 9, B and C, represent the stage reached after 12 hours, D, E and F after 20 hours, and G and H that reached after 45 hours.

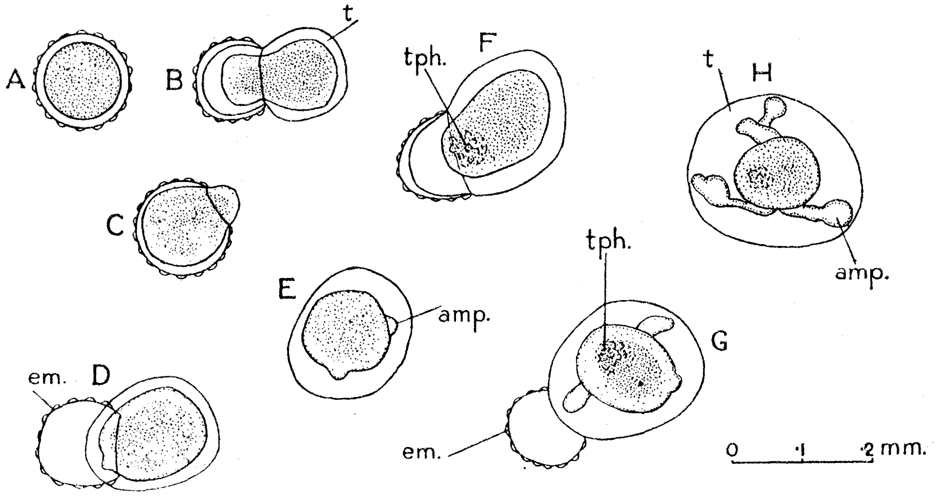

FIG. 9.—Development of *Molgula arenata*. A, egg; B, C, D and F, embryos hatching; E, G, H, hatched embryos. *amp.*, ampulla; *em.*, egg-membrane; *t*, test; *tph.*, tail phagocytes.

Hatching is by means of membrane rupture through the swelling first of the embryo and shortly after by the rapidly growing larval test. The ectodermal ampullæ appear relatively late and are somewhat slow in growth. Hatching occurs in many forms after about 10 to 12 hours, but, as may be seen from the figures, its completion may be delayed until 20 hours have elapsed. The embryo retains throughout its rounded form, except during the act of hatching when it becomes distorted into a dumb-bell shape by the tough membrane. No otocyst is developed, but the characteristic mass of refractive cells appears after about 15 hours and seems to lie in the same relative position as in the previously described species.

From unpublished notes and drawings made by Dr. A. M. LUCAS in 1925 and very kindly placed at my disposal, in addition to confirming the above observations, it seems that the final condition of the ampullæ is typical, two arise from a common base on one side of the body and three from a common base on the other. The large refractive cells are also shown very clearly (see fig. 10).

15. *Molgula robusta*, Van Name, 1912.

Syn. *Caesira robusta*, Van Name, 1912.

Habitat and Distribution.—Recorded only from Woods Hole and the adjacent Vineyard Sound. The species is typically unattached and lives on sandy or muddy bottoms in about 10 fathoms.

Source of Material.—Permanent preparations of larvæ and sections thereof, made in 1925, and lent by Dr. A. M. Lucas.

Dimensions.—Maximum size apparently about 38 mm. greatest dimension.

Remarks.—A short account of the development of this form has been given by Lucas in 1927, but the following description and figures are made entirely from the notes and permanent preparations made by the above author, very kindly placed at my disposal.

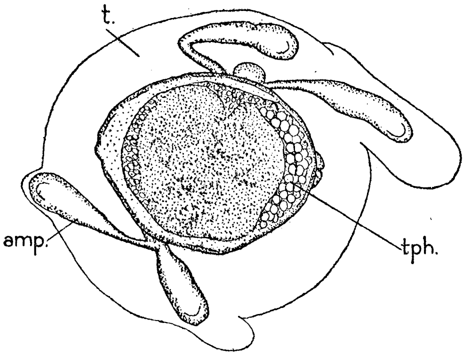

Fig. 10.—Development of *Molgula arenata* (after Lucas), showing final position of ampullæ and also the phagocytosed tail rudiment. *amp.*, ampulla; *t.*, test; *typh.*, tail phagocytes.

Molgula robusta, on the basis of adult structure alone, was considered by Hartmeyer to be closely allied to *Molgula manhattensis*, though both Van Name and Christie-Linde consider it to be a distinct species. The type of development, however, makes this a certainty.

Development.—The eggs are purplish and the inner follicle cells obvious. After about two days, four short tubes and one long tube (ampullæ) are seen extending beyond the embryo beyond the test and attaching it to a sand grain. Hatching presumably is by rupture, while there is no sign of any sense organs. After 67 hours the tubes were short and fat (temperature 15–20° C.), after 90 hours the embryos were attached, while at 117 hours the siphons were evident as a pair of lips at opposite ends of the larva. See fig. 11.

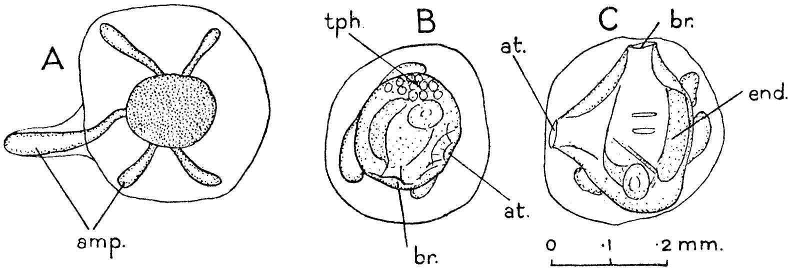

Fig. 11.—Development of *Molgula robusta*. A, larva after 92 hours showing long fixatory ampulla and four short ones (from drawing of A. M. Lucas); B, C, two stages of development from preparation of seven-day embryos, lent by A. M. Lucas; note "tail" phagocytes in B. *amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *end.*, endostyle; *tph.*, tail phagocytes.

16. *Eugyra pilularis*, Verrill, 1871.

Syn. *Molgula pilularis*, Verrill, 1871.

Bostrichobranchus manhattensis, Traustedt, 1882.

Bostrichobranchus pilularis, Van Name, 1912; Hartmeyer, 1923.

Habitat and Distribution.—Lives unattached embedded in sand or mud, and occurs abundantly from the Gulf of St. Lawrence to Florida.

Source of Material.—Numerous individuals were dredged in muddy sand in about 5 fathoms in West Quoddy Bay, near Lubec, Maine, in September, 1927. Permanent preparations of larvæ collected at Woods Hole, 1925, were lent by Dr. A. M. Lucas.

Dimensions.—The maximum size of this species seems to be about 30 mm. greatest dimension. Sexually mature forms were found of 15 mm.

Development.—The eggs of this species are small (0·11 mm.) and the cleavage was seen to be typical of ascidian eggs in general. The embryo retained a rounded form throughout the course of development and eventually hatched through the rupture of the egg-membrane by a long ectodermal ampulla.

All the figures, except fig. 12, E and F, illustrating this species are made from notes and preparations of Dr. A. M. Lucas.

17. *Eugyra arenosa*, Ald. and Hanc.

Habitat and Distribution.—European waters from Spain to the Norwegian Sea; lives free in sand or mud usually below 10 fathoms.

Source of Material.—A few specimens taken dredging in shell gravel off Stoke Point and the Eddystone at Plymouth, August, 1929.

Dimensions.—The largest individuals found were about 14 mm. greatest dimension; mature forms were found measuring about 10 mm.

Development.—The species is oviparous. Artificial fertilisation was successful only after many attempts made in the course of several summers. The eggs are very small, the diameter of the ovum usually being 0·11 mm., but on one occasion eggs of about two-thirds the volume were seen mixed with the normal ones and they developed in an apparently normal manner.

At 16° C. the eggs gastrulated after about seven hours, and after thirty-six hours many embryos had hatched. These were typically anural and the hatching occurred through the swelling of the embryo itself rather than through the formation of test material or the outgrowth of ampullæ. The efficiency of this method may be due to the presence of a relatively small perivitelline space, so that a minimum extent of swelling is effective. The subsequent course of development proceeded slowly and small functional ascidians were formed after three weeks. No otolith could be seen, but tail phagocytes or their equivalent were readily identified (see fig. 12, A–D).

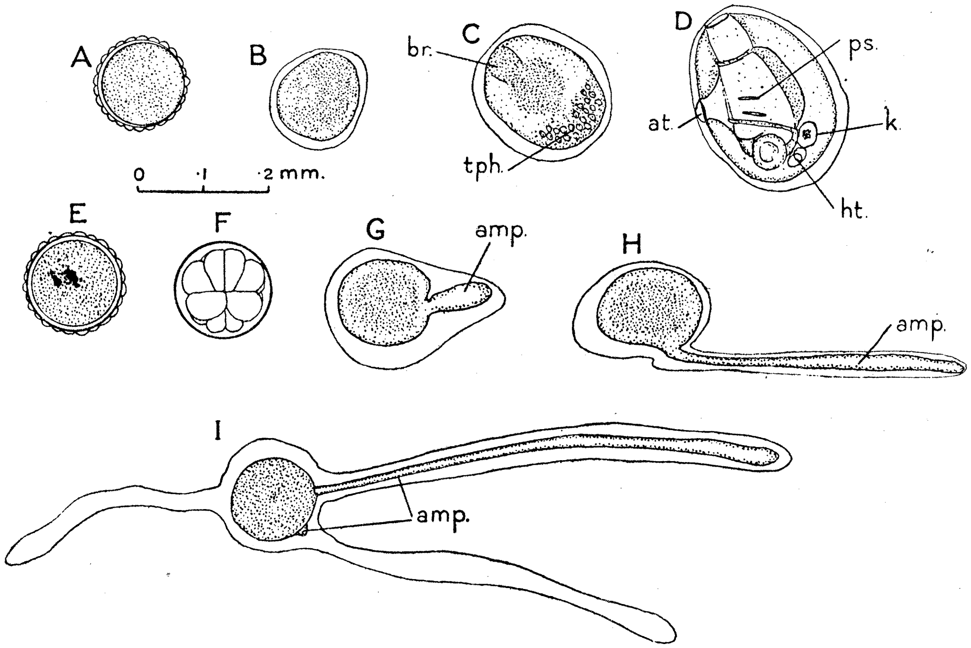

Fig. 12.—Development of *Eugyra arenosa* (A, B, C and D) and of *Eugyra pilularis* (E, F, G, H and I). A to F inclusive are original and are to scale, G, H and I are from drawings of Dr. A. M. Lucas, and the scale is only approximate. A, egg; B, embryo just hatched (36 hours); C, more advanced larva showing "tail" cells; D, completely metamorphosed individual showing siphons, protostigmata, heart and renal vesicle; E, egg; F, cleavage; G, H, larvæ shortly after hatching showing primary ampulla; I, later stage showing absorption of first and second ampullæ and outgrowth of third. *Amp.*, ampulla; *at.*, atrial siphon; *br.*, branchial siphon; *k*, renal vesicle; *ht.*, heart; *ps.*, protostigma; *tph.*, tail phagocytes.

18. *Molgula solenota*, Lacaze-Duthiers, 1877.

Syn. *Anurella solenota*, Lacaze-Duthiers, 1877.
Molgula oculata (part), Hartmeyer, 1923.

Habitat and Distribution.—Has been recorded only by Lacaze-Duthiers, and from dredging alone, from gravel bottoms north of l'Isle de Batz, and at Astan, near Roscoff.

Dimensions.—The maximum size seems to be from 15 to 20 mm. greatest dimension.

Remarks.—Lacaze-Duthiers stated that from embryos encountered within the body chamber of the parent there was no doubt that the development is anural. No confirmation has yet been made, and therefore the statement cannot be accepted without reservation since his similar statement concerning the development of *Molgula oculata* has proved to be incorrect.

19. *Molgula macrosiphonica*, Kupffer, 1872.

Syn. *Molgula manhattensis* (part), Hartmeyer, 1923.
? *Molgula simplex*, Kupffer, 1872.

Habitat and Distribution.—Recorded only from south-western Baltic Sea, and once from off Ostende (Damas, 1905), embedded free in sand, or attached to marine algæ.

Dimensions.—The species attains a size of 20 mm. diameter, according to KUPFFER.

Remarks.—There is reason to believe that this species includes also the form described by KUPFFER as *Molgula simplex.* (See A. CHRISTIE-LINDE and also this paper under *Molgula simplex.*)

Development.—According to KUPFFER (1872) the embryos of this species develop in the water outside the parent, and no tadpole stage is ever formed.

II. *Viviparous.*

20. *Molgula bleizi,* LACAZE-DUTHIERS, 1877.

Syn. *Molgula oculata* (part), HARTMEYER, 1923.
Anurella bleizi, LACAZE-DUTHIERS, 1877.

Habitat and Distribution.—This species was found by LACAZE-DUTHIERS attached side by side with his *Molgula echinosiphonica* on *Styelopsis grossularia* in grotto roofs at Roscoff. No records from other localities are known.

Source of Material.—Numerous individuals were obtained from the exact locations in the grottoes at Roscoff described by LACAZE-DUTHIERS, during September, 1926.

Dimensions.—Individuals are sexually mature at 4 mm. greatest dimension, but may attain a maximum size of about 15 mm., though very rarely.

Remarks.—HARTMEYER (1923) listed this species also under *Molgula oculata* incl. *M. occulta,* but with even less justification. It is a small attached form, whereas the preceding two species lie free in sand or gravel, and the test is never covered with adherent particles. It is a species, however, that belongs to the *Molgula oculata-occulta* group, and conceivably may have evolved from *Molgula occulta.* *Molgula occulta* seems to be most abundant at Roscoff, and occurs in sand within a few yards of the rocky grottoes in which is to be found *Molgula bleizi.* *M. bleizi* has only been recorded living side by side with *Molgula echinosiphonica* on clusters of *Styelopsis grossularia* in the roof of such grottoes at Roscoff and still may be found there. The species is small and viviparous.

Development.—The egg is much larger than that of *Molgula occulta,* while the outer follicle cells are greatly flattened out over the surface of the chorion. There is never any sign of the development of either a tail or an otocyst, and the embryo throughout retains a rounded form.

The ectodermal ampullæ seem to appear rather before the larval test, but both appear well before hatching occurs. Hatching is invariably by means of rupture of the membrane and occurs at a stage relatively very late compared with the case in *Molgula occulta.* The membrane is ruptured at a stage between that shown in fig. 13, C and D, and it is at about this later stage that the embryos are usually ejected from the atrial cavity of the parent. A single atrial siphon develops as in the other Molgulids, the ampullæ eventually form two clusters, one of three to four on one side of the body, and one of four to five on the opposite side.

Yet again it is seen (fig. 13, D) that a mass of highly refractive cells appears close alongside the stomach. The internal development is described later.

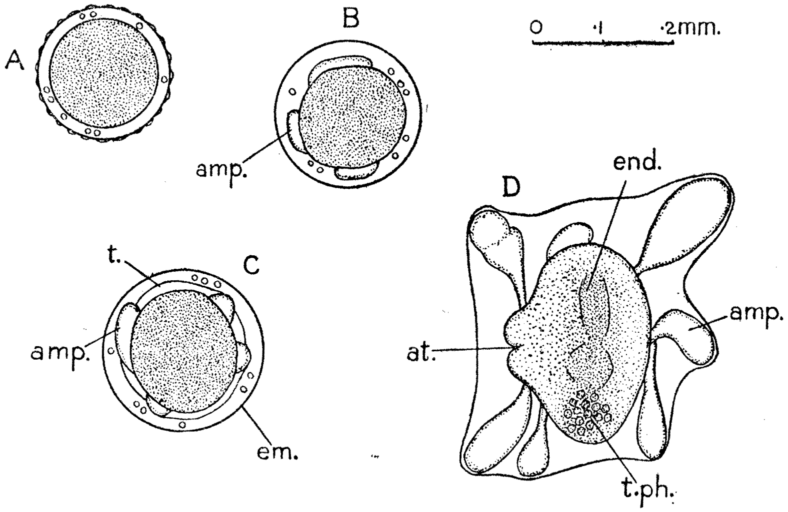

Fig. 13.—Development of *Molgula bleizi*. A, egg; B, C, embryos still within the egg-membrane; B, showing ampullary outgrowths; C, showing secretion of test as well; D, hatched embryo shortly after liberation from atrial cavity of parent, showing tail phagocytes, siphon and grouping of ampullæ. *amp.*, ampulla; *at.*, atrial siphon; *em.*, egg-membrane; *end.*, endostyle; *t.*, test; *tph.*, tail phagocytes.

21. *Molgula kolaensis*, Ãrnbâck-Christie-Linde, 1928.

Habitat and Distribution.—One specimen dredged with *Molgula retortiformis*, Styelids. etc., in 15 fathoms, Lumbowski, Kola Peninsula, 1877, attached to a piece of shell. The test is covered with a coat of fine sand.

Dimensions.—Height 7 mm., breadth 9 mm.

Source of Material.—Embryos extracted from the above specimen and lent by Dr. Ãrnbâck-Christie-Linde.

Remarks.—From the figure given by Ãrnbâck-Christie-Linde (Pl. 1, fig. 27), the attachment to a shell fragment seems to be accidental and unimportant, and the impression is given that the species is typically a sand-living form and unattached.

Development.—Development takes place within the atrial cavity, and considerable accumulation of yolk within the eggs is evident. Cleavage is typical of ascidian eggs in general. The embryo develops without ever losing its rounded form, or forming larval sense organs, and hatches while within the parental atrial chamber by rupture of the egg-membrane. The most advanced embryo encountered shows the atrial and branchial invaginations, the ectodermal ampullæ, and the caudal rudiment (see fig. 14, D).

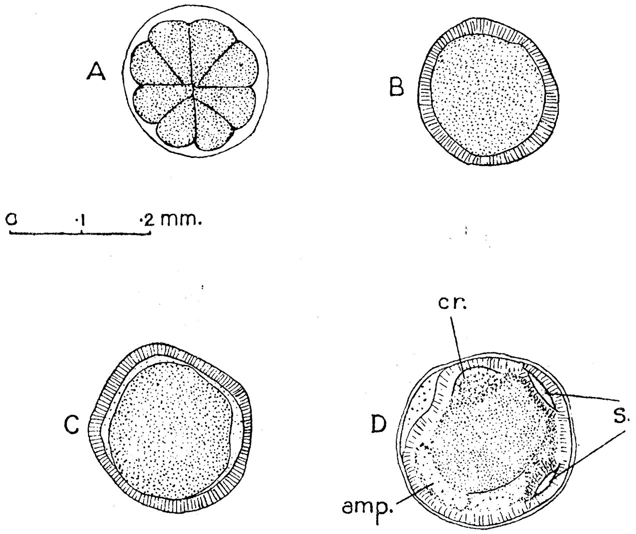

FIG. 14.—Development of *Molgula kolaensis*. A, cleavage; B, embryo apparently hatched; C, more advanced embryo; D, most advanced stage found within atrial cavity, showing atrial and branchial invaginations, the commencement of ampullary outgrowth, and a distinct caudal rudiment. *amp.*, ampulla; *cr.*, caudal rudiment; *s.* siphon invaginations.

(*c*) DISCUSSION.

Assuming that the development of such oviparous forms as the free-living *Molgula oculata* and the attached *Molgula tubifera* is the primitive type, it is seen from the preceding accounts that there has been a departure from it in two directions. On the one hand, there has been a suppression of the tadpole stage of development, structurally as well as functionally, more or less associated with the free-living mode of existence. On the other hand, to be associated rather with viviparity, there has been an increase both in size and in differentiation of the tadpole larvæ.

Both these departures present the same phenomenon, namely, a dissociation or independence between the development of the permanent organs in the trunk region, and the tail and nervous system or transient larval organs. In the one case the trunk region develops perfectly without the tail being formed at all, in the other a relatively high degree of differentiation of the trunk region is attained without affecting the development of the tail.

4. ORIGIN OF VIVIPARITY AND YOLK ACCUMULATION, AND THEIR EFFECT ON DEVELOPMENT.

Since within the family Molgulidæ both oviparous and viviparous species occur, it becomes possible to determine the factors leading to the retention of the developing embryos within the atrial cavity, and also the influence of such retention upon the course of development.

Any of the following changes from a typical oviparous form will *tend* to produce viviparity—a reduction in size of the adult resulting in a decrease in the rate of the exhalent water current,* an increase in the size of the eggs thereby lowering their surface volume ratio, and a reduction in size or number of the buoyant outer follicle cells of the eggs. Teleological reasons for the existence of viviparity are for the moment ignored.

From the preceding section (pp. 286 to 310) or from Table I, the following relationships become evident.

(*a*) Viviparous species are all small, with eggs and larvæ that are usually large.
(*b*) Oviparous species are all relatively large, with eggs and larvæ usually small.
(*c*) The two exceptions to the above generalisations are *Molgula platei*, a small viviparous species possessing small eggs and larvæ, and *Molgula retortiformis*, a large oviparous species possessing large eggs and larvæ.
(*d*) The outer follicle cells of the eggs of oviparous species have a hydrostatic function and are always round and vacuolated, those of viviparous species are always stretched and flattened over the egg-membrane (see fig. 15).
(*e*) Compared with the tadpole larvæ of oviparous species, those of viviparous species are not only larger, and therefore faster swimmers,† but at the time of hatching have undergone a definite growth in size from that of the egg, possess both branchial and atrial invaginations, and the first beginnings of the respiratory ectodermal ampullæ, all of which do not appear in the development of oviparous forms until the tail has been absorbed.

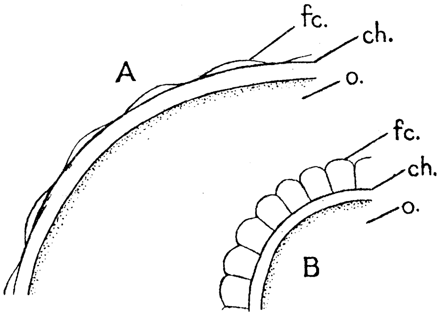

FIG. 15.—Diagram illustrating the influence of viviparity upon the outer follicle cells of the egg. A, large egg of viviparous form; B, small egg of oviparous form. *fc.*, follicle cells; *ch.*, chorion or egg-membrane; *o.*, ovum.

These relationships may be accounted for in the following manner.

It is generally agreed‡ that the family Molgulidæ is typically adapted to a life immersed in and loosely anchored by hairs to sand, and that the whole organisation is adapted

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* See p. 314.

† See Table II.

‡ "In spite of numerous secondary readaptations, these ascidians (Molgulids) from their entire organisation, appear to be essentially and primarily inhabitants of spacious sand-flats in which they live immersed, loosely anchored by their coating of adhesive hairs."—GARSTANG and GARSTANG, 1928, p. 40.

"Body (of *Molgula*) usually unattached because the animal lives on sandy or muddy bottoms, but sometimes is attached to rocks, occasionally pedunculate."—RITTER and FORSYTH, 1917, p. 441.

"Body, usually of globular form, attached or free, the animal living buried in sand or mud, at the bottom."—CHRISTIE-LINDE, 1928.

TABLE I.—Habitat, Development, etc., of the Family Molgulidæ.

Species.	Distribution.	Average Size of Adult.	Diameter of Egg.	Method of Hatching.
(*a*) OVIPAROUS, URODELE—				
ATTACHED—		mm.	mm.	
Molgula tubifera	E. Atlantic wide, 0–3 fms.	20	0·11	Enzyme.
,, *manhattensis*	W. Atlantic wide, 0–3 fms.	15	0·11	,,
,, *simplex*	E. Atlantic local, 20 fms.	18	0·11	,,
,, *socialis*	E. Atlantic local, 20 fms.	25	—	—
UNATTACHED—				
Molgula oculata	E. Atlantic wide, 9–70 fms.	40	0·11	Enzyme.
(*b*) OVIPAROUS, ANURAL—				
UNATTACHED—				
Molgula occulta	E Atlantic wide, 0–70 fms.	30	0·11	Rupture.
,, *solenota*	E. Atlantic local, 0–5 fms.	17	—	—
,, *macrosiphonica*	E. Atlantic wide, 15 fms.	20	—	—
,, *arenata*	W. Atlantic local, 8 fms.	15	0·11	Rupture.
,, *robusta*	W. Atlantic local, 8 fms.	30	0·11	,,
Eugyra pilularis	W. Atlantic wide, 5–20 fms.	18	0·11	,,
,, *arenosa*	E. Atlantic wide, 10–50 fms.	13	0·11	,,
ATTACHED—				
Molgula retortiformis	Circumpolar, 2–50 fms.	40	0·18	,,
(*c*) VIVIPAROUS, URODELE—				
ATTACHED—				
Molgula complanata	E. and W. Atlantic, 2–10 fms.	3–8	0·25	Rupture.
,, *morgatæ*	E. Atlantic local, 1 fm.	12	0·2	,,
,, *lanceplaini*	E. Atlantic local, 1–2 fms.	5	0·23	,,
,, *cooperi*	E. Pacific local, 0·1 fm.	12	0·18	,,
,, *verrucifera*	E. Pacific local, 0–5 fms.	9	0·14	,,
,, *citrina* (1)	E. Atlantic wide, 0–5 fms.	7	0·21	,,
,, *citrina* (2)	W. Atlantic wide, 2–5 fms.	12	0·21	,,
,, *heydmanni*	S.E. Atlantic local	3	0·2	,,
,, *platei*	W. Atlantic wide	12	0·10	(? enzyme)
(*d*) VIVIPAROUS, ANURAL—				
ATTACHED—				
Molgula bleizi	E. Atlantic local, 0–1 fm.	7	0·16	Rupture.
PROBABLY UNATTACHED—				
Molgula kolaensis	E. Arctic local, 5 fms.	8	0·18	,,
(*e*) UNATTACHED, PROBABLY OVIPAROUS—				
Molgula apoploa, HUNTSMAN	E. Pacific, 5–20 fms.	15	Development not known.	
,, *hecateia*, HUNTSMAN	E. Pacific, 15 fms.	25		
,, *oregonia*, RITTER	E. Pacific, 45 fms.	20		
,, *pannosa*, VERRILL	W. Atlantic, 2–70 fms.	25		
,, *rotunda*, OKA	N.W. Atlantic, 72 fms.	14		
,, *japonica*, HARTMEYER	W. Pacific, 10 fms.	30		
,, *aidæ*, OKA	W. Pacific	45		
,, *hartmeyeri*, OKA	W. Pacific, 3 fms.	20		
,, *xenophora*, OKA	W. Pacific	25		
,, *siphonalis*, SARS	Arctic, 10–70 fms.	30		
,, *lutulenta*, VAN NAME	W. Atlantic, 60 fms.	15		
,, *singularis*, VAN NAME	W. Atlantic, 14–27 fms.	11		
ATTACHED, PROBABLY OVIPAROUS				
Molgula crystallina, MOLLER	Arctic, 5–90 fms.	20		
,, *intumescens*, VAN NAME	W. Atlantic, 39 fms.	20		
,, *pacifica*, HUNTSMAN	E. Pacific, 0–? fms.	14		
,, *redikorzevi*, OKA	N.W. Pacific, 40 fms.	35		
,, *vannamei*, OKA	W. Pacific	16		

to such a habitat. From personal observations such forms are characteristically ovoid in shape and live with but their siphons projecting above the surface of the sand, and there is little doubt that the larger individuals are safer from engulfment by the shifting sand or mud than the smaller ones.

The attached species of shallower water and the littoral zone are colonists from the sand flats, a view already expressed by GARSTANG (1928). This view is supported by the fact that the majority of all Molgulids live unattached, a few are anchored merely by a slender stalk, and the majority of attached forms retain an adherent covering of sand grains, while even in others typical test hairs are present. The adaptation for attachment to a three-dimensional surface (sand-immersed) tends to unfit an ascidian for attachment to a two-dimensional surface (rock or weed), the ideal shape of the first being a sphere, of the second a film.

When a sand-living Molgulid colonizes rocks, etc., the settling is commenced as larvæ. As these assume the characteristic ovoid shape of the adult those attached to a flat surface will have a less secure anchorage than those attached in hollows, since fewer can obtain a holding, and they will therefore become more readily detached. The attachment in any case will become less and less secure as growth proceeds if the ovoid shape be retained, as actually it is. For a race to survive in such a habitat it is essential that some individuals attain sexual maturity before becoming dislodged. Therefore there will be a selection of those individuals that mature early rather than late. This does not in itself imply a limitation of the maximum size attainable. Detachment will eventually limit that size in the case of each individual though not for the race as a whole, *i.e.*, there is selection not of forms having a small maximum size but of forms having an early sexual maturity.

Early sexual maturity, however, will result in the existence of dwarf forms on the assumption that a decrease in the growth-rate occurs when the drain of germ-cell production commences. Assuming that the growth-rate before maturity and after show a constant ratio throughout the family, earlier maturity will result in smaller adults for a given age, as is shown in fig. 16. Where Molgulids live in aggregates they will in effect be increasing their base but not their height and the majority of individuals will again have a three-dimensional attachment. When this is so they may attain a relatively larger size before being broken off, and early maturity in such forms will only lower the average growth-rate for the whole life. Thus it becomes understandable that *Molgula citrina* of European waters, living solitary, is much smaller than *Molgula citrina* of eastern American waters, which lives in aggregates.

CONKLIN (1898 and 1912) for *Crepidula* and PAINTER (1928) for rabbits have shown that reduction in body size means reduction in cell number not in cell size. Therefore if eggs and embryos developing freely in the sea are eliminated at the same rate, smaller animals producing fewer eggs have greater need of reducing the rate of elimination as by means of viviparity.

Oviparity, however, is dependent upon the ability of the exhalent water current to carry the eggs out through the atrial chamber, *i.e.*, dependent on the strength of the current and on the buoyancy of the eggs. Therefore reduction in either factor will encourage any tendency towards egg retention or viviparity. The current-rate is lowered when the body size is reduced, for reduction and simplification of the stigmata-covered branchial sac exceeds relatively the reduction of area across the atrial siphon, so that there will be a tendency from this alone to retain the least buoyant eggs. These eggs would be the ones with least vacuolated outer follicle cells, and of the largest size (since their surface-volume ratio decreases with increase in size). Thus eggs will tend to become larger and their follicle cells to lose their vacuolisation and consequently fewer embryos still will be produced.

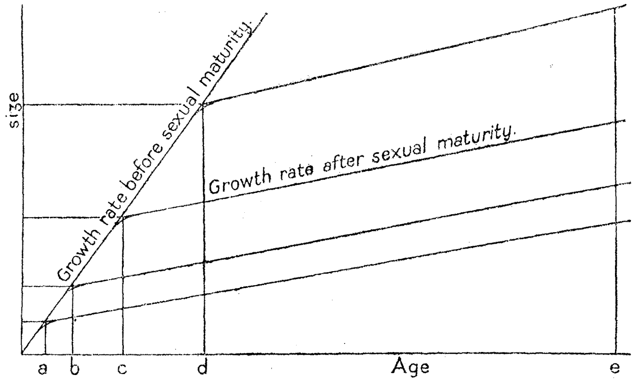

FIG. 16.—Diagram illustrating the influence of precocious sexual maturity upon adult size. The results shown depend on the belief that there is no definite breeding season in these forms, but that sexual maturity is reached after the attainment of a certain critical size and that eggs and sperm are produced practically continuously until death. *a*, *b*, *c*, *d*, ages (and sizes) at which sexual maturity is attained; *e*, maximum age showing comparative adult size.

Fewer but larger eggs and embryos will be an asset *as long as* they have the protection of the parent throughout development, for otherwise they will have the same rate of elimination as the numerous and small eggs of the large oviparous species, *i.e.*, viviparity *must* precede in small species marked increase in size of the egg. This is supported by the existence of viviparous forms having small eggs, *e.g.*, *Molgula platei*, *Boltenia echinata* and *B. hirsuta*, *Polycarpa rustica*, *P. pomaria* and *P. comata* (*fibrosa*).

The eggs of oviparous species undergo development for a period from six hours to one or two days, according to the temperature, and drift for that time in the bottom currents. On hatching, the tadpoles (if such a stage exists) swim surfacewards and sink again after about ten hours to become attached when the tail has started to degenerate. In other words dispersion is of primary importance, and selective activity on the part of the tadpoles at the time of settling almost negligible. In viviparous species the parent expels its offspring as active tadpoles that may have a free swimming period of but a few minutes only. Moreover, the most suitable region in which to settle is usually within a few feet of the parent. That is, dispersion is subordinated to selection of habitat, and large tadpoles being faster swimmers than small tadpoles are more independent of water movements and consequently have a more efficient selective activity (see Table II).

TABLE II.—Table to show Relative Speed of Various Ascidian Tadpoles.

Species.	Length of		Speed (approx.).	Stroke.
	Trunk.	Tail.		
	mm.	mm.	cm. per sec.	per sec.
Distomus variolosus	1·0	2·2	1·5–2·0	8–10
Styelopsis grossularia	0·6	2·0	1·0	10–15
Botryllus gigas	0·6	1·8	2·0	15–20
Morchellium argus	0·5	1·3	1·0	15–20
Trididemnum tenerum	0·5	1·5	1·0	15–20
Perophora listeri	0·6	1·4	1·2	15–20
Molgula complanata	0·4	1·2	0·5–1·0	15–20
Ciona intestinalis	0·3	0·9	0·4	25–35
Ascidiella scabra	0·3	0·9	0·4	25–35
Ascidia mentula	0·25	0·75	0·3	25–35
Molgula tubifera (*ampulloides*)	0·17	0·5	0·2	25–35

As far as can be determined from optical evidence alone (see Part I, p. 54, BERRILL, 1929) all four oviparous species examined produce embryos that hatch through the digestion of the egg-membrane by an enzyme. The tadpoles thus liberated normally swim for a period up to 12 hours, occasionally for longer periods, but almost always for at least several hours, before they settle and commence metamorphosis. As the tail becomes progressively phagocytised and absorbed, the primary and secondary ectodermal ampullæ grow out from the trunk region, and aid in respiration and fixation. The mouth and atrial invaginations occur approximately at the time of hatching. It should be noted that in these forms there is virtually no growth in the size of the embryo until after it has been liberated from the egg-membrane, and almost none until after metamorphosis.

Development in the case of viviparous species, however, differs markedly from the above. There is first an actual growth during the embryonic phase proper, causing the egg-membrane to be stretched and finally ruptured, this being the normal method of hatching, the digestive enzyme apparently having been lost or discarded.

The mouth and atrial invaginations that commence about the time of hatching in the oviparous forms, commence well before hatching; further, the tail of both types of tadpoles attains its full length and structure when the above invaginations are commencing, and before much increase in size has occurred. In other words it is the time of hatching that has been altered. In viviparous forms, however, following this stage of differentiation is a marked elongation and expansion of the trunk region in an anterior direction, associated with the outgrowth of the terminal, primary ampulla, while almost immediately afterwards the bulgings of the lateral ampullæ appear at the sides. This means that in the larvæ of these viviparous forms at the time of hatching, while the tail is fully developed and functional, the trunk region has developed to a stage that in the larvæ of oviparous ascidians is not attained until the tail has been phagocytised and absorbed, and the tadpole when liberated has much in common with those of Ascidiella and Phallusia in which tail absorption had been practically inhibited by hyper-alkalinity of the sea-water (Part I, page 63). In these the further development of the trunk region was merely retarded and not inhibited.

It is believed, on the basis of the experiments recorded in Part I, pages 60–63, that the factor determining the onset of tail absorption is a critical concentration in the tissues concerned of carbon-dioxide or hydrogen ion, and that the gradual attainment of this concentration in the tail tissues of tadpoles retained within the atrial cavity eventually limits the period of their existence there as such, and that in those tadpoles that are liberated and become active swimmers, as the result of their activity, this concentration is rapidly reached, the more so the longer the tadpoles had been kept within the parent. So that in viviparous forms there is a tendency for metamorphosis to commence before the escape of the larvæ from the parent,* the free-swimming period of those that escape as tadpoles is usually very short (see Table III), while the permanent structures of the larvæ are at a relatively high degree of differentiation at the moment of settling and fixation.

Among the cultures of *Molgula citrina* made at St. Andrews, however, there was commonly found, *i.e.*, from 2 to 5 per cent., larvæ which underwent all the normal post-metamorphic growth changes and differentiation including fixation and rotation associated with the trunk region without there being any absorption of the tail. That is, phagocytosis and absorption of the tail, and continuous growth and development of the trunk may be completely dissociated, and this fact will be seen to be of fundamental importance when the acceleration of development occurring in compound ascidians comes to be discussed in the third part of these studies. In the present connection, however, it is of interest to compare this apparently normal miniature adult with its persistent tail, with the similar type commonly occurring among those cultures of *Ascidiella* and *Phallusia* already referred to that had been reared in hyper-alkaline water. Therefore, in general, hyper-alkalinity preventing accumulation of acid or carbon dioxide in the case of larvæ of the Ascidiidæ and viviparity in the case of larvæ of the Molgulidæ seem to produce the same larval modifications. In both cases there is delayed metamorphosis resulting in tadpoles the trunk region of which has grown in size and possesses ectodermal ampullæ, and also a tendency for complete metamorphosis of the trunk to occur without there being any absorption of the tail.

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* The tendency to metamorphose before liberation from the parent has been seen in *M. lanceplaini*, *M. complanata*, *M. cooperi*, *M. verrucifera*, and *M. citrina*, and in fact is not confined to species of *Molgula*, but is common to most viviparous ascidians (see Part I, p. 64).

TABLE III.—Table to show Influence of Viviparity on Tadpole Larvæ.

Species.	Diameter of Egg.	Condition of Larvæ at Liberation.	Length of Free-swimming Period.	
			hours.	
Molgula complanata	0·23	*b*	0–2	Viviparous
,, *cooperi*	0·18	*b*	0–?	
,, *verrucifera*	0·14	*b*	0–?	
,, *citrina* (W. Atlantic)	0·21	*b* and *c*	0–3	
,, *citrina* (E. Atlantic)	0·21	*b* and *c*	0–1	
Polycarpa comata	0·16	*a*	8–12	
,, *rustica*	0·18	*a* and *c*	12 or 0	
,, *pomaria*	0·15	*c*	0	
Styelopsis grossularia	0·48	*a*	0–24	
Tethyum pyriforme americanum	0·26	*a* and *c*	8–16 (or 0)	Oviparous
Molgula tubifera	0·11	*a*	6–12	
,, *manhattensis*	0·11	*a*	4–10	
,, *simplex*	0·11	*a*	6–18	
,, *oculata*	0·11	*a*	4–12	
Styela partita	0·15	*a*	3–8	
Ciona intestinalis	0·16	*a*	10–24	
Ascidia mentula	0·14	*a*	6–12	
Ascidiella aspersa	0·17	*a*	8–24	
Phallusia mammillata	0·15	*a*	8–24	

The figures given for the duration of the free-swimming period are for rough comparison only.

a represents tadpoles with perfect tails and with atrial and mouth invaginations just commencing. *b*, the same, but with invaginations and ectodermal ampullae well started. *c*, larvæ hatched and liberated after completion of tail absorption.

Therefore, among viviparous species of *Molgula* compared with oviparous species, there seems to be a tendency to shorten the free-swimming period and to develop the degree of differentiation of the permanent organs at the time of settling at the expense of the tadpole stage, a tendency that reaches complete expression in the case of the European forms of *Molgula citrina*.*†.

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* As already described on p. 293, over 80 per cent. of the larvæ of this form hatch from the egg-membrane after the tadpole stage has ceased to exist. A similar suppression of the tadpole stage occurs in *Polycarpa pomaria* and frequently in *Polycarpa rustica*. (See Part I, p. 57.)

† CASWELL GRAVE suggests (1926, p. 456) that while the structure of the tadpole larva of *Molgula citrina* is without apparent deficiencies, the extreme brevity of the free-swimming period denotes the approach in this form to its complete suppression, and is an early stage in a continuous degeneration process; it is by no means certain, however, that the short free-swimming period is not essential in at least a percentage of cases, and further there is no indication that progressive curtailment of the active phase leads eventually to anural development; all that can be said is that if the environment tolerates complete suppression of larval activity, then suppression of the larval structures associated with that activity may also occur, but not unconditionally. Further, brevity of the free-swimming period, as distinct from the absence of a tadpole stage altogether, is to be correlated with viviparity, whereas anural development is associated rather with oviparity. (See Table I.)

5 (*a*). Direct (Anural) and Indirect (Urodele) Development.

With regard to the main issue of this paper, namely, the suppression of the tadpole stage, certain generalisations may now be made.

1. In no case where a tail is present in the larva is an otolith absent; in no case where a tail is not formed is an otolith ever present.
2. Those tailed larvæ developing from eggs containing considerable yolk accumulation hatch by rupturing the egg-membrane. Those developing from small eggs hatch by digesting and dissolving the egg-membrane. In every case of anural development, whether there be yolk accumulation or not, the method of hatching is by rupture and never by digestion.
3. In both types of development, where it has been followed for sufficiently long periods, an aggregation of cells of relatively high refractive index, is seen in the region of the larval stomach, which cells, in the case of the urodele type, can readily be traced to be those responsible for the phagocytosis of the tail.
4. In both cases ectodermal ampullæ appear at about the same stage in development and eventually form a cluster on either side of the body.
5. In both urodele and anural development the early cleavage is the same, and those blastomeres responsible for the tail musculature in the former type are readily recognisable in the equivalent stage of development of the abbreviated type.

In endeavouring to account for these great differences in development there are two distinct problems to be considered. There is first the analysis of the failure to form a tail in terms of developmental mechanics and physiology, that is to say, *how* it is the tail is not formed; and second, the nature of the inducing factor or factors, *i.e.*, *why* it is the tail is not formed. In the first case one is dealing with the relation one to another of the constituent cells of the organism; in the second, the relation between the organism as a whole and its environment, present and past.

5 (*b*). Loss of Tail in Terms of Developmental Mechanics.

I. *Cleavage.*

Cleavage in ascidians is determinate; that is, from the first division the right and left sides of the future embryo are delimited, then the dorsal from the ventral half, and so on. Moreover, apart from such topographical determination, there is a corresponding separation of the various inclusions of the egg, both quantitatively and qualitatively. Therefore failure to develop a tail could be due to several things, atypical cleavage resulting in the absence, dislocation, or abnormality of those cells which should have been set aside for tail production, or the failure of those cells, in spite of their normal composition to cohere together to form a co-ordinate structure.

The only account of the early cleavage of the egg of an ascidian with anural development is that given by LACAZE-DUTHIERS in 1874. Admittedly he made his observations on the development of the small egg of *Molgula occulta* (his *Anurella roscovita*) at a time when ascidians were yet thought to be most closely related to the Mollusca and when the characteristic cleavage forms were unknown, but the fact that he stated that the first division was invariably unequal, and that cleavage resulted in the formation of translucent micromeres and yolky macromeres made it possible that he was correct and that in this peculiarity lay the explanation of the abbreviated or anural development. The observations of DAMAS on the development of *Molgula bleizi* commenced at too late a stage to be of any value in this respect. Therefore, the cleavage of the eggs of *Molgula occulta* was examined with very great care when this species was cultured at Roscoff during the summer of 1926. The specimens were obtained through following the directions as to locality given by LACAZE-DUTHIERS, and the cleavage of eggs was examined in cultures made on about six different occasions, and from living material. *Molgula oculata* was cultured at the same time to act as a control.

In the first place it can be stated with all assurance that unequal cleavage in the case of the first division is a rarity and equal division is as typical of the first two cleavages as of any other ascidian egg. In the second place, the cleavage of the egg, as far as was followed, differs in no way from that described by CONKLIN for *Styela* and other forms, and the micromeres and macromeres of LACAZE-DUTHIERS must have been the four small relatively yolkless posterior blastomeres* and the more anterior and yolkier blastomeres respectively, formed at the fourth cleavage. These may be seen in fig. 17, and the pair derived from the vegetative hemisphere are those responsible for the formation of tail muscle cells when the tadpole stage exists. There is, in fact, no difference at this stage between these two species of *Molgula*, although they represent the two types of development, also the developmental course so far is identical with that described by CONKLIN and CASTLE for other families and orders. So that as an explanation or cause of the abbreviation of development, the course of early cleavage may be eliminated. The cleavage of the eggs of *Molgula retortiformis*, *Molgula arenata*, and *Bostrichobranchus* (*Eugyra*) *pilularis* was followed in cultures made at St. Andrews and Woods Hole during the summer of 1927, and it was seen that as far as the 16-cell stage at least, it was perfectly typical and took the course just described.

Gastrulation was observed in the case of *Molgula occulta* and *Molgula retortiformis*, and while opacity of the eggs made it hard to follow, it seemed to be typical and similar to that of *Molgula oculata* and *Molgula citrina* that were used for comparison in the two cases respectively.

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* Corresponding to the cells $B^{5\cdot 2}$ $\mathrm{B}^{5\cdot 2}$ $b^{5\cdot 4}$ $\mathrm{b}^{5\cdot 4}$ of *Styela* as figured by CONKLIN.

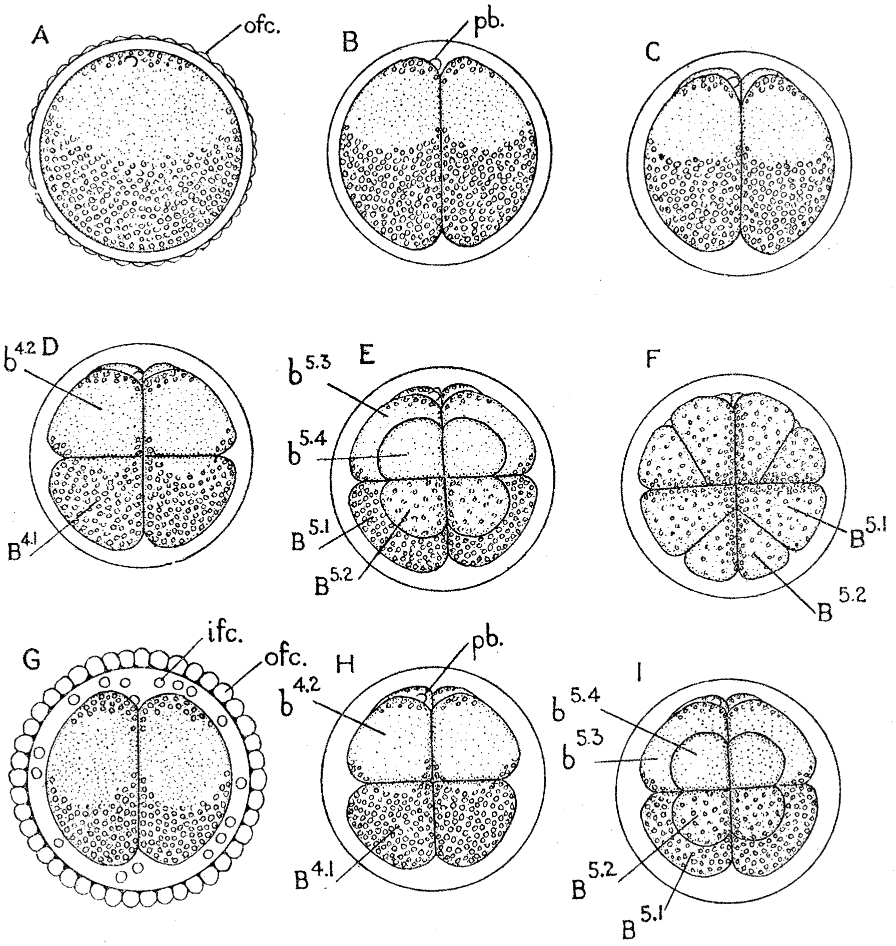

FIG. 17.—Cleavage of the egg of *Molgula occulta* (*roscovita*) and *Molgula oculata*. A, 1-cell; B, 2-cell; C, 4-cell; D, 8-cell; E and F, 16-cell stages of *M. occulta*; E, posterior; F, ventral aspects of 16-cell stage; G, 2-cell; H, 8-cell, and I, 16-cell stage of *M. oculata*. The blastomeres are labelled according to the scheme used by CONKLIN for *Styela*. *ifc*, inner follicle cells; *ofc*., outer follicle cells; *pb*, polar bodies.

II. *Tail Formation in Typical Ascidians and in Anural Molgulids.*

In order to understand in what ways during the later development of the embryo the cells concerned may fail to form a typical tail, it is necessary to examine the course followed under normal circumstances.

This was worked out in detail by CONKLIN for *Styela* and by VAN BENEDEN and JULIN for *Clavelina*, and the two accounts are in very close agreement, in spite of the wide systematic separation of these genera. Fig. 18 illustrates the essential points.

The development of the egg of *Styela* up to gastrulation has already been described in Part I, and all that need be said in this connection is that at the end of gastrulation a shallow cup is formed, at the anterior rim of which is an arc both of presumptive neutral cells and chordal cells, while at the posterior rim there is an arc of mesodermal cells. The wide blastopore is closed through the posterior growth of the anterior rim (or lip), and at the same time the chordal cells are enrolled by the neural cells to lie beneath the latter, and at this stage may be seen to form two rows of eight cells curved into an arc. This may be considered to be the starting point of the present discussion.

From this stage two changes occur together. The posterior rim (or ventral lip of the blastopore) grows dorsally and lifts the mesodermal cells to the level of the chordal cells, while the two arcs of chordal cells, 16 in all, slide in to form four columns of four cells each (five in the case of *Clavelina*), in line with the main axis of the embryo. At this stage there are yet but 16 chordal cells, the blastopore is on the point of closure, and the neural groove appearing. Growth in length now commences. The neutral folds first appear at the posterior end and fuse dorsally to form the neural canal, and for a very short while this is connected by the neurenteric canal with the archenteron. The connection, however, is rapidly obliterated by the posterior growth of the notochord.

The whole of the growth in length posterior from the blastopore, in fact, seems wholly to be dependent on the notochord, and it becomes desirable therefore to see how it is effected. As already described, at the moment of closure of the blastopore there exist 16 chordal cells, arranged in four rows of four and one cell deep (see fig. 18, H, J). From the figures of both CONKLIN and VAN BENEDEN and JULIN the final number of notochordal cells seems to be from 60 to 64, and they become eventually arranged into a single column. Therefore, during the elongation of the notochord and the formation of the tail, several changes occur. Two divisions of each notochord cell must occur to change the first number of 16 into about 64, and if the figures already referred to are examined it will be seen that this process is coincident with the sliding movements that result in the formation of a single and long column of cells. During the first division of the notochord cells, which is a horizontal cleavage, the upper and the lower four rows of four cells thus formed slide and interdigitate to form an upper and a lower two rows of eight cells each, *i.e.*, a column in the major axis of the embryo four cells in cross section (see fig. 18, K, and Part I, fig. 6, C and D).

The neural folds at this stage have fused to form the canal throughout the greater part of their length. As the second cleavage of chordal cells proceeds (plane unknown, probably vertical at right angles to the major axis), the cells of the ventral half of the column interdigitate with those of the upper half, so that the notochord viewed from the dorsal surface appears to consist of two rows of cells, one on either side of the middle line, while viewed from the side appears to be a single row. As the second cleavage is completed, the right and left row of cells interdigitate to form a long single column, the cells of which gradually each form a vacuole that grows and progressively increases the turgidity of the whole structure.

The only obvious difference between the development of *Styela* and *Clavelina* seems to be that in the latter division of the notochord cells is for the most part completed before the interdigitating process has progressed to any extent, while in *Styela* they

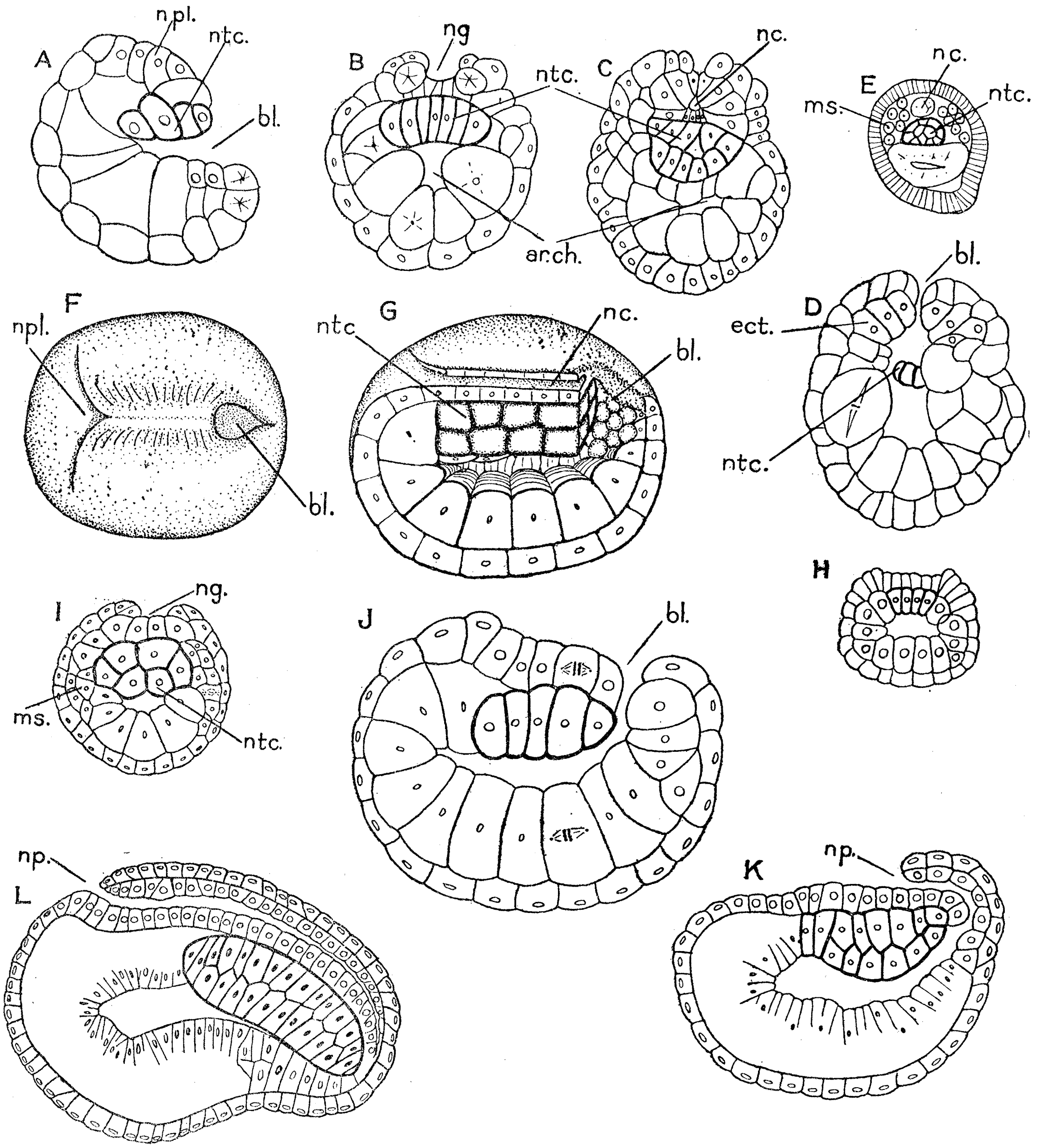

Fig. 18.—The development of *Molgula bleizi*, *Styela partita*, and *Clavelina rissoana* to show the notochord and neural tube. A, B, C, D, E, sections of embryos of *M. bleizi* (after Damas); F, G, diagrammatic reconstructions of embryos of *M. bleizi* (original); H, I, *Styela partita* (after Conklin); J, K, L. *Clavelina rissoana* (after Van Beneden); notochordal cells shown in thick outline in each case; A (G), J, K, L are all longitudinal sections; B, C, D, E, H, I, transverse, and F represents a dorsal surface view. For further description see text. *arch.*, archenteron; *bl.*, blastopore; *ect.*, ectoderm; *ms.*, mesoderm; *ng.*, neural groove; *nc.* neural canal; *np.*, neuropore; *npl.*, neural plate; *ntc.*, notochord cells.

are practically co-extensive. In both forms the neurenteric canal becomes obliterated through the backward pressure of the notochord cells.

The extension of the notochord posteriorly must depend on a relative increase in volume of the chordal cells, rather than any increase in their number. That is, mere multiplication of the primary 16 cells to form 64 will not produce any pressure on the surrounding tissues unless there happens to be at the same time an associated increase in aggregate volume. In the case of *Styela* this increase commences with the first of the two divisions, while in *Clavelina* it commences rather with the second. If fig. 18, I, J, K, L, are examined it will be seen that the chordal cells are continuous with and blocked anteriorly by large yolky endoderm cells, so that any pressure in that direction will be resisted. Moreover, for the greater part, the rows of chordal cells are packed tightly by the surrounding sheaths of mesodermal and ectodermal cells, so that the diameter of the notochord as a whole tends to remain constant. Therefore, the one point where there is relatively little resistance and where the thrust of the notochord resulting from its increase in volume will be most effective, is at the anterior lip of the blastopore. That is, the notochord may be considered to be a mass of cells enclosed within a cylinder closed at one end and with rigid walls, and therefore any increase in volume of the enclosed cells will be expressed as an extension towards the open end of the cylinder.

The starting condition, however, consisted of four rows of four large cells. Increase in volume of these would result in pressure and extension posteriorly, and finally in a single row of 16 cells. That is, with extensibility in one direction alone, sliding and interdigitating movements would be expected and explained, but as the ratio $\frac{\text{length}}{\text{width}}$ of each cell probably could not exceed a certain value, the column of 16 cells would be short and wide compared with that actually formed, either expanding or rupturing the surrounding tissue sheaths. But with each successive division of the chordal cells the length and width of the whole column would be doubled and halved respectively, the $\frac{\text{length}}{\text{width}}$ ratio for each cell remaining constant. Therefore the outgrowth and formation of a tail seems to be dependent primarily upon the swelling of individual notochord cells, while the length the tail may attain depends upon the number of cells present. In other words, the force is derived from increase in cell volume, while its effect is limited by cell number.

The tail thus formed, after the larva has undergone a period of free-swimming life of variable extent, becomes destroyed and stored by phagocytes in the trunk region. The phagocytosis commences at the tip of the tail and progresses in an orderly manner toward the trunk, changes first appearing in the muscle cells and later in notochord and ectodermal cells. There are therefore two very different ways in which the tail may fail to appear. There may be lack of swelling and division of the chordal cells, which alone would be sufficient explanation, or phagocytosis of the tail-forming cells may commence before posterior extension becomes appreciable, *i.e.*, premature phagocytosis.

The only account of the development made from the study of sections of a Molgulid with anural development is that given by DAMAS for *Molgula bleizi*. This species affords the material most readily obtained owing to its viviparity, and moreover the egg is considerably larger than those of any other, with the single exception of *Molgula retortiformis*.

Embryos and larvæ of *Molgula occulta* have been sectioned, though since they are relatively so much smaller than those of *Molgula bleizi*, the results merely confirm many of DAMAS' observations without extending them. It had been hoped to obtain material of *Molgula retortiformis* for this purpose, since the eggs and embryos exceed in size those of *Molgula bleizi* as much as the latter do those of *Molgula occulta* and most other forms. As the species in question, however, is oviparous, it is a matter of some difficulty, and after the first culture had been made successfully and the course of development in living embryos followed, further attempts were unsatisfactory and an examination of the development of this species from sectioned material will have to be left for a future occasion.

The description and figures of DAMAS, however, are excellent, although his interpretations are very much to be doubted (see fig. 18, A-G).

DAMAS' description commences properly with the gastrula already formed, and his Stage III is shown in fig. 18, A. His figures of earlier stages show a somewhat greater tendency toward epibolic gastrulation than might be expected, but if the embryos of *Clavelina*, *Styela*, *Ciona* and *Molgula bleizi* shown here are compared and Part I, fig. 4, it will be seen that that shown by fig. 18, A, is perfectly typical. There is a mid-dorsal neural plate, and archenteron of reduced size owing to the presence of voluminous endoderm cells, and notochord cells enrolled to lie in the roof of the archenteron and consisting of several columns three cells long extending back to the anterior lip of the blastopore. The blastopore at this stage is wide open. To attain Stage IV, shown by fig. 18, B, it seems that the ventral or posterior lip of the blastopore grows dorsally as in *Styela* and so lifts up the posterior ectoderm and mesoderm cells to the level of the neural plate. At this stage the neural groove and folds are well formed, while beneath them the notochordal cells extend backwards as a plate one cell deep and consisting of at least four rows. That the figures show up to six rows is conceivably due to obliqueness of the section plane, previous orientation of these embryos being almost an impossibility. The neural groove, together with the rows of chordal cells, ends posteriorly at the anterior lip of the blastopore. The latter is still open, though the neural folds extend along its borders and meet together behind in the median plane. At a slightly later stage (fig. 18, C) it will be seen that the single plate of notochord cells have divided into an upper and a lower, as in *Clavelina* and *Styela*, and that the neural folds above this region have fused mid-dorsally to enclose the neural canal. It is also seen, however, that the notochord cells have not extended posteriorly, and that behind them the neural canal is in communication with the archenteron through the neurenteric canal. Further, above the latter the neural folds do not meet together dorsally (fig. 18, D). That is, the blastopore is still open, while the neural folds extend laterally along its borders. DAMAS considers that in the stages just described (fig. 18, C–G), the notochord cells also extend posteriorly along the sides of the blastopore in the same way as the neural folds.* The cells of the notochord proper he describes as containing as much yolk as the endodermal cells, but those he considers to form a posterior forked extension of the notochord he himself describes as being considerably clearer, so that it is more than possible that the cells in question are to be associated with the mesoderm and not the notochord. At the next stage described, the notochord consists of a rounded mass situated exactly in the median plane (fig. 18, E), while the nervous system consists of an anterior vesicle continuous in front with the mouth invagination, and behind as a tubular prechordal part and a part above the notochord. The cloacal invagination commences as a single median process between the last and middle parts of the nervous system, over which it divides. There is anterior mesoderm (mesenchyme) lateral and dorsal to the sides of the digestive tract, and posterior mesoderm of larger and fewer cells, at the sides of the notochord (muscle plates). Further, at this stage there is present a very delicate test. The large " muscle " cells possess vacuoles, granules and compact nuclei, *i.e.*, signs of degeneration.

The caudal elements are later found at the side of the gut, and the yolky notochord and clearer muscle cells are long recognisable. The above account is entirely of *Molgula bleizi*. Sectioned material of *Molgula occulta* has been found to be of little value for the earlier stages, but all the details of this last stage of DAMAS were confirmed, including the disaggregations of the caudal elements and their final storage alongside the gut.

DAMAS sums up his account of caudal degeneration as follows (page 630) :—

1. There exists at a given moment a notochordal rod, " muscle " plates, and a caudal part of the nerve tube. They degenerate finally.
2. This caudal region never elongates. Its precocious disaggregation is characteristic of the anural embryo.
3. In anural embryos, as in urodele, the disaggregation of muscle plates (active organ) precedes that of the notochord (passive organ).

DAMAS therefore regards the development of the anural embryo as follows :—Development is for the greater part typical, but there is precocious formation of a medullary tube, preceding the closure of the blastopore, there is no caudal elongation but precocious disaggregation of the caudal elements, which commences at the same time as test secretion, and there are no larval sense organs. He also considers both

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* There is a certain resemblance between this stage and the *spina bifida* of the trout and frog embryos of HERTWIG, but the similarity seems to be merely superficial, for in those forms the phenomenon seems to be rather a modified and incomplete form of twinning than anything analogous to the condition in anural Molgulid embryos.

the notochord and neural folds to divide posteriorly and line the sides of the blastopore. There is, however, in his account no explanation why the tail is not formed, other than the obscure statement that because the nervous system is formed before the closure of the blastopore, the embryo retains its rounded form throughout development.

Damas, in talking of precocity of formation of the neural tube and disaggregation of the caudal elements, has apparently assumed that it is the time of closure of the blastopore and rate of development of the notochord that are normal. This would also imply that the secretion of the test is precocious as well, but since Damas did not determine the absolute times for the various stages of development, which is practically impossible in this viviparous form, it is equally justifiable to assume that the formation of the neural tube and degeneration of caudal elements occur normally and that the closure of the blastopore is delayed. This theory is not only supported by experimental data given below, but, moreover, affords an explanation of the failure of tail formation, which Damas' theory does not.

On this assumption development may be described in a way very different from that just given for Damas.

Development proceeds in a typical manner until there is formed a gastrula, the opening of which is more or less closed by the posterior growth of the anterior lip of the blastopore. At this stage (Stage III of Damas), notochordal cells form at least four rows, each of three cells, in the archenteron roof, and underlying the neural plate, and with this last extend back to the anterior lip of the blastopore. The ventral lip grows dorsally and lifts the mesodermal cells up to the level of the notochord. That is, a stage is reached practically identical with that which in *Styela* and *Clavelina* immediately precedes the outgrowth of the tail. The notochord cell plate divides into an upper and a lower layer, corresponding to the first chordal cell division in *Clavelina* and *Styela*, while neural folds appear and meet dorsally and posteriorly to enclose the neural canal, leaving a neurenteric canal to connect it with the archenteron. *But*, while the notochord cells have undergone the first cleavage, they have not, individually, or in aggregate, increased in volume, so that there has been a negligible tendency to extend posteriorly and obliterate the neurenteric canal, complete the closure of the blastopore, and allow the lateral ectoderm forming the neural folds in this region to meet and fuse dorsally one with the other. That is, since there is no or little increase in total volume of the notochord, there is no tendency for the cells to slide and interdigitate and produce the backwardly growing tail. Moreover, since they, and also the neural plate cells immediately above them, form a broad edge at least four cells across at the anterior lip of the blastopore, upward extension of the posterior and lateral lips will necessarily have great difficulty in closing the blastopore except in the posterior region farthest away from the anterior broad edge, and therefore the prolonged existence of the blastopore as an opening broad in front and converging behind is to be expected. It is also seen that the neural folds posterior to the notochord and lining the lateral limits of the blastopore extend inwards and ventrally to below the level of the notochord.

Had the notochord existed in this region, this downward growth would have been arrested at a certain stage and the folding process transformed into a tilt toward the middle line and fusion of the two folds would result.

Therefore, the whole of the dissimilarity existing between anural and urodele development can be explained if the notochordal cells fail to swell, and consequently fail to commence the sliding and interdigitating process, and this is believed to be the true explanation.

Further, for DAMAS' interpretation to be correct, disaggregation of the caudal elements and the appearance of the test would have to be precocious, and this has been disproved experimentally.

TABLE IV.—Comparison of Times of Appearance for Various Stages of Anural and Urodele Molgulid Embryos.

	Stages of Development.					
	Blastopore Closed and Tail Bud Visible.	Hatching.	Test Secretion.	Caudal Degeneration.	Outgrowth of Ampullæ.	Species.
		hours.	hours.	hours.	hours.	
19° C.	4 hours	8–11	18–24	18–24	18–24	*M. manhattensis.*
	?	15–20	12–18	?	25–	*M. arenata.*
18° C.	5–6 hours ...	10–12	18–25	18–25	18–25	*M. oculata.*
	?	12	10–12	?	14–15	*M. occulta.*

(Urodele species, *M. manhattensis*, *M. oculata*; anural, *M. arenata*, *M. occulta*).

III. *Comparison of Time Factor in Development of Urodele and Anural Molgulids.*

The eggs of *Molgula manhattensis*, *M. arenata*, *M. oculata* and *M. occulta* are all of approximately the same diameter, all four species are oviparous and, as far as could be judged, the rate of segmentation and development are the same in all. Therefore the various stages of development reached at any given time, environmental conditions being equal, are strictly comparable.

Molgula oculata and *Molgula occulta*, obtained from one and the same dredging at Roscoff, were cultured under identical conditions with regard to water and temperature, and at the same time. The stages in development noted were :—The time of hatching, of appearance of the test, the outgrowth of ectodermal ampullæ, and the phagocytosis of caudal elements. At a water temperature of 18–19° C., hatching of the tadpole of *Molgula oculata* occurred from 10 to 12 hours from fertilisation, the onset of phagocytosis of the tail and first appearance of ampullæ occurred after 18 hours from fertilisation and was completed in most at 24 hours. In the development of *Molgula occulta* under the same conditions, hatching occurred after about 12 hours from fertilisation, *i.e.*, slightly though definitely later than the tadpole of *M. oculata*. The ampullæ became recognisable as such from 14–15 hours from fertilisation, while the highly refractive "caudal" cell mass became visible after 20 hours. The later appearance of these phagocytised cells may be due to the fact that in anural embryos they do not become by any means so highly refractive as the corresponding cells in urodele embryos, probably as the result of the non-vacuolisation of the chordal cells. Therefore, in comparing the development of these two species it is seen that there is but very little difference between the time of attainment of the various stages of development in the two forms. Hatching occurs a little later in the anural form, but as this is effected by the swelling of the larval test, and as the swelling also marks the onset of degeneration of the "caudal" elements and appearance of ampullæ, these last two phenomena are somewhat earlier than in the urodele form. But they are at least as late in development as the fully formed and hatched tadpole of *Molgula oculata.* That is, there is no question of precocity of development of caudal degeneration or test secretion in the anural form, other than that accounted for by the absence of a larval free-swimming period and as, according to DAMAS, precocity of neural tube formation is to be correlated with precocity of the above phenomena, the elimination of the one precludes the other. A similar comparison was made between the development of *Molgula arenata* and that of *Molgula manhattensis*, and it is seen that the rate of development and time of attainment of the more easily recognisable stages are almost identical in the two types.

If the accounts of the development and metamorphosis of various genera given in Part I are referred to, and also the experimental control of metamorphosis, it will be seen that there is normally a very definite correlation between several phenomena. This is shown very clearly in the development of *Styelopsis grossularia.* The tadpole swims actively for a while and then settles on some solid substratum. At this moment five changes are initiated. Phagocytosis commences at the tip of the tail, there is a sudden and marked secretion of test material, there is a sudden migration of ectodermal and mesodermal cells into the test substance, and there is a development and prolongation of the 25 or so anterior ampullæ. There is also a marked change in body shape and colour. If the experiments referred to are examined, it will be seen that while phagocytosis of the tail can be practically inhibited, there is relatively little inhibition of the development of test and ampullæ, and that this phenomenon occurs normally among urodele viviparous Molgulids. But while there is a certain degree of separation in development between the tail and trunk when the process as a whole is retarded, no acceleration can be induced that does not affect all the metamorphic changes just mentioned. That is, any "precocity" must affect equally the absorption of the tail, the onset of test secretion and the outgrowth of ampullæ. Therefore, since in the development of the anural species *Molgula arenata* and *Molgula occulta* there is no significant precocity in the appearance of these last two, there is no ground for supposing that there is precocity in "caudal" degeneration, and since DAMAS associates all these with his precocious development of the neural tube, this precocity is also eliminated.

Table V indicates the relative times at which the blastopore closes and a complete tadpole is formed in some other ascidians.

TABLE V.—Showing Relative Times of Development for Various Stages.

Stage Reached.	Species.					(*Corella willmeriana*, CHILD, '27).
	Phallusia mammillata.			*Ascidiella aspersa.*		
	hrs.	hrs.	hrs.	hrs.	hrs.	
32-cell	$4\frac{1}{2}$	$3\frac{3}{4}$	5	$2\frac{3}{4}$	—	4 hours (64-cell).
Early gastrula	—	5	$7\frac{1}{2}$	—	6	—
Blastopore on point of closing	8	$6\frac{1}{4}$	9	5	$7\frac{1}{2}$	12 hours (tail elongate).
Eye-pigment appearing	—	—	19	—	—	—
Hatching	26	19	32	17	24	25 hours.
Temperature	$16\frac{1}{2}$	18	$15\frac{1}{2}$	$18\frac{1}{2}$	$17\frac{1}{2}$	18–20° C.

5 (*c*). EXPERIMENTAL.

The main conclusions reached in the last sections are that the failure to develop a tail in anural embryos is due to the failure of the notochord cells, which come to lie in the correct starting position, to imbibe water, swell in consequence and by sliding and interdigitating, thrust their way posteriorly and so form a tail; and in the case of *Molgula bleizi*, at least, that failure causes a marked delay in the closure of the blastopore. Therefore, the primary problem, from the point of view of developmental mechanics, is to what is due the progressive imbibition of water and formation of vacuoles by normal notochord cells? Secondary problems concern the dependence or otherwise on the normal development of the notochord of the formation of larval sense organs and an enzyme for the purpose of hatching. These problems necessarily involve the whole question of the mechanics of chordate development.

The following observations, however, may help to clarify matters. While investigating the development of *Phallusia*, the main account of which is contained in Part I, it was noted that tadpoles hatching out in normal sea-water (p_{H} 8·1) almost invariably retained much of the tail curvature originally produced by the confinement of the embryo within the egg-membrane, and were feeble swimmers in consequence. Under identical conditions this was not found to occur among tadpoles of *Ascidiella aspersa*, *Ascidiella scabra*, *Ascidia mentula*, *Ascidia conchilega*, or *Ciona intestinalis*. If, however, the water was made hyper-alkaline with NaOH (to p_{H} 9·0) this curvature disappeared at the moment of hatching, *i.e.*, the turgidity of the notochord was greater, and this must have been due to greater imbibition. This is supported by an experiment of a converse nature, in which *Phallusia* eggs in the two-cell stage were allowed to develop in sea-water of p_H 5·0, produced by the addition of HCl and subsequent aeration to bring the CO_2 tension again into equilibrium with the atmosphere, for about one hour and then returned to normal water again. That is, the developing eggs were subjected to relatively high acidity during just that period in which the primary 8 or 16 chordal cells became delimited, and the result of this treatment was that tadpoles were formed apparently normal except that their tails were but half the normal full length, and were relatively wide. In fact, they had exactly the appearance of normal tails that were half grown, and in which there were still two parallel columns of chordal cells. The sense organs in these larvæ were apparently normal and fully differentiated. Therefore, not only does an increase in alkalinity produce an increase in the degree of imbibition but a decrease causes a corresponding reduction.

Child has made certain observations and experiments recently (1927) on the development of *Corella willmeriana* of very great interest in connection with anural development. This paper has already in part been discussed (Berrill, 1929) in connection with the onset of metamorphosis. *Corella willmeriana* is viviparous and the embryos are liberated from the parent as fully formed tadpoles. However, if the eggs are extracted from the parent and caused to develop in ordinary sea-water, all degrees of differential inhibition of development occur, and this was found to be due to the alteration of the critical tension of CO_2 characteristic of the water of the parental atrial chamber and which is necessary for normal development. Variation from this concentration in either direction produces marked inhibition of tail development, but except in the more extreme cases hatching and metamorphosis may occur.

The abnormal forms may be classed into three groups, though in reality they grade one into the other. There are those that are normal except that the tail is kinky or twisted, those in which the tail remains short and stumpy, and those in which the tail is never more than a rounded mass.

The first two types of inhibited forms seem to be due, as indicated by Child's figures and text, not so much to the failure in varying degree of the notochord to elongate fully, as to the failure of the ectoderm to extend posteriorly under its influence. That is, the tail will only be formed properly if the ectoderm at the tip is stimulated to grow by the notochord and give way before it, *i.e.*, in effect, become stretched by it. If the "stretching" should happen to be uneven, the notochord will become bent or kinked. If greatly reduced, the notochord will of necessity become coiled and bent back on itself within a confined region, and give rise to the more inhibited forms described by Child where the tail remains more or less rounded but which can be seen to contain a variously bent and coiled notochord. Similarly, if the ectoderm fails to extend posteriorly at all, the notochordal cells will fail to form a coherent structure and form merely a posterior swollen mass, but even in this case the swollen part produces a certain elongation of the embryo as a whole. That is, it is implied that the increase in volume, and maybe number, of the notochord cells is normal, but that the failure to develop a tail is due primarily to the resistance of the posterior ectoderm; in the simile used before, the extensibility of the cylinder at one end is diminished.

The differential inhibition just described for *Corella* may, however, be seen in other forms. In *Corella* normal development apparently occurs only within the atrial chamber of the parent. In such forms as *Ascidiella* and *Phallusia* where development occurs in the sea-water outside, such abnormal development is usually to be found in a certain percentage of every culture (see fig. 19), the percentage as a rule being very small, but varying with the temperature and condition of the eggs (see Part I, page 73).

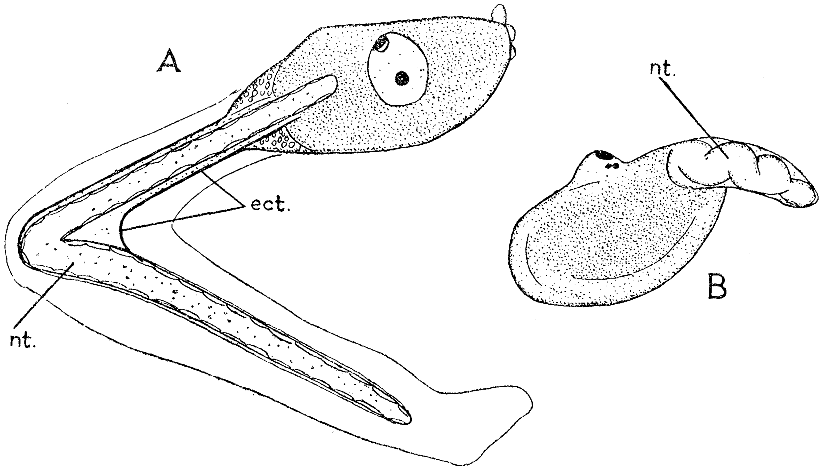

Fig. 19.—Abnormal development of tadpoles in *Ascidiella aspersa*. A, normal tadpole except uneven growth of tail ectoderm; B, more modified form with coiled notochord within short tail ectoderm, and abnormal sense organs; both types can metamorphose and continue development. *ect.*, ectoderm; *nt.*, notochord.

If, however, anural development in the Molgulidæ was due not to the failure of the notochordal cells to increase in volume but to the effective resistance of the ectoderm to their pressure, a certain bulging, posteriorly at least, would be visible, and the typical spherical shape of the embryo would be lost. Moreover, in the case of *Molgula bleizi*, the only one so far examined in this connection, there would be no tardiness in the closure of the blastopore.

While investigating the origin of variability in the eggs and larvæ of *Phallusia*, etc., described in Part I, last section, there was observed a development in *Phallusia* remarkably similar to the anural development of *Molgula*. The eggs were extracted from the oviduct and divided among six finger bowls, according to the position along the oviduct where they were taken. The same sperm suspension was used for fertilisation of all. On one occasion the culture derived from eggs from the middle part of the oviduct, on a second occasion eggs derived from the distal part of the oviduct, gave rise to anural embryos. About 20 per cent. were normal tadpoles, the rest for the most part tailless, while in the neighbouring cultures, the ratio of tailed to tailless was reversed. No tail elongation could be seen, nor sense organs, but at the time when the normal tadpoles were metamorphosing, *i.e.*, the tail being phagocytised and ampullæ appearing, a certain region became highly refractive and a single translucent swelling appeared at the opposite end of the body. That is, changes possibly to be interpreted as phagocytosis of caudal elements and the outgrowth of ectodermal ampullæ. The ampullæ in the case of *Phallusia* are in any case too weak to rupture the egg-membrane should the digestion method fail (see Part I, p. 57), and therefore in this abnormal development enforced confinement within the membrane may have been the cause of the arrest of development at this stage. The main reason for supposing this striking abnormality in *Phallusia* development to be due to lack of pressure from the notochord cells rather than the non-extensibility of the ectoderm is that in all known cases where the latter occurs, *i.e.*, *Corella*, *Phallusia* and *Ascidiella*, it has been found in all degrees, *i.e.*, varying from almost perfect larvæ to those with little sign of a tail. In this other *Phallusia* type either a fairly typical tadpole was formed or else completely inhibited, while, moreover, no sign of notochord cells could be seen, either as individual cells or as a somewhat bulging mass.

If this interpretation is correct, and there is a tendency in *Phallusia*, at least, to produce anural embryos, since it seems to involve an inactivity of the hatching enzyme, the fact that the outgrowth of ectodermal ampullæ and test in this form is invariably ineffective as an alternative hatching mechanism may be sufficient to keep it a rarity.

5 (*d*). Occurrence of Anural Development within the Tunicata.

The tailed larval stage has been eliminated completely from the development of *Salpa* and *Pyrosoma*, and modified in the case of *Doliolum*, but as far as the Ascidiacæ are concerned, no instance of its suppression is known outside the Molgulidæ. The distribution among ascidians of tailed and tailless larvæ is shown in Table VI, and it will be seen that the whole ten species with anural development out of the total of 135 is confined to the Molgulidæ. The number of species of known development for each family is probably sufficiently large to be representative, with the exception of the Pyuridæ. Naturally, a typically viviparous order as the Aplousobranchia is much better known in this respect than the other two, where most of the information has been derived from artificial culture methods.

The confinement of the anural development to the Molgulidæ is sufficiently striking, however, for it to be possible that it is some characteristic of this family that is responsible for its presence, though it is necessary first to determine whether it has occurred on one or several occasions within this family.

5 (*e*). Occurrence of Anural Development within the Molgulidæ.

There have been several attempts to discover a sound basis for the classification of the Molgulidæ, none of which can be said to be completely satisfactory.

TABLE VI.—Showing Occurrence of Anural Development within Class Ascidiacea.

(Species with Anural Development underlined; all others produce tadpole larvæ.)

STOLIDOBRANCHIATA Lahille.

MOLGULIDÆ.

Molgula citrina	v	*Molgula occulta*	*
,, *complanata*	v	,, *solenota*	38
,, *cooperi*	v	,, *bleizi*	v
,, *heydmanni*	41v	,, *robusta*	40
,, *oculata*	*	,, *arenata*	*
,, *simplex*	*	,, *kolaensis*	v
,, *tubifera*	*	,, *retortiformis*	*
,, *manhattensis*	*	,, *macrosiphonica*	38v
,, *socialis*	23	*Eugyra pilularis*	*
,, *platei*	v	,, *arenosa*	*
,, *verrucifera*	v		

PYURIDÆ.

Tethyum pyriforme americanum	v	*Boltenia echinata*	v
		,, *hirsuta*	v

STYELIDÆ.

Styela partita	*	*Styelopsis grossularia*	v
Polycarpa comata (*fibrosa*)	v	,, ,, var. *carnea*	v
,, *rustica*	v	*Stolonica socialis*	v
,, *pomaria*	v	*Distomus variolosus*	v
,, *tenera* (*gracilis*)	v	*Polyzoa falclandica*	24v
Polyandrocarpa tincta	22v	*Symplegme viride*	v
		Goodsiria placenta	26v

BOTRYLLIDÆ.

Botryllus schlosseri	v	*Botrylloides leachi*	v
,, ,, var. *polycyclus*	v	...	

PHLEBOBRANCHIATA.

RHODOSOMATIDÆ.

Corella parallelogramma	*	*Corella willmeriana*	8v

PHALLUSIIDÆ.

Ascidia mentula	*	*Phallusia mammillata*	*
,, *virginea*	*	*Ascidiella aspersa*	*
,, *conchilega*	*	,, *scabra*	*
,, *prunum*	*	*Perophora listeri*	v
,, *obliqua*	*	,, *viridis*	v
,, *atra* (*Ph. nigra*)	22	,, *annectens*	v
Ecteinascidia turbinata	51v		

CIONIDÆ.

Ciona intestinalis	*	*Diazona violacea*	*
		Tylobranchion speciosum	v

TABLE VI (continued).

APLOUSOBRANCHIATA.

POLYCITORIDÆ. v.

Clavelina lepadiformis	—	*Distaplia clavata*	—
,, *rissoana*	3	,, *magnilarva*	6
Chondrostachys oblonga	56	,, *rosea*	—
,, *enormis*	24	,, *bermudensis*	56
,, sp.	7	,, *valii*	55
Podoclavella molluccensis	55	,, *lubrica*	16
Pycnoclavella aurilucens	—	*Cystodites durus*	39
Archiascidia neapolitana	31	,, *draschii*	28
Archidistoma aggregata	—	,, *aucklandicus*	44
Colella (Sycozoa) claviformis	29	*Polycitor (Eudistoma) clarus*	56
,, *gaimardi*	28	,, *crystallinum*	16
,, *pulchra*	28	,, *mucosum*	16
,, *thomsoni*	28		
,, *pedunculata*	7		
,, *incerta*	7		
,, *cerebriformis*	7		

SYNOICIDÆ. v.

Polyclinum globosum	29	*Amaroucium obesum*	26
,, *complanatum*	29	,, *glabrum*	56
,, *fungosum*	28	,, *galeritum*	28
Sidnym (Parascidia) elegans	39	,, *roseum*	6
,, ,, *turbinatum*	—	,, *colelloides*	28
Synoicum pulmonaria	6	,, *pallidulum*	28
Morchellium argus	—	,, *nordmanni*	—
Euherdmania claviformis	48	,, *pellucidum*	20
Atopogaster gigantea	28	,, *constellatum*	20
,, *aurantia*	28	,, (*Psammaplidium*) *exiguum*	29
,, *informis*	28	,, ,, *solidum*	29
Polyclinoides diaphanus	16	,, ,, *lobatum*	29
Placentela crystallina	—	,, ,, *fragile*	29
Aplidium pallidum	—	,, ,, *incrustans*	29
,, *exile*	56		
,, *fallax*	28		
,, *despectum*	28		

DIDEMNIDÆ v.

Didemnum albidum	—	*Sarcodidemnoides misakiense*	46
,, *auranticum*	28	*Polysyncraton lacazii*	39
,, *quincunciale*	41	,, *paradoxum*	41
,, *psammatodes*	41	,, *amethysteum*	53
,, *braueri*	41	*Leptoclinum perforatum*	39
,, *karlae*	41	,, *densum*	44
,, *velans*	41	*Diplosoma listerianum*	39
,, *conglomerans*	41	,, ,, var. *gelatinosum*	—
,, *sycon*	41	*Coelocormus huxleyi*	28
Trididemnum cereum	—		

Viviparous species or families ... v. Oviparous species reared by artificial fertilisation ... *.

Reference number given only where no personal observations.

Lacaze-Duthiers, in 1877, having discovered several species of Molgulids with anural and with urodele development, made the type of development the basis for classification and grouped all species with anural larvæ under the genus *Anurella*. In so doing, however, he ignored completely adult organisation.

Of the classification of the family Molgulidæ based upon adult structure there are three of outstanding importance, all of fairly recent date.

Huntsman (1922) bases his classification mainly upon the organisation of the gonads, and splits the family on this ground into three sub-families and each of these into sub-groups. It is only when these are again sub-divided into genera that other structures such as those of the branchial sac are considered. The other feature of this scheme is that nineteen genera are maintained instead of six or seven as in the others, and mostly as the result of the breaking up of the original genus *Molgula*.

This classification, greatly abbreviated and including only those species the development of which is known, is shown below (Table VII), and it is seen that the species with anural larvæ are widely separated one from another. Therefore, if the structure and arrangement of the gonads be a satisfactory basis for relating the various species, then anural development must have arisen on at least four separate occasions, and conceivably more.

Hartmeyer (1923), however, instead of promoting species to form new generic types, included frequently many previously recognised species under one specific name, and in many cases quite unjustifiably (see Berrill, 1928). His classification is based primarily upon the structure of the branchial sac, and but secondarily upon that of the gonads, and here again it is seen from Table VII that the species with anural development are scattered. Moreover, in two outstanding cases, namely, his *Molgula manhattensis* and *Molgula oculata*, it will be seen that both urodele and anural development are to be found among the forms included under each of these two species. Therefore, if the structure of the branchial sac be an indication of interspecific relationship, anural development must have arisen at least four or five times.

The most recent classification is that of Ärnbäck (1928), and here, where the sub-division of the family is based equally upon the structure and arrangement of both the branchial sac and of the gonads, the species with anural development are again widely separated. Therefore, if the adult structure is used at all to group the more closely related species together (and apart from the character of the branchial sac and gonads there are left to be used only the less satisfactory character of the dorsal tubercle, etc.), the conclusion is unavoidable that anural or abbreviated development in the Molgulidæ is polyphyletic and has arisen on at least four different occasions. The alternative to this conclusion is that it is monophyletic and therefore similarity in adult organisation, of any type, is no indication whatever of phyletic relationship; that is, within the family Molgulidæ there has not only been extensive parallel and convergent evolution of the branchial structure, as Huntsman claims and which he uses as his justification for ignoring that structure in favour of that of the gonads, but also in the

TABLE VII.—Classification of the family Molgulidæ.

(Species with anural development underlined.)

1. According to HUNTSMAN, 1922 (classification based entirely upon structure of gonads).

Sub-family Eugyrinæ	*Eugyra pilularis.*
	,, *arenosa.*
,, Cystingiinæ, Group 1	*Anurella bleizi.*
	Euritteria cooperi.
	,, *arenata.*
	Molgula oculata.
	,, *occulta* (*roscovita*).
	,, *solenota.*
,, ,, Group 2	,, *citrina.*
	Gymnocystis tubifera (*ampulloides*).
	,, *manhattensis.*
	,, *simplex.*
	,, *robusta.*
,, ,, Group 3	*Cystingia retortiformis.*
	Lithonephria complanata.
	,, *canadensis.*
	,, *morgatæ.*
,, Caesirinæ	*Syphonotethys verrucifera.*

2. According to HARTMEYER 1923 (classification based upon branchial sac).

Branchial sac without folds	*Eugyra pilularis.*
	,, *arenosa.*
,, with 6 folds	*Molgula arenata.*
	,, *robusta.*
	,, *manhattensis* (including *simplex*, *tubifera* and *macrosiphonica*).
,, with 7 folds	*Molgula complanata.*
	,, *citrina.*
	,, *oculata* (including *occulta*, *bleizi* and *solenota*).

3. According to ÅRNBÄCK-CHRISTIE-LINDE, 1928 (classification based upon branchial sac and gonads).

Stigmata few but of great length, branchial sac without folds	*Eugyra pilularis.*
	,, *arenosa.*
Stigmata many, branchial sac with folds—	
Testis and ovary united to form hermaphrodite gland—	
6 folds	*Molgula tubifera.*
	,, *manhattensis.*
	,, *robusta.*
	,, *arenata.*
7 folds	*Molgula oculata.*
	,, *occulta.*
	,, *citrina.*
	,, *bleizi.*
	,, *kolaensis.*
	,, *complanata.*
Testis more or less separated from ovary	*Molgula retortiformis.*
	,, *simplex.*

gonads themselves. That convergent or parallel evolution has probably occurred is admitted, but the extent which is implied if anural development is assumed to be monophyletic, until much more positive evidence is produced, is too enormous to be considered.

It is therefore concluded that the less unreasonable of these alternatives is that anural development has been evolved on several, if not many, occasions within the family Molgulidæ, in spite of the fact that among the ascidians it seems to be confined to that family.

5 (*f*). Dependence of Development of Sense Organs upon Development of Tail.

It has been seen that in the development of *Molgula* there is a correlation between the formation of a larval sense organ, otolith alone in this case, and the presence of a tail. In no case is the one known to be present without the other.* This can mean one of two things. Either the formation of an otolith is directly dependent upon the development of a tail, *i.e.*, the tail or some region or activity thereof acts as a "organiser," or else in every case where the tailed larva has been suppressed the otolith has disappeared through disuse.

The absence of the otolith in all anural Molgulid larvæ in spite of the polyphyletic origin of the latter and its presence in every other case, whether the tadpole possess a prolonged free-swimming period or not, suggests that the correlation is ontogenetic. This is supported by the fact that such sense organs are present in all ascidian larvæ that bear tails, and are absent, in addition to the anural Molgulids, in all ascidiozoids produced by budding, whether from the adult as in *Stolonica socialis* or in the tailed tadpole as in *Diplosoma*. In this last the zooid connected directly with the tail and controlling its movements possesses both an eye and an otolith; the zooid connected with the tail only indirectly by way of the visceral mass of the other possesses no sense organs. Finally, in *Salpa* and *Pyrosoma*, both anural forms, no larval sense organs

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* Garstang and Garstang (1928, p. 38) visualise the attainment of anural development in the following series :—

"1. Tadpole with large thin-walled expansion of cerebral vesicle enclosing large otolith; the right wall undergoes disintegration with the loss of the otolith at metamorphosis, *e.g.*, *M. citrina*.

2. As above, but otolith smaller. Cerebral wall remains intact at metamorphosis, and retains otolith in the wall of neural gland, *e.g.*, *M. tubifera* (*ampulloides*).

3. Tailless embryos, with cerebral cavity but slightly enlarged, and its wall remains intact. No otolith is developed, *e.g.*, *M. bleizi*.

The short-tailed larva of *M. morgatæ* (*Lithonephria*) is expected to fall into the series between 2 and 3, but details are wanting as to the metamorphosis."

There is no degeneration implied in the relative reduced size of the otolith of *M. tubifera*, merely that the egg, whole larva and its constituent cells are all about half the size of those of *M. citrina*. Also *M. citrina*, apparently in common with all Molgulids possessing such, retains the otolith in the wall of the neural gland. There is, moreover, no need to postulate an intermediate form between urodele and anural larvæ, let alone one that has a short-tailed and functional larva as has *M. morgatæ*.

are ever formed, the eye of *Salpa* having been shown by METCALF to be a new development.

According to CONKLIN, DRIESCH, CHABRY and CHILD, in partial or teratological embryos of various ascidians, sensory pigment may appear in spite of there being no tail development, though it seems invariably to be abnormal and usually diffuse; but while DRIESCH found that the otolith, as distinct from eye-pigment, is usually suppressed, CONKLIN working with *Styela* found it to be present, though by no means normal. It seems possible therefore that the disappearance of the otolith in anural Molgulids is the direct result of the failure of the tail to develop in each ontogeny, but this is a question that can be decided only by direct experiment with the embryos of other Molgulids.

5 (*g*). ENVIRONMENT.

The confinement of anural development to the family Molgulidæ among ascidians, and its polyphyletic origin within this group, makes it probable that it is some peculiar characteristic of this family which is responsible for its existence. HARTMEYER'S suggestion that it is to be correlated with viviparity is invalid for several reasons, for viviparity in *Molgula* is to be correlated rather with the presence of urodele development, and, moreover, anural development is unknown among the one order that is universally viviparous. There remains, however, the question of the distribution of the numerous species, or in other words, their relation with the environment. In one sense distribution may be eliminated, in that the genus *Molgula* possesses species in both north and south hemispheres, in polar and tropical waters, in shallow shore regions and deep-sea basins and, as may be seen from Table I, there is no connection between such distribution and the occurrence of anural development.

There is left distribution of another kind, namely, the type of habitat, rather than its geographical and bathymetrical range. In this connection there appears a fact of possibly great significance, that whereas all compound ascidians and the Ascidiidæ, Cionidæ, Styelidæ and Pyuridæ are typically attached firmly to stones, rock, wood, etc., and only one or two isolated cases are known where this is not so, of the 52 species of Molgulids here investigated, 31 are unattached and live free in sand, mud or shell-gravel (see Table I). Moreover, the average size of the free-living forms is about three times that of the attached forms, and the former are typically oviparous, as distinct from the latter.

The free forms usually become embedded in the sand and anchored more or less firmly by means of the very numerous extensions of the test to which sand grains adhere. Of the attached forms listed in Table I, *i.e.*, 21, at least 5 have their outer surface covered with sand, two are anchored by long slender stalks, and many others possess numerous fine test extensions.

These facts taken together suggest that the primitive habitat of the Molgulidæ is that of the open sand and mud flats, and that the relatively large ovoid individuals, oviparous and unattached, colonised the rocky shore regions. In so doing, as has already been argued, their attachment to hard and relatively smooth surfaces exposed to wave action, involved a reduction in size, and consequent viviparity.

It is therefore assumed that the Molgulidæ are to be considered typically as dwellers of the sand flats, a view already put forward both by RITTER (1913) and by GARSTANG (1928).*

Since viviparity, accumulation of yolk in the egg, geographical distribution of the adult (*i.e.*, its exposure to varying conditions of salinity and temperature), its bathymetrical distribution (*i.e.*, its exposure to varying conditions of pressure and light intensity), have been shown to have no significance with regard to the occurrence of anural development, and since such development seems to be confined to this family and its origin to be polyphyletic, it is very probable that it is to be correlated with the outstanding peculiarity of the family, namely, its unattached sand-flat habitat and its adaptation to such an existence.

If Table I is consulted it will be seen that of the nine species of known development that live unattached, eight have anural development. Of anural species that are attached there are but the small viviparous *Molgula bleizi*, recorded only from Roscoff, and the large oviparous *Molgula retortiformis*, with its relatively large eggs. Of the species that produce urodele larvæ, all but one out of the fourteen listed here are attached forms. Such facts are more briefly summarised below :—

Of viviparous forms 8 species are urodele, 2 anural.
Of oviparous forms, 5 species are urodele, 8 anural.
Of attached forms, 13 species are urodele, 2 anural.
Of unattached forms, 1 species is urodele, 7 anural.

There is accordingly not only a correlation between shore-living attached forms and viviparity on the one hand, and sand-flat free-living oviparous forms on the other, but there seems to be a corresponding association between the presence of urodele larvæ among the former and anural larvæ among the latter. The agreement is not absolute, as GIARD thought, yet is very much closer than the opponents of his view considered, and when the development of more species that are free-living and oviparous is known, it may become closer still.

Summarising the principal facts and conclusions so far reached :—

(*a*) Anural development is confined to Molgulidæ within the Ascidiacea ;

(*b*) a sandflat free-living habitat is typical of the Molgulidæ and of no other group ;

(*c*) apart from " *b* " there seems to be no other peculiarity in the mode of life of the family ;

(*d*) the shore-living forms become attached, viviparous, and with two exceptions retain the tadpole larva ;

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* See note to page 311.

(*e*) viviparity is a rarity among the free-living forms, and that all but one of these possess anural larvæ;

(*f*) the origin of anural development is polyphyletic;

(*g*) of the two attached forms with anural development there is strong reason to believe that *M. bleizi* is a species derived directly from the anural *M. occulta*, and that the other, *M. retortiformis*, shows more markedly than most attached forms its free-living origin; *i.e.*, in both species anural development conceivably arose while they were yet free-living sand forms.

It is concluded therefore that anural development is to be correlated with the sand-flat habitat. It is not considered that such a habitat is directly responsible for its origin, but that conceivably through a lessening of the rigour of natural selection, those species in which it does arise are allowed to survive, whereas among attached shore forms they are not.

From the accounts given above of anural and teratological development it is clear that the normal process of tail formation in Tunicates is one that is very easily disturbed. It is dependent upon extensibility of the ectoderm posteriorly (cp. *Corella* and *Ascidiella*), closure of the blastopore (cp. *Pyrosoma*), swelling and vacuolisation of the notochordal cells (anural Molgulids), and several other factors of unknown stability.

It is therefore more than probable that development of a tail frequently fails to occur throughout the Ascidiacea, but except among the Molgulidæ such failure is incompatible with survival of the species. That such a structure as the tail may be lost with impunity will depend upon at least three things.

1. Failure to develop a tail must not interfere with the ability of the embryo to develop into a normal adult.
2. Either the hatching mechanism must be left unaffected or else an efficient alternative method must exist.
3. The adult habitat must be of such a nature that not only a tail for swimming but also larval sense organs are inessential for its discovery.

Unless these three conditions, at least, are fulfilled, any tendency in a species to undergo anural development will be eradicated, or if persisted in will result in the death of that species.

The third condition alone is probably sufficient to account almost completely for the confinement of anural development among the Molgulidæ.

Selection of habitat is necessarily predominant in compound ascidians of whatever order,* and most other forms, including those of the Molgulidæ, that are viviparous.

Simple ascidians in general (*i.e.*, the Pyuridæ, most of the Styelidæ, Phallusidæ, Cionidæ, and Rhodosomatidæ) are attached to some solid structure and are scattered sparingly over wide areas, though an exceptionally favourable region may become

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* Owing to the relatively small number of larvæ produced and the specialised nature of the habitat.

thickly populated by any given species. In such forms dispersion over wide areas is of primary importance, though a certain amount of selection is probably desirable. The attached oviparous tadpole-producing species of *Molgula* fall within this last category.

Among the sand-flat forms the need for selection disappears, and since relatively wide areas can be thickly populated, dispersion becomes of less importance as well. There is no need for the larvæ to rise from the bottom currents, it is of no consequence that dispersion is approximately halved through the elimination of the active phase, and there is literally no opportunity for selection of habitat on the part of a tadpole, should such a form exist.

Therefore among sand-living species there is no reason why those with anural development should not survive as readily as those with urodele. In fact, the type of dispersion occurring among such forms, *i.e.*, more nearly confined to the bottom currents, and extensive but not too extensive, should be definitely advantageous. The history of the family Molgulidæ may accordingly be visualised as follows :—

The primitive Molgulids were inhabitants of extensive, submerged sand-flats. They had a spherical or ellipsoid body of average size 25 mm., with test processes for attachment to sand grains. The majority remain there, are typically oviparous and have small eggs, and all species with one exception, where development is known, have lost the tadpole stage from development.

A minority moved to the shore and became attached to rocks and weed, though retaining much of their free sand-living adaptations. These, with two exceptions, retain the tadpole stage in development. The majority, owing to their poor equipment for an attached existence, became sexually mature at a relatively early age and are therefore dwarfed ; those attaining sexual maturity when less than 8 mm. diameter became viviparous. The reduction in size and the development of viviparity led to a reduction in number and increase in size of the eggs and embryos, and also to a modification of the method of hatching and in the type of the tadpole larvæ.

In two cases sand-living forms assumed an attached mode of life after they had lost the tadpole stage from development, with a resulting limitation of distribution in the one case, and increase in embryo size in the other.

While the occurrence of anural development both within and without the Molgulidæ may be explained more or less satisfactorily upon the basis of adult habitat, it is left uncertain whether that habitat is directly responsible for the existence of that type of development or is merely a controlling factor.

5 (*h*). HATCHING MECHANISMS.

It has been shown that the primitive method of hatching of ascidian larvæ is by digesting the egg-membrane with a proteolytic enzyme, and that this enzyme is very unstable.* Its presence is typical, however, of embryos forming from very small eggs rather than from those where there has been marked accumulation of yolk; so that its absence in all embryos developing anurally, whether from eggs small or large, is a fact needing explanation.

There are two possibilities. Any disturbance in the general course of development, such as the breakdown of the tail structures, may involve an inactivation of the enzyme. In this case only those species with anural development survive that possess an efficient alternative method of hatching, and that have a habitat of such a nature that a free-swimming phase is unnecessary.

Alternatively the enzyme may become inactive or lost, when only those species survive that are able to hatch by means of the rupture method, and probably where an active free-swimming larval existence again is unnecessary. However, loss of the enzyme does not necessarily imply loss of the tadpole stage since the viviparous forms with large eggs hatch by rupture and yet have succeeded in retaining the tadpole stage.

These alternatives may be expressed more clearly as follows :—

(*a*) Enzyme inactivated or lost.	(*b*) Tadpole stage lost.
—	—
Species survives only if rupture method of hatching efficient.	Species survives only if— (1) Free-swimming selective phase unnecessary. (2) Enzyme having been lost with loss of tail, rupture method of hatching efficient.
—	
Tadpole stage retained if necessary, completely eliminated if inessential.	

Molgula oculata has identically the same habitat and distribution as *Molgula occulta*, which is typical of the anural type, but alone of the sand-flat dwellers the former possesses tadpole larvæ. Therefore, as far as environment is concerned, there has been no need for the retention of that stage. The larval ampullæ and test-growth, however, are insufficiently vigorous to rupture the egg-membrane should the enzyme method fail, and accordingly, if loss of the tadpole stage involves in some way an inactivation of the enzyme, the tadpole stage *must* be retained for the species to survive.

If loss of the enzyme is a precursor in anural development to loss of the tail, then the existence of the somewhat feeble tadpole stage in *Molgula oculata* is an anomaly,

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* Willey (1900, p. 149), commenting on the occasional confinement of the tadpole of *Molgula manhattensis* within the egg-membrane until part way through metamorphosis, says, "This indication of precocious metamorphosis is of importance in comparison with the development of other Molgulids, in which the urodele stage is omitted from the ontogeny." Since the time factor was ignored, however, it is as probable that the phenomenon is the result of delayed hatching as it is of precocious metamorphosis, and is but another instance of the instability of the hatching enzyme in these forms. Moreover, the final conclusion reached in the present paper is that the absence of a hatching enzyme in anural development is a result and not a cause of that type of development.

and since almost anything that disturbs the normal development of structure, however slight, tends to inactivate the enzyme, it seems probable that the absence of a hatching enzyme among the anural Molgulids is a result and not a cause of their peculiar development.

6. Summary and Conclusions.

1. An account is given of the development of 16 species of *Molgula* and 2 of *Eugyra*; that of 3 others is discussed, pp. 286–310.

2. Ten species have been found to be viviparous, and a discussion of viviparity and its origin within the family Molgulidæ is given, together with an account of its influence upon development, pp. 310–317.

3. It is concluded that viviparity is due for the most part to a reduction in adult size resulting from an early sexual maturity following the colonisation of relatively exposed rocks, etc., by large sand-living forms. Reduction in adult size lowers the number of germ cells in the body, viviparity offers a reduction in the rate of elimination of the eggs, and reduction in adult size lessens the force of the exhalent water current and encourages any tendency to retain the least buoyant eggs; such eggs are the relatively larger ones with the least vacuolate follicle cells.

4. Increase in egg size, or else the mere fact of retention within the atrial chamber, has resulted in the development of a secondary method of hatching, replacing the primitive and early digestion method, namely, that of rupture, and also in a more highly differentiated and more active tadpole larva of relatively short free-swimming period.

5. Nine species have been found to have direct or anural development, four of which are reported for the first time. Two species, *Molgula oculata* and *Molgula simplex*, previously said to have anural development, have been found to possess tadpole larvæ, pp. 299–310.

6. The cleavage of eggs leading to anural development is quite typical of ascidian eggs in general, contrary to the statement of Lacaze-Duthiers, pp. 318–319.

7. The course of development in all species producing anural larvæ seems to be fundamentally the same. The embryo retains a spherical shape throughout development, there is at no time any sign of a tail nor of larval sense organs, and hatching is always by membrane rupture and never by digestion. Post-larval development, however, seems to be the same as that of urodele larvæ. Ectodermal ampullæ for respiration and fixation grow out in exactly the same manner and, where development has been followed for a sufficient length of time, an aggregation of cells of relatively high refractive index is seen in the region of the larval stomach, and in the case of urodele larvæ are those resulting from the phagocytosis of the tail, p. 318.

8. An analysis is made of the processes leading to the formation of a tail in more typical ascidian larvæ and the conclusion reached that the most important factor is the increase in size of individual notochord cells through the imbibition of water, usually preceding the formation of internal vacuoles, pp. 320–323.

9. The histological account of the development of *Molgula bleizi* given by Damas is

reinterpreted in the light of the above and through the introduction of the time factor, which had previously been ignored. Instead of there being precocity in the formation of the neural tube, caudal disaggregation, secretion of larval test, and outgrowth of ectodermal ampullæ, as Damas concludes, it is believed that these all are normal in their time of appearance, and that it is the closure of the blastopore, which Damas considers normal, that is delayed. This delay is considered to be a direct result of the failure of the notochord cells to extend posteriorly, pp. 324–329.

10. A relation is shown to exist between the degree of imbibition by notochord cells and the alkalinity or acidity of the surrounding medium, pp. 329–332.

11. Anural or direct development is shown to occur in the Tunicata apart from Molgulids only in Thaliacea and in *Pyrosoma*. Out of 139 members of the Ascidiacea the only species possessing anural development are the ten Molgulids already described, p. 332.

12. Within the family Molgulidæ the occurrence of anural development is shown to have no relation whatsoever to the adult organisation, *i.e.*, its origin within the family is polyphyletic, pp. 332–337.

13. The otolith, the only larval Molgulid sense organ, is absent in all anural larvæ, present in all urodele larvæ. There are indications that its absence is a direct ontogenetic result of the failure of the tail to develop, *i.e.*, that the tail or some part of it acts as an " organiser " in this respect, pp. 337–338.

14. The relation of the members of the Molgulidæ to their environment is examined, and it is concluded that the family is fundamentally adapted for an existence embedded and loosely anchored in submerged sand-flats, and that the attached species of the shore regions are secondarily adapted to such an habitat.

15. There is shown to be a correlation between the presence of anural development and relatively large oviparous, free-living species on the one hand, and urodele development and relatively small, viviparous, attached species on the other, pp. 338–341.

16. All anural embryos hatch through the rupture of the egg-membrane, even when hatching by means of membrane digestion is expected, and it is concluded that the apparent loss of the hatching enzyme is a result and not a cause of anural development, although it is considered to be a limiting factor, pp. 341–343.

17. The shore-living Molgulids are believed to have been derived from the sand-flat dwellers before the latter had existed as such long enough for the majority to have lost their tadpole stage.

18. The general conclusions concerning anural development are that the sand-embedded habitat is such that the possession of active tadpole larvæ no longer has any survival value, that development of a tail can readily be disturbed in all ascidians, and its complete absence in anural Molgulids results from the failure of notochord cells to swell, and that since such disturbance involves the loss or inactivation of the hatching enzyme, the presence of anural development is confined to those sand-living species in possession of an alternative method of hatching.

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