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Studies in Tunicate Development. Part III. Differential Retardation and Acceleration

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VIII—Studies in Tunicate Development

Part III—Differential Retardation and Acceleration

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I—Introduction

Accumulation of yolk within the egg has played an important part in the evolution of many groups of animals. This is especially true of the vertebrates, but comparative investigations in this series involve the study of rather widely separated types in which other evolutionary factors may have obscured the issue. In the ascidians there is such a wealth of closely related forms that it is possible to determine more or less accurately the influence of yolk accumulation, both where it is evenly distributed and where it tends to be confined to the vegetal hemisphere, and also the nature of certain factors of great significance in development and evolution unconnected with increase in egg-size.

This paper contains a general account of the origin of viviparity in ascidians, the significance of cell-size, a survey of development in each family of ascidians, and a discussion of the abbreviation and telescoping of development resulting from the retarding effect of accumulation of yolk and of other factors.

The observations have for the most part been made, upon living organisms, at the marine biological stations at Plymouth and Bermuda and also at the Bermuda Government Aquarium. Examination of preserved material was carried out in the Department of Zoology, McGill University.

Grateful acknowledgments are made to the staffs of the Plymouth and Bermuda laboratories, and to Louis L. Mowbray, Esq., of the Bermuda Aquarium, for facilities and much assistance in procuring material, also to the British Museum of Natural History for the privilege of examining rare material of Tylobranchion.

II—Material and Methods

The descriptions made in this paper are based upon investigations into the development of forty-six species representing thirty-five genera.

All but six species were obtained in the vicinity of the biological stations at St. Georges, Bermuda, and at Plymouth ; three species are common at St. Andrews, Bay of Fundy, while another is not uncommon at Kristineberg, Sweden. All were examined in the living condition, with the exception of Tylobranchion, although prepared material in most cases was subsequently investigated.

Since a detailed fauna list has recently been published for the ascidians of the Plymouth district, and a separate account of the ascidian fauna of Bermuda has also been made, all that is deemed necessary here is Table I showing the best localities and approximate breeding seasons of the species under discussion.

The development of oviparous forms was determined, as in previous studies, by artificial fertilization. Difficulty was encountered only with *Diazona violacea*, the only known example of an oviparous colonial ascidian. This species has a relatively limited breeding season, on an average, of six weeks, the onset of which may be from late June to early September ; moreover, the adult cannot be kept alive in

Table I—Forms Investigated Arranged in Order of Description

The locality is that where a species is most readily obtained, while the breeding season in any case may be more extended than that stated.

Genus and Species	Locality	Breeding Season
Corella parallelogramma	Plymouth	July.
Ciona intestinalis	Plymouth ; Mill-bay docks	April–Sept.
Diazona violacea	,, Mewstone ledge	Aug.–Sept.
Ascidia mentula	,, ,, ,,	Not seasonal.
Ascidiella scabra	,, Eddystone grounds	Not seasonal.
Phallusia mamillata	,, Mewstone ledge	Not seasonal.
Boltenia echinata	Kristineberg ; Gulmarfjord	July–Sept.
,, *hirsuta*	St. Andrews, Fundy ; low-water	July–Sept.
Tethyum pyriforme	,, 1–10 fathoms	July–Sept.
Molgula tubifera	Plymouth ; Mill-bay docks	March–Oct.
,, *complanata*	,, Asia shoal, etc.	April–Oct.
,, *citrina*	,, Roscoff ; St. Andrews	April–Oct.
Styela partita	Woods Hole	Aug.–Sept.
Polycarpa rustica	Plymouth ; Salcombe	May–Oct.
,, *obtecta*	Bermuda ; low-water	May–Sept.
Polyandrocarpa tincta	,, ,,	May–Aug.
Styelopsis grossularia	Plymouth ; below laboratory	March–Oct.
Distomus variolosus	,, Mewstone and Sound	July–Sept.
Stolonica socialis	,, Queen's grounds	July–Sept.
Symplegma viride	Bermuda ; Causeway stones	July–Aug.
Botryllus gigas	Plymouth ; Mewstone ledge	May–Sept.
,, *schlosseri*	,, Asia shoal	April–Sept.
Botrylloides leachii	,, Salcombe	April–Sept.
Perophora listeri	,, Duke Rock	Aug.–Sept.
,, *viridis*	Bermuda ; Causeway stones	July–Sept.
Ecteinascidia turbinata	,, ,, ,,	June–Sept.
,, *conklini*	,, ,, ,,	July–Sept.
Tylobranchion speciosum	British Museum specimen	—
Clavelina lepadiformis	Plymouth ; Salcombe	July–Sept.
,, *oblonga*	Bermuda ; Causeway stones	May–Sept.
,, *picta*	,, wreck, Castle Harbour	July–Aug.
Pycnoclavella auriculens	Plymouth ; Mewstone ledge	Aug.–Sept.
Distaplia rosea	,, New Grounds	July–Sept.
,, *clavata*	St. Andrews ; shallow water	Aug.–Sept.
,, *bermudensis*	Bermuda ; Causeway stones	May–Sept.
Archidistoma aggregata	Plymouth ; Duke Rock	Aug.–Sept.
Morchellium argus	,, Salcombe	June-Sept.
Sidnyum turbinatum	,, ,,	June–Aug.
Polyclinum sabulosum	,, Sound and Salcombe	June–Aug.
Amaroucium nordmanni	,, Salcombe	June–Aug.
Didemnum gelatinosum	,, Mewstone ledge	July–Sept.
Trididemnum cereum	,, Asia and New Grounds	July–Sept.
Polysyncraton amethysteum	Bermuda ; Inlet and Causeway	May–Aug.
Diplosoma gelatinosa	Plymouth ; Aquaria walls	April–Oct.

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aquaria for any length of time, and accordingly fertilization of the eggs becomes a matter of urgency after a colony has been dredged.

Of viviparous forms the larval and post-larval development is fairly readily examined. Colonies on compression will usually extrude embryos at all stages of development, when those tadpoles exhibiting signs of activity may be isolated and their subsequent history observed.

The introduction of the time-factor in development being of paramount importance to this investigation, the ease with which the post-larval development in viviparous species may be studied is counteracted by the great difficulty of making the necessary observations upon embryonic stages. This difficulty could not completely be overcome, but in a number of representative species sufficient data have been obtained.

In *Perophora*, *Tethyum*, and *Clavelina lepadiformis*, embryos will continue to develop in a normal manner outside the parent. *Botryllus* colonies contain embryos, if any, all at one stage of development, and therefore samples may be cut from time to time and the stage determined. *Diplosoma* colonies are sufficiently transparent for the embryonic development to be studied within the parental tissues. Large colonies were cut into pieces which were allowed to become reattached to pieces of glass, which they readily do, so that each piece contained but two or three embryos. The development of these could best be followed by inverting the glass on supports in a small trough of water and making an examination from the base of the colony.

Developing eggs and embryos of *Molgula citrina*, *M. complanata*, *Polycarpa*, *Archidistoma*, *Didemnum*, *Trididemnum*, *Morchellium*, *Distaplia*, *Styelopsis*, *Distomus*, and *Stolonica* were examined in two ways. The mouth of a thistle funnel was closed with medium bolting-silk, the embryos dropped in the open end on to the silk, a T-piece attached to that end, and the whole submerged in water. Air-bubbles forced through one end of the T-piece will draw a gentle stream of water through the silk. This serves two purposes ; it supplies oxygen to and removes waste products from the region of the embryos, and at the same time agitates them very quietly so that no one spot can become a centre for infection.

The other method involved the use of the small reattached partial colonies of *Diplosoma*. Such pieces almost invariably develop each a single oscular opening into the common cloacal cavity to allow the exhalent current to emerge. In this way an ideal incubatory chamber is formed, and eggs and embryos of other species if inserted will continue to develop perfectly. These two methods are illustrated in fig. 1.

The two species of *Ecteinascidia* are also sufficiently transparent for development to be examined within the living parent. The atrial chambers of individuals of this form may be used as incubators for eggs and embryos as in *Diplosoma*, and were used as such for those of *Clavelina picta* and *Polysyncraton amythysteum*. The one danger in the use of these living incubators is the possibility of a sudden contraction causing the exhalent current to carry the embryos out of the colony or individual, as the case may be.

Embryos and larvae were preserved in Kleinenberg's picro-sulphuric, Bouin's fluid, and formalin (neutralized) ; they were stained with alum-carmine or with diluted Delafield's haematoxylin as used by CONKLIN.

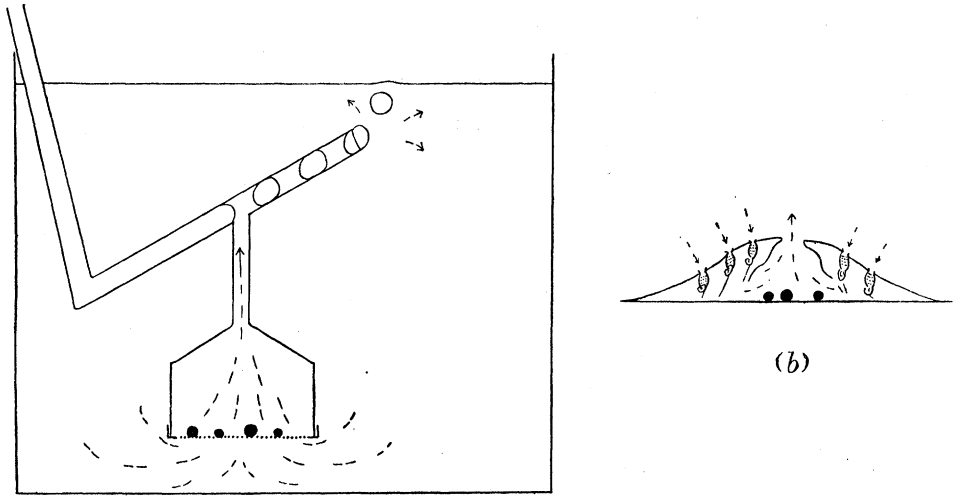

FIG. 1—Methods for rearing ascidians with yolky eggs through embryonic development. (*a*) artificial incubator ; (*b*) natural incubator formed by reattached fragment of colony of *Diplosoma listerianum*

III—CLASSIFICATION OF ASCIDIANS

The accepted classification of the ascidians divides that class into three orders. These are the Phlebobranchia, Stolidobranchia, and Aplousobranchia of LAHILLE, or the orders Ptychobranchia, Dictyobranchia, and Krikobranchia of SEELIGER. The three orders in the two cases are the same ; since LAHILLE easily possesses priority and there is no real merit in SEELIGER's names over his, the first three order names should be used. In a subsequent publication in this series a proposal will be made, and supported with considerable evidence from embryology and comparative anatomy, that there should be fundamental changes in the classification of ascidians.

Each of the prevailing orders are composed of from three to four families, and in these investigations representative genera and species of each have been examined.

The Stolidobranchia and Aplousobranchia each form fairly well-defined groups, but the Phlebobranchia is very heterogeneous. A diagram, fig. 2, shows roughly the relationships one with another of the different families, and indicates the viviparous and oviparous, large- and small-egged, budding and non-budding genera investigated.

IV—ORIGIN OF VIVIPARITY AND YOLK ACCUMULATION

Viviparity is usually to be correlated with the presence of large eggs, and there is no doubt that the one tends to induce the other. Large eggs are more likely to be retained than small, and yolk is more likely to be accumulated in eggs developing within the protecting parental cavities.

At the same time viviparity alone is a very great asset, but accumulation of yolk in unprotected eggs is, if anything, detrimental. The former results in an enormous decrease in the rate of elimination of the developing larvae, although often at the expense of their degree of dispersion. The latter implies a decrease in egg-number without any compensating decrease in rate of elimination.

Supporting the contention that viviparity precedes marked accumulation of yolk are the facts that such accumulation above the average for the genus occurs among

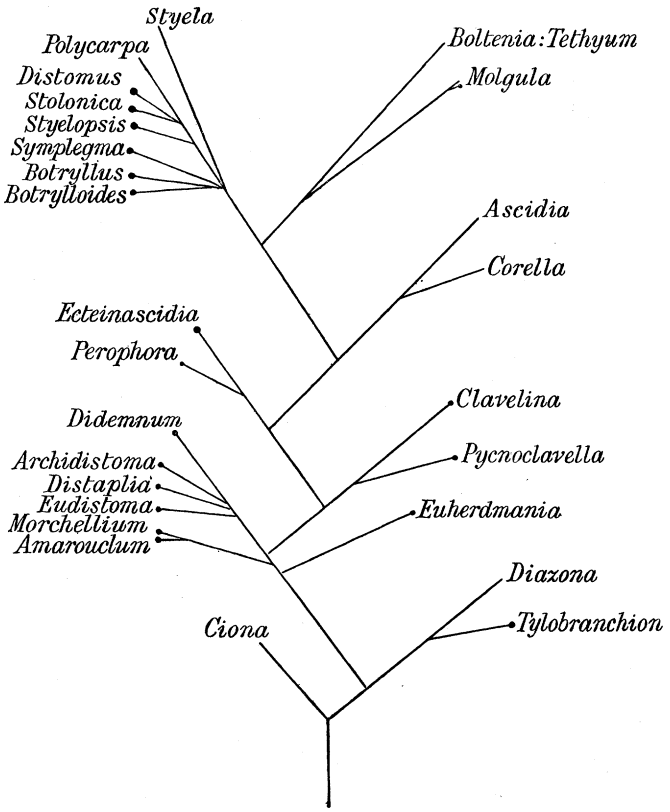

FIG. 2—Tentative phylogenetic tree of the ascidians to indicate their mutual relationships as expressed by adult structure. Those with large eggs have capitate terminals. The small-egged forms are oviparous with the exception of *Polycarpa*, and *Boltenia*, the large-egged forms all being viviparous. Asexual reproduction occurs in all the large-egged forms excepting *Styelopsis* and *Molgula*; *Diazona* alone of the oviparous and small-egged forms is able to bud

oviparous forms only in *Tethyum pyriforme* and *Molgula retortiformis*, and in these species the increase does not exceed four times in volume; on the other hand, viviparity does occur in a number of species that has small eggs, *Molgula platei* with eggs 0·10 mm diameter, *Boltenia hirsuta* and *B. echinata* with eggs 0·15 mm, most if not all species of *Polycarpa* and *Polyandrocarpa*, with eggs 0·17 mm, and *Corella willmeriana* with eggs 0·17 mm.

In oviparous species a large number of eggs is necessary, not only for dispersion but to ensure a reasonable number surviving to maturity. In viviparous species

dispersion is in any case curtailed while the embryonic period is passed through in safety, so that egg-number may decrease with impunity. Other conditions being equal, the chances of any one egg surviving to maturity increases with its store of yolk. Accordingly in viviparous forms there is every inducement for egg-size to increase at the expense of egg-number. Natural selection prevents such a tendency from being carried too far.

Yolk accumulation may therefore be said to be the result of long-established viviparity. Viviparity, itself, however, may arise in several ways.

In Molgulids, as demonstrated in Part II, p. 313 (BERRILL, 1931), it seems to depend upon a reduction in body-size, in itself the result of precocious sexual maturity. In the genera *Polycarpa* and *Polyandrocarpa*, both with small eggs, viviparity is due primarily to the liberation of eggs by polycarps lining the atrial sac at a considerable distance from the exhalent siphon. Apparently eggs must be liberated very close to the siphon for the water current to be strong enough to carry them out.

From the polycarpid stock there probably arose the polystyelids *Stolonica* and *Distomus*, on the one hand, and the botryllids (including *Symplegma*) on the other. It is quite probable that the viviparity of these last two groups is inherited from the first. Otherwise it is a case of convergence, for in each group the main factor is the liberation of eggs too far from the atrial siphon for them to be carried out by the current of water. In all but the polycarpids, increase in egg-size ensures such viviparity.

Viviparity in the Perophoridae has a somewhat obscure origin so far as the origin of the perophorids themselves is obscure, but there is little doubt that the extreme shortness of the oviduct is primarily responsible.

In the remaining groups, Diazonidae and Aplousobranchia, viviparity may be of single or multiple origin, although forming apparently a natural series they will be treated as such.

Ciona and the diazonids *Rhopalea* and *Diazona* are all oviparous, possess gonads that lie in the intestinal loop and an oviduct that accompanies the sperm duct close to the atrial siphon. The eggs are small (0·16 mm) and are produced in large numbers ; the condition in these forms is therefore primitive.

Linking the above genera with the synoicids is *Tylobranchion*, a rare diazonid of the South Atlantic. Not only is the adult an intermediate type but also the nature of its viviparity is, in some respects, very primitive.

In oviparous ascidians the eggs are stored in the ovary or oviduct until a fair number has accumulated. These eventually are shed in one or several batches, together with sperm so that a large number of tadpoles develop at once.

Tylobranchion, in spite of viviparity and the possession of very large eggs, has departed hardly at all from this condition, a batch of eggs grow and mature together in the ovary, pass up the oviduct and become fertilized simultaneously in the atrial cavity. Retention in that cavity, while encouraged by the difficulty of extruding such large eggs (0·74 mm), is undoubtedly due to the ending of the oviduct at a considerable distance from the atrial siphon, as in perophorids.

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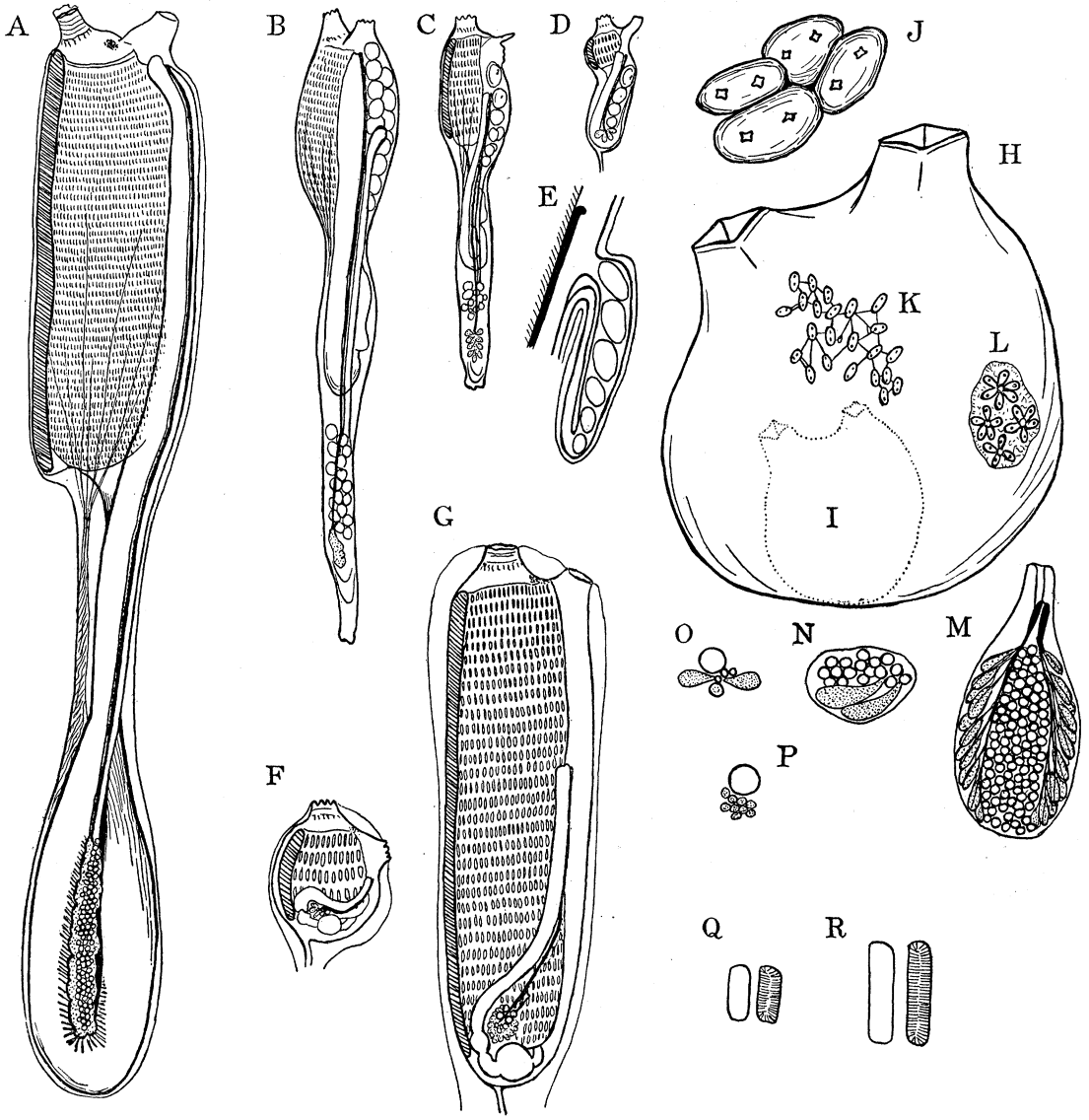

FIG. 3—A comparison of body-sizes. A, B, C, and D (× 5) illustrate the relationship between size of zooid and the number of stigmata rows, the shortening of the oviduct, and the formation of a post-abdomen. A, *Diazona*; B, *Tylobranchion*; C, *Morchellium*; D, *Eudistoma*; E, brood-pouch of *Distaplia*. F and G (× 7) illustrate the relationship between body-size of zooid and number of rows of stigmata for *Perophora* (F) and *Ecteinascidia* (G). H, I, J, K, and L (natural size) illustrate the relative body-sizes of various styelids—H, *Polycarpa obtecta*; I, *Polycarpa rustica*; J, *Polyandrocarpa tincta*; K, *Symplegma viride*; L, *Botryllus schlosseri*. M, N, O, and P illustrate the relative number of cell units in hermaphrodite gland or polycarp (× 10) of *Polycarpa obtecta* (M), *Polyandrocarpa tincta* (N), *Symplegma viride* (O), and *Botryllus schlosseri* (P.). Q and R represent the relative sizes of stigmata of *Polycarpa obtecta* and *Symplegma viride* respectively

The embryos in *Tylobranchion* are accordingly, in one zooid, all at the same stage of development. This is a very different state from that found in the four orders of the Aplousobranchia, where in one zooid embryos at all stages of development may occur. The condition in these forms may be derived from that in *Tylobranchion* either directly or as the result of parallel and extended evolution. The oviduct in all is even shorter than in *Tylobranchion* and only just reaches the base of the atrial cavity. This again is believed to be the primary cause of the viviparity, should this state have been independently acquired.

The presence of embryos at all stages of development, the formation of brood-pouches, and the occurrence of intra-oveducal development common in this order are to be associated with other factors.

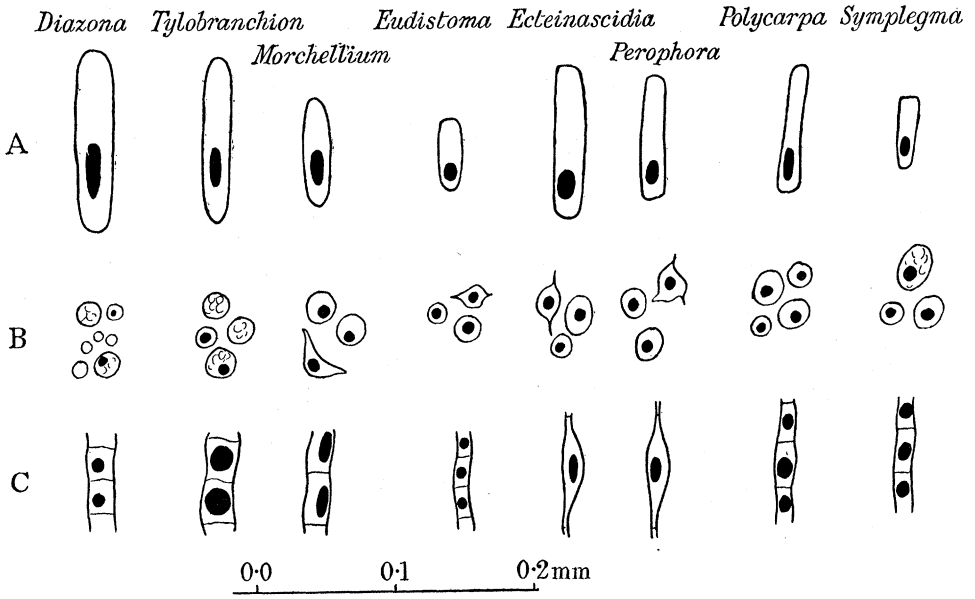

FIG. 4—Relative cell-sizes of various tissues of the ascidians shown in fig. 3. A, epithelial cells of the intestine; B, mesenchyme cells; C, epidermal cells

V—CELL-SIZE, BODY-SIZE, AND EGG-SIZE

The primary cause of viviparity is the relative shortening of the oviduct. Increase in egg-size, however, is undoubtedly an abetting factor. Such increase may be absolute or relative or both. If the body of the mature zooid should decrease in size without there being any reduction in egg-size, the eggs will be correspondingly more difficult to extrude since, relative to the parental structures, they have become larger.

This introduces the more general problem of cell-size and body-size. Inasmuch as the ascidians vary in size at maturity to a greater extent than occurs in most animal phyla, they form a very suitable material for investigation.

Epidermal, mesenchymal, and intestinal cells were measured and are illustrated in fig. 4; all drawn to the same scale. In fig. 3 the adult zooids are also shown; these form three groups, the scales differing as concerns the wholes but being

constant within each group. In the group *Diazona*, *Tylobranchion*, *Morchellium*, and *Eudistoma*, the adult body-size varies from 1 to 730 in volume ; in *Perophora* and *Ecteinascidia* it varies from 1 to 64 ; while in *Polycarpa*, *Polyandrocarpa*, *Symplegma*, and *Botryllus* the volume of the mature zooids varies from 1 to 18,000. The variation in volume of the cells, however, is seen to vary less than 1 to 8 with regard to those of the intestine, and is negligible in the other two cases.

Therefore, within the ascidians cell-size may be considered virtually to be constant, and accordingly any variation in adult size must find its expression in varying cell-number.

This is borne out in stigmata. The cells and cilia lining a stigma are as constant in size as any other type of cell. The size of a stigma is limited by the length of the cilia, and therefore the short axis at least can vary but little. So that unless the shape of stigmata be profoundly altered, reduction in body-size cannot be expressed as a reduction in size of the stigmata but only in their number.

The same reduction in number of an organ resulting from reduction in body-size is to be seen in those mixed gonads known as polycarps, so typical of Botryllids and most Styelids.

A few species of *Polycarpa* far exceed in size the majority. In those few, the number of polycarps on each mantle wall is much greater than in the others. The size of individual polycarps being relatively constant, there seems to be a certain maximum size for such organs, possibly controlled by the need of a definite ratio between the thickness of the organ and the total area of the sheet-like mantle wall to which it is attached.

On the other hand, in those polycarpids and botryllids that bud, the size of the individual zooids is naturally much smaller. In these the number of polycarps is correspondingly fewer. As such reduction proceeds, a reduction in cell-number appears within each polycarp, usually when the number of these organs has been reduced to single figures. Approximately the same proportion in cell-number is maintained between the eggs and spermatozoa.

Reduction in cell-number in the polycarps with decreasing body-size proceeds until only a single egg remains in each. In *Botryllus* there may be four such organs on each side, in *Botrylloides* only one ; in the latter a placental-like condition has been evolved. Compared with species of *Polycarpa*, the botryllids *Symplegma* and *Botryllus* possess very large eggs, while in *Botrylloides*, to be correlated with placentation, there is a secondary reduction in egg-size.

Conklin (1912) draws similar conclusions for various species of *Crepidula*. Not only is cell-size approximately constant in spite of variation in body-size from 1 to 125 in volume (and therefore differences in body-size are due to differences in cell-number), but also parts which are reduplicated, such as gill filaments, lobules of liver, kidney, ovary, testis, etc., are more numerous in large individuals than in small ones. As in ascidians, also the smaller species form the larger eggs.

In discussing variation in body-size the difficulty is encountered of distinguishing between causes of reduction and of increase in size. The available evidence points

to a fairly plausible conclusion, that the primitive body-size for Ascidians is neither the very large nor the very small. Certain characters of the larger families can be explained as adaptations to increase in size, in particular the presence of well-defined folds in the branchial wall of most Stolidobranchia is almost certainly an adaptation to maintain a constant ratio between the water-filtering area of the branchial sac and the mass of the whole organism.

A rapid rate of budding results in smaller, though more numerous, zooids than a slower rate. At the same time a rapid budding rate is probably more specialized and less primitive than a relatively slow rate, so that colonies with very small zooids have probably been derived from ones with larger zooids.

Among the Diazonidae and most Aplousobranchiate families, all colonial and in possession of epicardial chambers, the colonies tend to be massive rather than sheet-like. Colonies of *Diazona* may be 750 mm diameter and 200 mm high, colonies of *Eudistoma* may be but 12 mm diameter and 7 mm high, while between the two all sizes may be found.

Diazona zooids have about fifty rows of stigmata, *Morchellium* 10, and *Eudistoma* 3. Accordingly, there may have been an increase or decrease in size from an extreme or both from the mean. This may be determined by a study of surface-area and volume relationships.

The zooids of *Diazona* resemble the larger zooids of *Rhopalea*, and the individual of *Ciona* in possessing small eggs that develop from an ovary bordered by testes and situated in the loop of the intestine, and in having an oviduct and sperm duct that extend close to the atrial siphon. These are all undoubtedly primitive features.

With reduction in size of the colony the mass decreases faster than the surface, and therefore if there is reduction in zooid size rather than in number, such zooids will become crowded together toward the base of the colony. This crowding should be overcome in several ways, by a relative as well as absolute shortening of the zooids, by the development of a stalk into which parts of a zooid, such as gonads and heart, may descend and so make it longer but narrower, or by a reduction in number of zooids.

Eudistoma combines the first and last method, *Tylobranchion* and *Morchellium* the second. In this last the necessary adaptation is a fundamental tapering of the zooid basally into a fine extension—the post-abdomen.

These forms, with the exception of *Diazona*, are all viviparous. In each the sperm-duct accompanies the intestine along its whole length, to the atrial siphon in *Diazona* and *Tylobranchion*, or well short of that in *Eudistoma*, *Morchellium*, and other members of the order.

In all except *Diazona*, *i.e.*, in all the viviparous forms, the oviduct stops well short of the siphon, and it is the result of this that the eggs are retained. The condition is not, however, uniform.

In *Tylobranchion* the oviduct stops well short of the ending of the intestine and sperm-duct, as already described, and a large batch of eggs reaches the atrial cavity to be fertilized together.

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In *Morchellium* and *Eudistoma* the whole set of structures, intestine, sperm-duct and oviduct, is short, and it is possible that viviparity has been independently acquired. Instead of the oviduct turning into the atrial cavity at the middle of that chamber, it blends with its base so that there is no obvious junction. In *Clavelina*, a colonial form almost as large as *Diazona*, the same condition holds. Eggs on maturity are passed up the oviduct to the atrial chamber, where they are fertilized and accumulate ; consequently a large number of embryos at all stages of development are to be found.

Not only is cell-size relatively constant in spite of extreme reduction in body-size, but eggs tend to increase in size as their number becomes markedly reduced, culminating in single-egged ovaries as in *Botryllus*. Consequently, the developing eggs and embryos frequently tend to exceed in bulk the parental zooid. Atrial chambers which originally could accommodate a large number of embryos, as in *Clavelina*, can in *Morchellium* and *Amaroucium* hold only a few and in linear series. In these forms, the upper end of the oviduct is wide, the eggs can be fertilized while still within it, and consequently the row of embryos extends well below the atrial chamber.

In *Eudistoma* reduction in body-size is carried much farther, yet with eggs and embryos even larger than in *Morchellium*. Here the process of oveducal development is even more pronounced. The eggs are fertilized as soon as they enter the oviduct, the sperm having to find its way from near the atrial siphon. The result is that the embryos develop entirely within the oviduct and below the atrial and branchial chambers, the oviduct forming a true brood chamber.

In the closely allied genus *Distaplia* this process is developed still further, as determined by Bancroft (1900). The oviduct bulges out from the zooid proper to form a brood pouch which eventually becomes constricted off. *Collella* apparently is similar. In *Distaplia* the parental zooid degenerates after the pouch separates off. A pouch still attached is shown in fig. 3.

VI—Ascidian Development

In the following section the development in each family and its range of variation will be discussed in turn. The type of development considered to be most primitive is first described, and this is used as a standard for comparison in other families for the more or less modified development of the egg, resulting from accumulation of yolk in most species, indirectly from viviparity in others.

1—*Family Cionidae and Diazonidae*—Fig. 5

With the exception of *Tylobranchion*, all members of these families are oviparous, producing eggs of about 0·17 mm diameter. Development was followed in *Ciona intestinalis* (Linn) and *Diazona violacea* (Savigny) and at 16° C. Gastrulation commences 7–8 hours after fertilization and at the 64-cell stage ; sensory pigment first

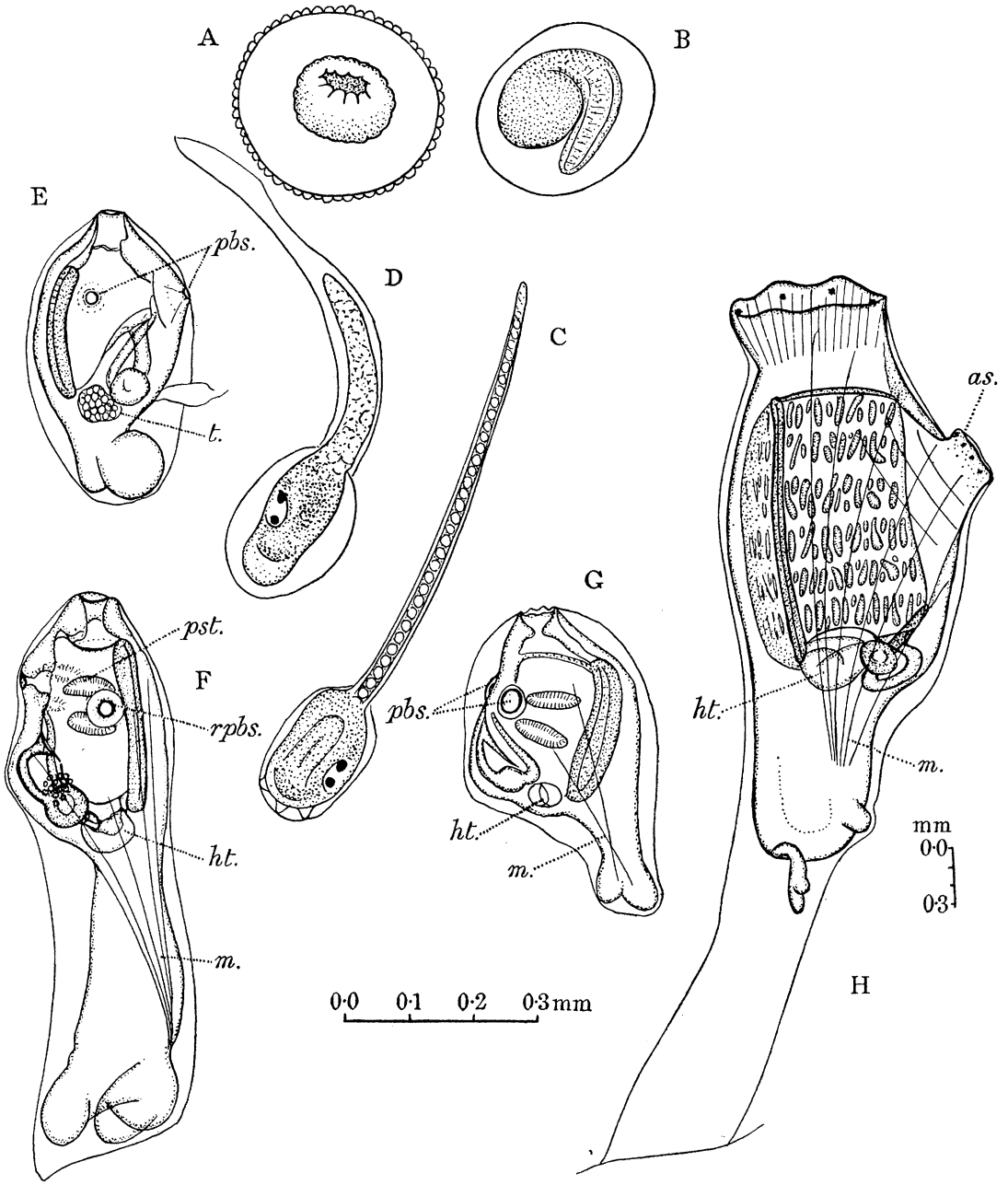

FIG. 5—Development of *Ciona intestinalis* and *Diazona violacea*. A, Gastrula of *Diazona*, showing also perivitelline space and follicle cells ; B, early tadpole stage of *Diazona* ; C, fully formed tadpole ; D, metamorphosing tadpole, and E, completely metamorphosed form of *Diazona* ; F, later stage with functional heart and stigmata ; G, stage in development of *Ciona* corresponding to stage F of *Diazona* (note the pair of peribranchial siphons in each genus) ; H, a relatively late stage in the development of *Ciona*, in which six protostigmata have become subdivided into six rows and the two peribranchial siphons have fused to form the single atrial siphon. *as*, atrial siphon ; *ht.*, heart ; *m.*, muscle fibres ; *pbs.*, peribranchial siphon ; *pst.*, protostigma ; *rpbs.*, right peribranchial siphon

appears after 19 hours, while hatching occurs after 25 hours by digestion of the egg membrane by a proteolytic enzyme (*cf.*, Part I, p. 55, BERRILL, 1929). During such embryonic development no growth in size occurs, merely division and differentiation of cells, excepting those forming the notochord, which increase markedly in volume (*cf.*, Part II, p. 320, BERRILL, 1931). The branchial and peribranchial invaginations commence somewhat before hatching, but the only functional organs of the tadpole are the tail and sensory structures. The free swimming period lasts from 6 to 36 hours, usually over 12. Settling then occurs, the tail is absorbed and stored within the trunk, while proliferation of the anterior lip rotates the mouth and peribranchial apertures to a dorsal position. Only after this stage does increase in body-size commence.

Immediately after settling, the anterior region of the tadpole grows out as the mental process or ampulla, the growth of which lifts the trunk away from the substratum. In effect it acts as a true stalk of fixation below the viscera and branchial sac.

The pericardium develops as two diverticula from the floor of the pharynx. Medial invaginations from these form the heart (SELYS–LONGCHAMPS, 1900), which at 16° C starts beating about 200 hours after fertilization ; from the first the regular reversal in direction of the wave is to be seen. At this stage the circulation is closed and does not include the primary body cavity. After about 250 hours from fertilization the siphons open and two protostigmata on each side of the branchial sac become functional ; later, when the absorbed tail material has entirely disappeared, a third protostigma appears posteriorly.

The development of *Ciona* and *Diazona* to this stage is the same except that the stalk or ampulla in *Diazona* is a more important structure. The subsequent development is known only in *Ciona*, but that of *Diazona* probably is identical except for differences apparent in the adult organization.

In *Ciona* the 3 protostigmata loop at the ventral ends to form 6. After 6 or 8 weeks at 16° C, and when considerable growth has taken place, two changes occur. Processes from the anterior or posterior edge divide the 6 protostigmata into 6 rows of definitive stigmata, while at the same time the right and left peribranchial siphons move together mid-dorsally to fuse and form the atrial siphon of the adult.

Somewhat later the circulation opens into the cavity of the stalk, and a septum, which eventually extends throughout the blood vessels of the test, produces an afferent and efferent current. At about the same time 2 posterior extensions of the branchial sac envelop the viscera and heart to form the right and left perivisceral cavities.

Diazona presumably follows the same general course, differing only in that the gut loop and heart descend far into the stalk while the pair of perivisceral cavities fuse to form the epicardium.

The development of the Ascidiidae differs from that of *Ciona* in but two particulars. Instead of a single ampullary outgrowth there are three or four ventral outgrowths

which are not necessarily homologous. More important is a midventral outgrowth from the pharynx to form the primary renal vesicle. There is good reason to consider this homologous with the epicardium of *Diazona* and others ; if true, there has not only been a developmental abbreviation independent of accumulation of yolk but also precocity, since it develops at about the same time as the heart.

A renal vesicle is similarly formed in place of an epicardium in the order Stolido (Dictyo)-branchia ; but in these forms a single dorsal invagination that bifurcates over the nervous system replaces the paired peribranchial invaginations. This is not in any sense an acceleration of the normal development but an abrupt change, resulting from the failure to divide on the part of what is presumably a medial rudiment.

Tylobranchion speciosum Herdman, while a member of the Diazonidae, probably has a more modified development than *Diazona* or *Rhopalea*. The eggs are as large as may be found within the Ascidiacea, but unfortunately the only colony known to contain embryos does not possess stages more advanced than one with the tail half-grown. Up to this stage no growth in size of the whole embryo has occurred, but so large a quantity of yolk is almost certain to cause a departure from the primitive development described above.

The primitive egg-size for ascidians is seen to be from 0·10 mm to 0·20 mm diameter, 0·11 mm in the Molgulids, 0·13 to 0·19 mm in Pyurids and Styelids, 0·14 to 0·18 mm in Ascidiids, and 0·16 to 0·17 mm in Cionids.

Accumulation of yolk must accordingly have occurred in members of these families the egg-size of which exceeds the above values.

2—*Family Pyuridae and Molgulidae*

Pyuridae—Accumulation of yolk has only occurred to a slight extent. Development was followed in two forms, *Boltenia* and *Tethyum* :

(*a*) *Boltenia echinata* (Linn), and *B. hirsuta* (Agassiz), egg 0·18 mm diameter, development is primitive, the tadpole hatches after 34 hours at 16° C, and has a free swimming period of about 10 hours, while 2 protostigmata develop on each side and are functional long before the rest appear, as in *Ciona*, *Ascidia*, and *Styela*.

(*b*) *Tethyum pyriforme americanum* (Rathke), egg 0·26 mm diameter. The tadpole hatches 60 hours after fertilization, at 16° C. Development is primitive except that, in place of the first two, the first four primary stigmata function together on each side (*see* Part I, pp. 48–50).

Molgulidae (*see also* Part II) :

(*a*) Species with small eggs, *i.e.*, 0·11 diameter, form tadpoles at 16° C in about 12 hours. Such tadpoles hatch by digestion of the egg membrane and have a free swimming period of at least some hours.

(*b*) Species with larger eggs, *i.e.*, 0·21 mm (*Molgula citrina* and *M. complanata* (Alder and Hancock)), at 16° C form tadpoles that hatch after about 150 hours from fertilization. Such tadpoles hatch by rupture of the egg membrane and have a free

swimming period usually of only a few minutes. The embryo attains a stage equivalent in structure to that which hatches in species with small eggs, about 30 hours before hatching.

The larval eye has been lost throughout the Molgulidae and in many Styelids. The otocyst is retained in all forms except the anural larvae of some Molgulids (*cf.* Part II). Correlated with increase in egg-size in Molgulids there is increase in duration of embryonic development, but this increase exceeds that to be expected from increase in egg-size alone (the larger egg of *Tethyum* developing in less than one-half the time). This excess increase seems to be correlated with a shortening of the free swimming period.

3—*Family Styelidae and Botryllidae*—Figs. 6, 7, 8, 9, 10

These two families form a single natural group, there being greater differences between members of the Styelidae than between some Styelids and the Botryllidae.

Species investigated were *Styela partita*, *Styelopsis grossularia*, *Polycarpa rustica* and *P. obtecta*, *Polyandrocarpa tincta*, *Distomus variolusus*, *Stolonica socialis*, *Symplegma viride*, *Botryllus schlosseri* and *B. gigas*, and *Botrylloides leachi*. The last 6 genera are budding forms. All but the first are viviparous.

a—*Styela partita* (Stimpson), egg about 0·15 mm diameter, development primitive, tadpole formed in 18 hours at 16° C, hatching by digestion; free swimming period lasts for some hours. The atrial invagination occurs somewhat before hatching, as in Pyurids and Molgulids. The otocyst is normally developed, but the larval eye is very small and may be degenerating. It has disappeared in the other 6 genera mentioned above, although light sensitivity may be reacquired (*cf.* Grave, 1932).

b—*Polycarpa rustica* (Linn), *P. obtecta* (Traustedt), and *Polyandrocarpa tincta* (Van Name) have eggs 0·19–0·24 mm diameter, and form tadpoles at 16° C in about 40 hours. Apart from the absence of a larval eye, the tadpoles and post-larval development are similar to those of *Styela*. Two protostigmata on each side function together about 280 hours after fertilization. Others are added as growth proceeds; subdivision into rows of definitive stigmata occurring when 6 or 7 protostigmata have been formed. The heart commences to beat at about 200 hours from fertilization.

N.B.—The eggs of these forms are of the same size as those of *Molgula citrina* and *complanata* and yet develop to the tadpole stage in one-quarter the time.

c—*Styelopsis grossularia* (van Beneden), egg 0·48 mm diameter. At 16° C the tadpole becomes active and is usually liberated about 160 hours after fertilization, although hatching from the egg membrane occurs through digestion 45 hours earlier. The free swimming period lasts from 5 to 30 hours. The atrial invagination occurs between hatching and liberation.

Protostigmata become functional about a week after settling, 4 on each side as in *Tethyum*. Of these the first is much elongated with traces of the processes that will divide it into a row of stigmata already in evidence, while the fourth is small and circular. This antero-posterior gradient is equally obvious in the later stage shown in fig. 6.

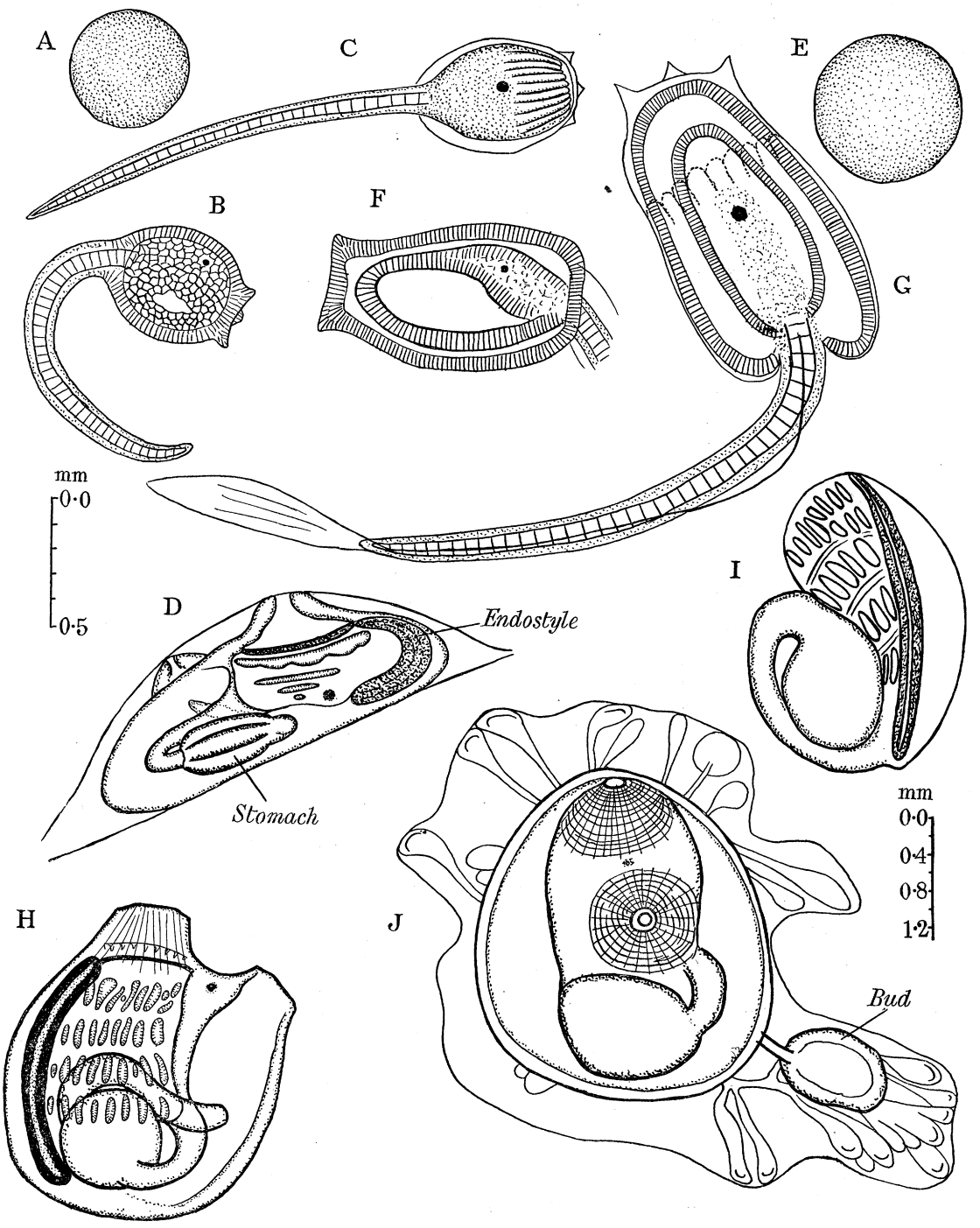

FIG. 6—Development of *Styelopsis grossularia* and *Distomus variolosus*. A, egg; B, early tadpole with trace of sensory pigment; C, fully formed tadpole of *Styelopsis*; D, metamorphosed *Styelopsis* with functional stigmata. E, egg; F, early tadpole with trace of sensory pigment; G, fully formed tadpole (shown in optical section to show cell sizes, etc.) of *Distomus*; H, metamorphosed *Distomus* with functional stigmata; I and J, much older individual showing subdivision of first row of stigmata and showing formation of first bud

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d—*Distomus variolosus* (GAETNER), egg 0·59 mm diameter. At 16° C the tadpole hatches from the egg membrane by digestion after 125 hours, becomes active and is liberated from the parent 190 hours from fertilization. Atrial invagination occurs

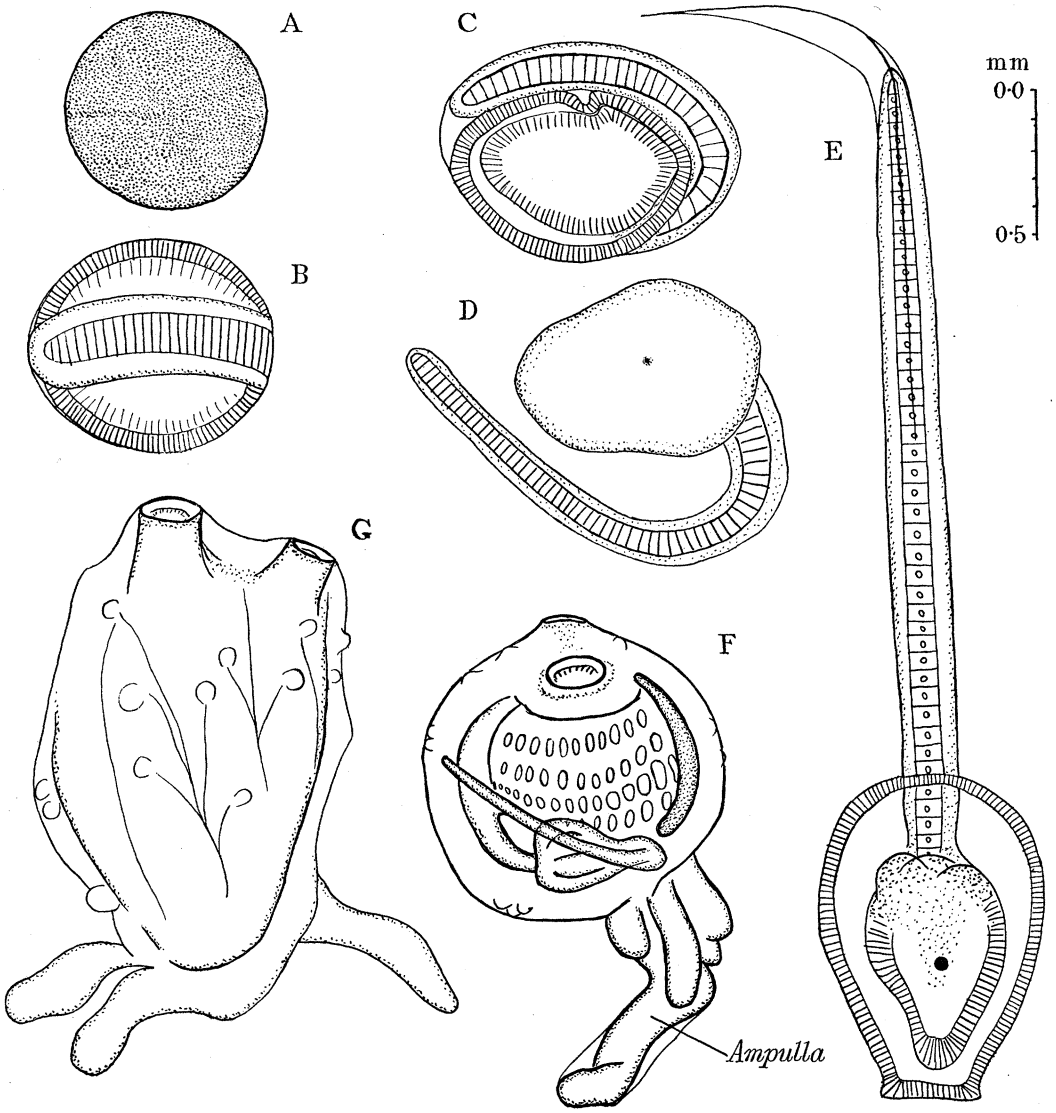

FIG. 7—Development of *Stolonica socialis*. A, egg ; B and C, early tadpole stages to show cell-numbers and sizes ; D, tadpole immature but with trace of sensory pigment and already hatched from egg membrane, though not liberated from parent ; E, mature tadpole shown in optical section ; F, metamorphosed form with functional stigmata ; G, somewhat older form showing expansion of basal ampullae and reduced nature of the more distal ones

between hatching and liberation. The free swimming period is about 18 hours. There is some degree of embryonic growth, due chiefly to the separation of the ectoderm and endoderm to form the primary body cavity.

The siphons open and currents (indicating stigmata activity) are established

11–12 days after settling. The post-branchial gut functions (as evidenced by faeces) after another 2 days, while the first bud appears after 2 months. The heart commences to beat about 360 hours from the beginning of development.

Protostigmata are not formed, 4 rows of definitive stigmata appear from the very beginning, and so far as can be determined develop as independent perforations of the branchial wall during the first few days after settling. Such development is more like that of a blastozooid than of an oozoid.

e—*Stolonica socialis* (HARTMEYER), egg 0·72 mm. diameter. At 16° C the tadpole hatches from the egg membrane by digestion after 130 hours, becomes active and is liberated after 240 hours from fertilization. Embryonic growth occurs to a greater degree than in *Distomus*. The atrial invagination commences shortly before liberation. The duration of the free swimming period is on the average 30 hours, but may be much longer or shorter. The heart first beats after 390 hours.

The stigmata develop exactly as in *Distomus*, 4 rows of definitive stigmata developing as independent perforations. They become active about 6 days after settling while the post-branchial gut functions after 11 days.

f—*Symplegma viride* (HERDMAN),* egg 0·44 mm diameter. At 16° C the tadpole becomes active about 140 hours after fertilization, although hatching from the egg membrane occurs somewhat earlier. There is a very slight degree of embryonic growth between hatching and liberation, during which time the atrial invagination commences. The free swimming period is from 3 to 12 hours.

On settling, 8 ampullae grow out radially from the middle region of the trunk, and in those individuals that fail to become fixed there is visible a single anterior extension corresponding to the " stalk " of *Diazona* ; this normally is pressed against the substratum by the lateral ampullae and becomes reabsorbed.

Forty-eight hours after settling, the trunk is expanding rapidly and the heart beat commences, emptying and filling the 8 ampullae alternately. These join to a common vessel before entering the body.

Seventy hours after settling, the siphons and stigmata function. Three protostigmata on each side first become active together ; a day later a fourth is added. During the next two days there is much growth of the oozoid as a whole, and the protostigmata necessarily elongate. Two or three days later these are found to have become divided into 4 rows on each side while 3 or 4 longitudinal bars appear internal to them. Therefore, while functional protostigmata develop, a stage is rapidly reached similar to the young oozoids of *Distomus* and *Stolonica*.

There are many varieties in colour and form of *Symplegma*, just as there are of *Botryllus* and *Botrylloides*, and the above development is typical of compact black-and-white colonies with very short budding stolons and of orange colonies in which the zooids are widely separated by long stolons. There are many other varieties similar in form to the black-and-white colonies but differing in colours, and from one of these, unfortunately not identified, were obtained tadpoles similar to those described above

* With *Symplegma* and all forms investigated at Bermuda (*cf.* p. 257) development was followed at 26° C. The developmental times have been doubled in order to correct for 16° C. (*cf.* fig. 18).

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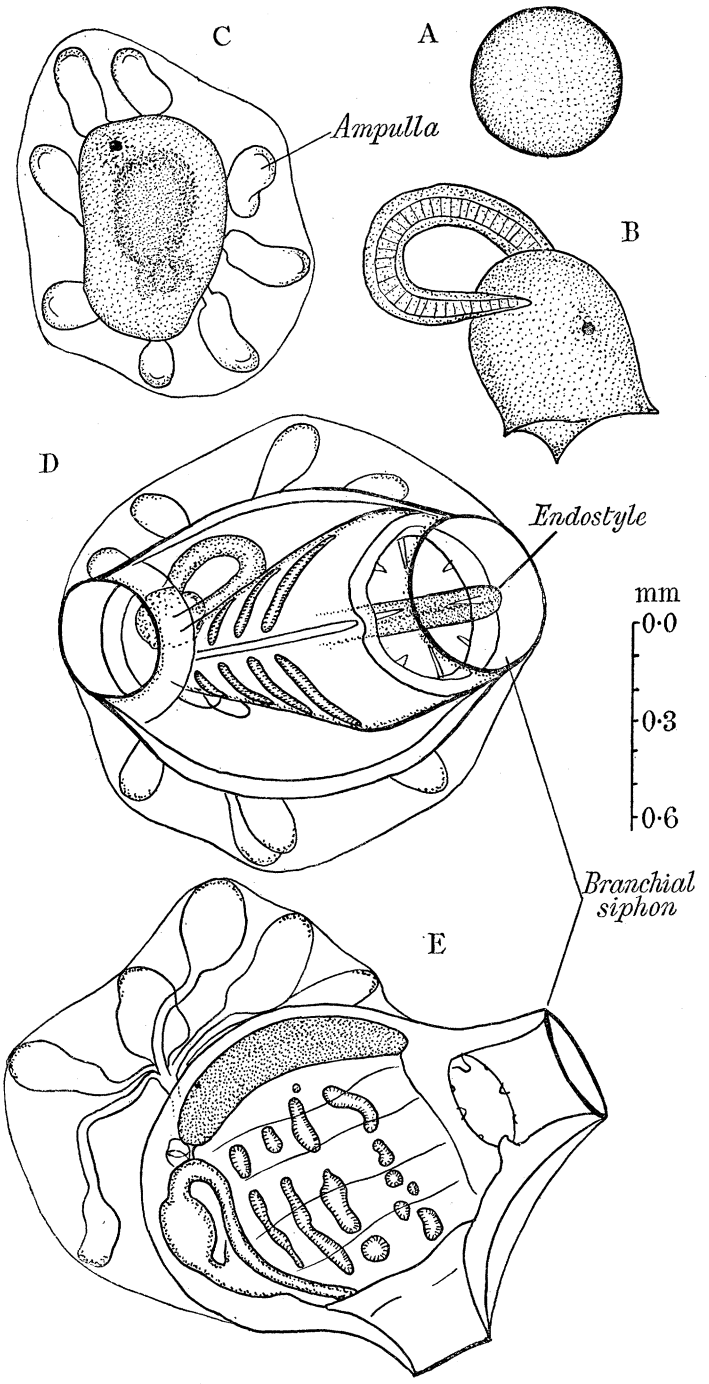

FIG. 8—Development of *Symplegma viride*. A, egg ; B, mature tadpole ; C, metamorphosing individual showing outgrowth of the 8 ampullae ; D, metamorphosed form with functional heart and stigmata, viewed from above ; E, somewhat older individual in which the 4 protostigmata have become subdivided into 4 rows

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but with a subsequent development that was dissimilar. Three protostigmata developed and became functional 3–4 days after settling, as in the first type. Instead, however, of a fourth being added and then all becoming subdivided, successive protostigmata appeared until 9 were functional on one side and 8 on the other, which, 18 days after settling, showed no sign of subdivision.

g—*Botryllus gigas* (HARTMEYER), egg 0·45 mm diameter. The embryo gastrulates after 60 hours at 16° C, hatches by digestion of the egg membrane after 145 hours, becomes active and is liberated from the parent 190 hours from fertilization. Sensory pigment first appeared after about 155 hours from fertilization. The free swimming period lasts about $2\frac{1}{2}$ hours.

The development of *Botryllus* differs markedly from that of the preceding genera. Compared with *Symplegma*, which has eggs of the same size, the development is prolonged. There is much embryonic growth, while the active tadpole has a highly differentiated trunk region with 8 large ampullae arranged around the anterior mental process (again the " stalk " of *Diazona*) ; there is also a well-developed gut, and a branchial sac with protostigmata in the form of 8 perforations on one side, 6 on the other. The trunk increases rapidly in bulk after settling, while the heart and stigmata function after about 48 hours, the post-branchial gut after another 60 hours. In the oozoid of *Botryllus gigas*, and *B. schlosseri*, as also in *Botrylloides leachi*, the protostigmata never became subdivided into definite stigmata. This is due probably to too short a life.

The first blastozooid is visible a few hours after settling but grows rapidly only after 12 days have elapsed. It then grows from one-third the size of the oozoid to its full size and becomes completely functional within 3 days, during which time the oozoid degenerates and is absorbed by the bud.

The first blastozooid is in turn replaced by the second after 4 days, and that by a third generation after about another 5 days.

In the first and second buds the stigmata develop as four rows of independent perforations, just as in the oozoids of *Distomus* and *Stolonica*. In later generations the number of rows tends to increase until more than 10 are formed in adult buds.

h—*Botrylloides leachi* (SAVIGNY), egg 0·26 mm. diameter. As in *Botryllus*, the inter-cleavage interval is about 4 hours, at 16° C. Gastrulation commences from 50 to 60 hours after fertilization, the blastopore closes after 80 hours, sensory pigment appears after 160 hours, and liberation of the tadpole about 220 hours after fertilization. The post-larval development of *Botrylloides* is in all essentials identical with that of *Botryllus*, differing in the final arrangements of the zooids but similar even to the onset and frequency of the successive bud generations.

The oozoid develops 7 protostigmata on one side, 9 on the other ; the first blastozooid has four rows of definitive stigmata on each side, while in the second blastozooid the first row on each side is doubled through half its extent as in the budding oozoid of *Distomus*.

The free swimming period of *Botrylloides* is about one hour, *i.e.*, shorter than that of *Botryllus*, but at the same time the swimming tadpole is somewhat better developed

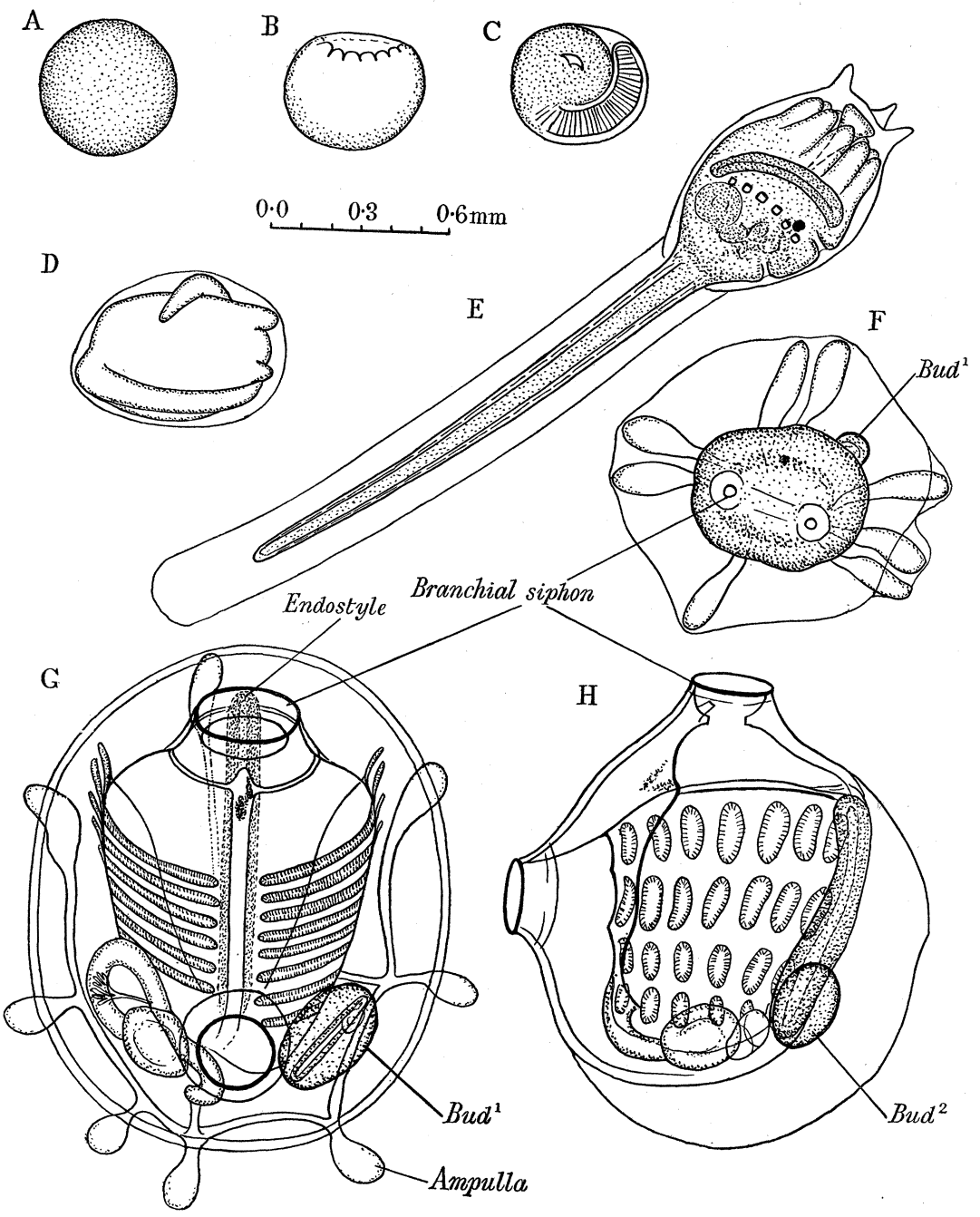

FIG. 9—Development of *Botryllus gigas*. A, egg ; B, gastrula ; C, very early tadpole stage showing delimitation of notochord cells ; D, later tadpole stage showing curling of tail over trunk ; and E, mature tadpole showing perforate but non-functional protostigmata ; F, metamorphosing form showing outgrowth of the 8 ampullae and of the first bud ; G, fully developed oozoid viewed from dorsal side and showing first bud and the unequal number of protostigmata on the two sides ; H, fully developed first bud (oozoid absorbed) showing the 4 rows of definitive stigmata and the rudimentary second bud

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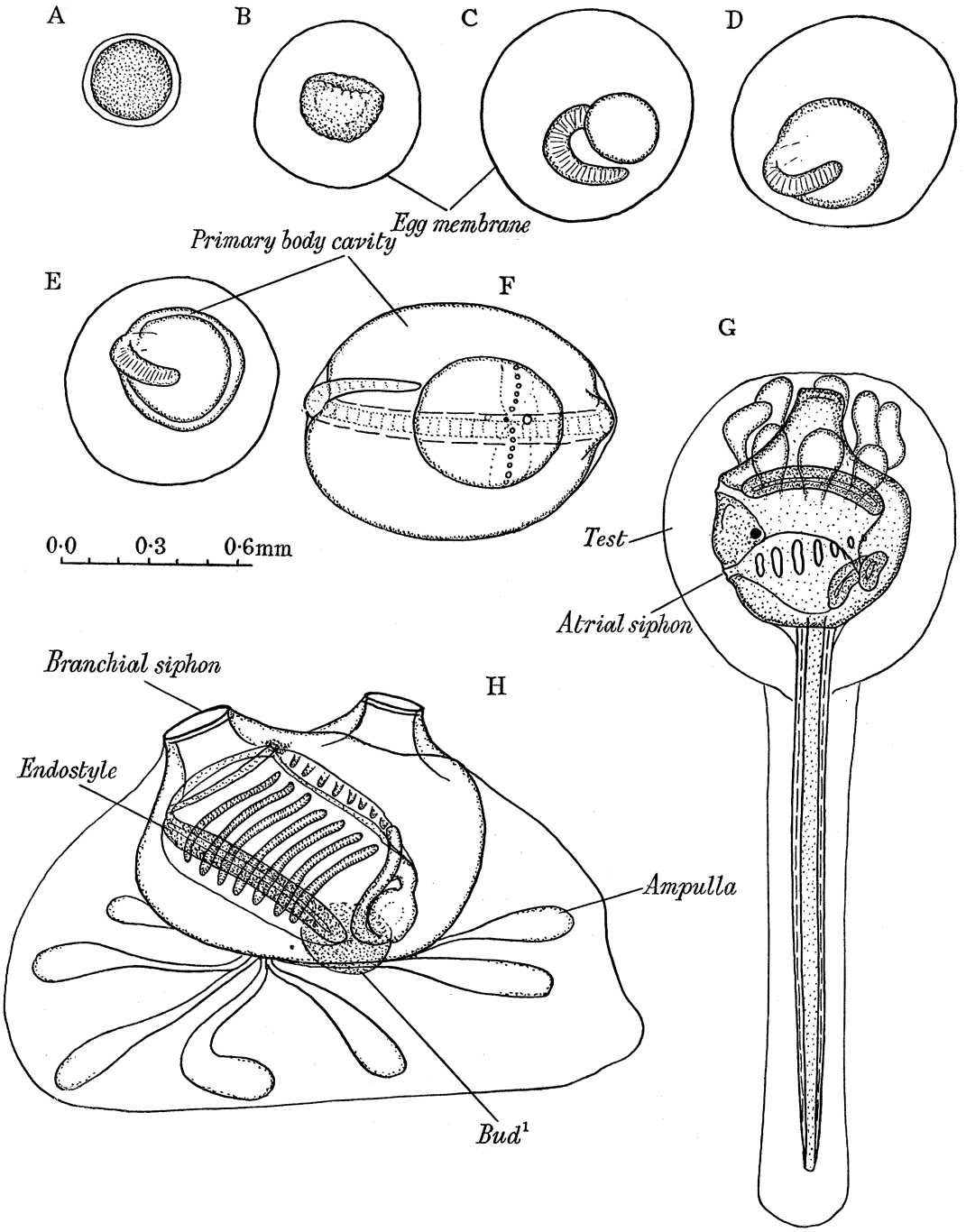

FIG. 10—Development of *Botrylloides leachii*. A, egg; B, gastrula showing growth of perivitelline space; C, young tadpole stage showing delineation of notochord cells and further growth of perivitelline space; D, E, young tadpole stages showing growth of trunk region and precocious appearance of primary body cavity; F, immature tadpole stage with enormous primary body cavity, with trace of sensory pigment, with perforate protostigmata, and stretching egg membrane; G, active tadpole with outgrowing ampullae, stigmata perforate but not functional; H, fully developed oozooid with rudimentary first bud

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in that the 8 ampullae are as well formed as they are in *Botryllus* shortly after the absorption of the tail.

The great difference between the two genera concerns their embryonic development, in spite of the similarity of larval and post-larval stages.

In *Botryllus* the embryos each rest in cup-shaped extensions of the peribranchial wall, from which they eventually escape through the atrial aperture into the common cloacal cavity.

In *Botrylloides* placental diverticula in the region of the gonads each contain a developing embryo, the escape of which into the cloacal cavity is effected by rupture of the placental chamber. Associated with such placental development is a secondary reduction in egg-size, as in *Salpa*, so that while the tadpole is almost identical in size and organization with that of *Botryllus*, it develops from an egg of one-fifth its volume.

The main features of the development are the great increase in size, due mainly to the extension of the primary body cavity, and that the major part of this growth occurs after a small tadpole in form has been evolved ; so that if there is considerable extra-cellular nutrition of the embryo, it is difficult to understand how absorption can take place evenly through the embryo as a whole except in the early stages.

For further discussion, *see* pp. 321–323.

4—*General*

The Styelids and Botryllids described above comprise a single natural group within which there has been considerable divergent evolution.

On the one hand there are the numerous species of the solitary genus *Polycarpa* and the budding *Polyandrocarpa*, both with small eggs. From this stock, by a process of increase in rate of budding and resultant decrease in body-size, the Botryllids, *Symplegma*, *Botryllus*, and *Botrylloides* may be derived, involving an increase in egg-size. On the other hand is the group *Styelopsis*, *Distomus*, *Alleocarpa*, and *Stolonica*, which conceivably may be derived from a stock such as *Symplegma*, or may be more closely related to the more primitive *Styela-Polycarpa* stock. In any case, they all possess eggs larger than those of the Botryllids and accordingly represent either a continuation of the process of egg growth or of yolk accumulation in that group, or an independent ovarian evolution. There is some embryological evidence, whatever may be that of adult structure, that indicates an essential unity.

Forms such as *Styela*, *Polycarpa*, and *Polyandrocarpa* possess small eggs from which tadpoles hatch by a process of membrane digestion to become immediately active. As yolk accumulates, the primitive digestion method of hatching is usually replaced by that of rupture at a later period by the swelling embryo, as in Molgulids and the Aplousobranchs. In *Styelopsis*, *Distomus*, *Stolonica*, *Symplegma*, and *Botryllus*, the embryos hatch by the digestion method, but at a time long before the tadpole is fully formed or active.

The significance of this will be discussed elsewhere, but in the present connection it indicates either a close relationship between the two groups or else is a good example of convergence.

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Accumulation of yolk usually obliterates what perivitelline space there may be in the egg, and if the accumulation be considerable the egg membrane itself may become stretched, and the follicle cells on its outer surface flattened out and separated. The result is that there is relatively little room in which the embryo can form a tail and tail fin. These consequently are flattened and compressed against the trunk around which they coil.

Styelids with small eggs, Molgulids, Cionids, and Ascidiids are alike in that the tail curves round the embryo in the sagittal plane and in having some freedom of movement in the perivitelline space. The tail and tail fin in these forms, when the tadpole emerges, are vertical and typical of chordates as a whole.

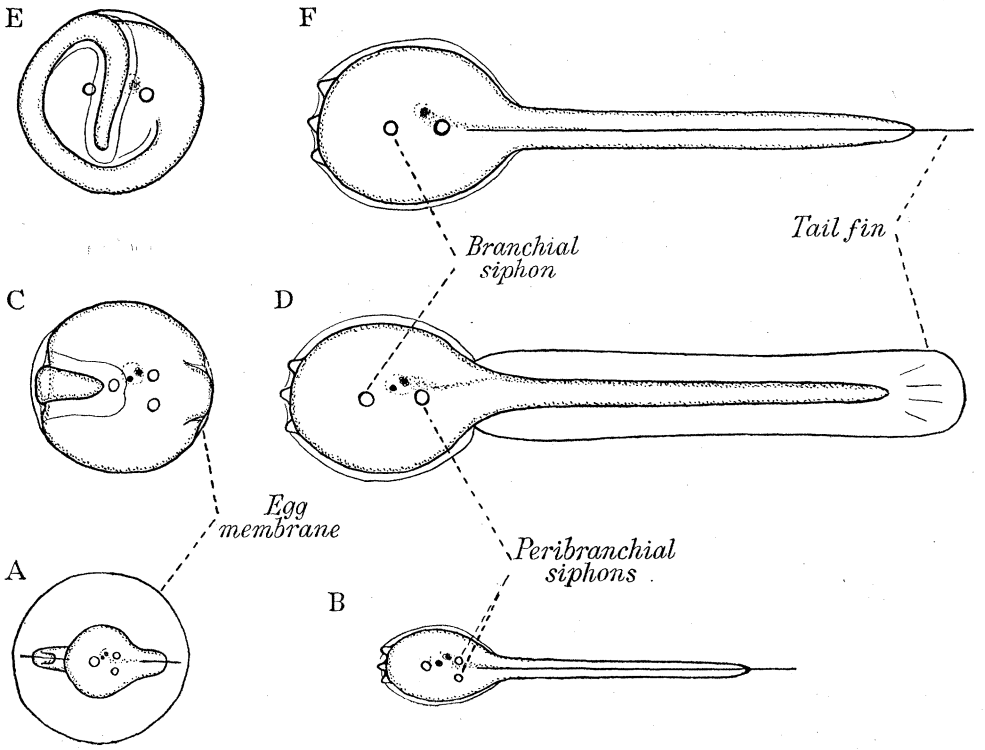

FIG. 11—Diagram to illustrate the two ways of coiling the tail around the trunk in embryos developing from large eggs with little or no perivitelline space. A, B, embryo and tadpole of primitive unmodified type with small-egg and large perivitelline space; C, D, large-egged forms with reduced perivitelline space but with tail still coiled round the sagittal axis of the trunk, resulting in a twist of 90 degrees; E, F, large-egged forms in which the tail is coiled round the transverse axis of the trunk so that the twist of the tail in the final tadpole is obviated

In the Aplousobranchia and Perophoridae the developing tail is still coiled vertically, but owing to compression it is twisted through 90 degrees so that the tail fin is flattened against the body of the embryo. Consequently, after hatching, the tadpole is seen to possess horizontal instead of vertical tail fins, while the nerve cord lies along one side instead of along the mid-dorsal surface.

In the Botryllids, and the Polystyelids that have large eggs, the tail is coiled not

vertically but horizontally around the embryo. On hatching, therefore, the tail and tail fin that were pressed against the body assume their proper vertical position. There is no twist.

Since in the more primitive Styelids and Polystyelids the tail is coiled vertically, this horizontal coiling can only be of the nature of an adaptation to the compressed condition. Accordingly the fact that both groups, Botryllids and Polystyelids (including *Styelopsis*), show the same adaptation is further evidence that there has been but one primary instance of marked accumulation of yolk within the family.

In any case there has been divergent evolution in development, from Styelopsis to *Distomus* and *Stolonica*, and from *Symplegma* to *Botryllus* and *Botrylloides*, whether or not the large eggs of *Styelopsis* and *Symplegma* represent related or parallel development among themselves.

In the change *Symplegma*——→*Botryllus* there has been what can only be called a telescoping (*cf.* MacBride, 1914) of development. This must be due either to a relative retardation of tail development or an acceleration of trunk development, without any important change in the nature of the developmental processes themselves.

In the development of the bud of *Botryllus*, compared with that of the egg, there is the usual difference that is found between regenerative and ontogenetic development, namely, an abbreviation eliminating all stages of phylogenetic interest. In the change from *Styelopsis* to *Distomus* and *Stolonica* there is the same difference regarding the mode of formation of the stigmata, the last structures to develop, as there is between that of the *Botryllus* oozoid and blastozooid.

An attempt to interpret such differences will be left until the development of the remaining two orders of Ascidians has been described.

5—*Family Perophoridae*—Figs. 12, 13

In this family is included the genus *Ecteinascidia*, although by some authors *Perophora* and *Ecteinascidia* are widely separated.

The forms studied were *Perophora listeri* and *P. viridis*, *Ecteinascidia turbinata*, and *Ecteinascidia conklini*. The chief differences between these two genera are in the number of rows of stigmata and in size ; the tadpole larvae differ also with respect to these two characters but are identical in all other important features. Considering the peculiar nature of the tadpole, this similarity is strong evidence for an extremely close relationship between these genera.

a—*Perophora listeri* (Forbes), and *P. viridis* (Verrill), eggs 0·24 mm diameter. The intercleavage interval at 16° C was about 4 hours. At 16° C gastrulation commences 45 hours after fertilization, the blastopore closes after 60 hours, sensory pigment first appears after 120 hours, while hatching by rupture of the distended egg membrane occurs after about 185 hours. The tadpole of *P. listeri* swims for about 3 hours, that of *P. viridis* (according to Grave, 1921) from 4 to 5 hours.

In the swimming tadpole the branchial sac is contractile though not open, while the heart not only is fully formed but exhibits the characteristic rhythmical reversal of beat. The stigmata function 10 hours after settling, at which time four ampullae

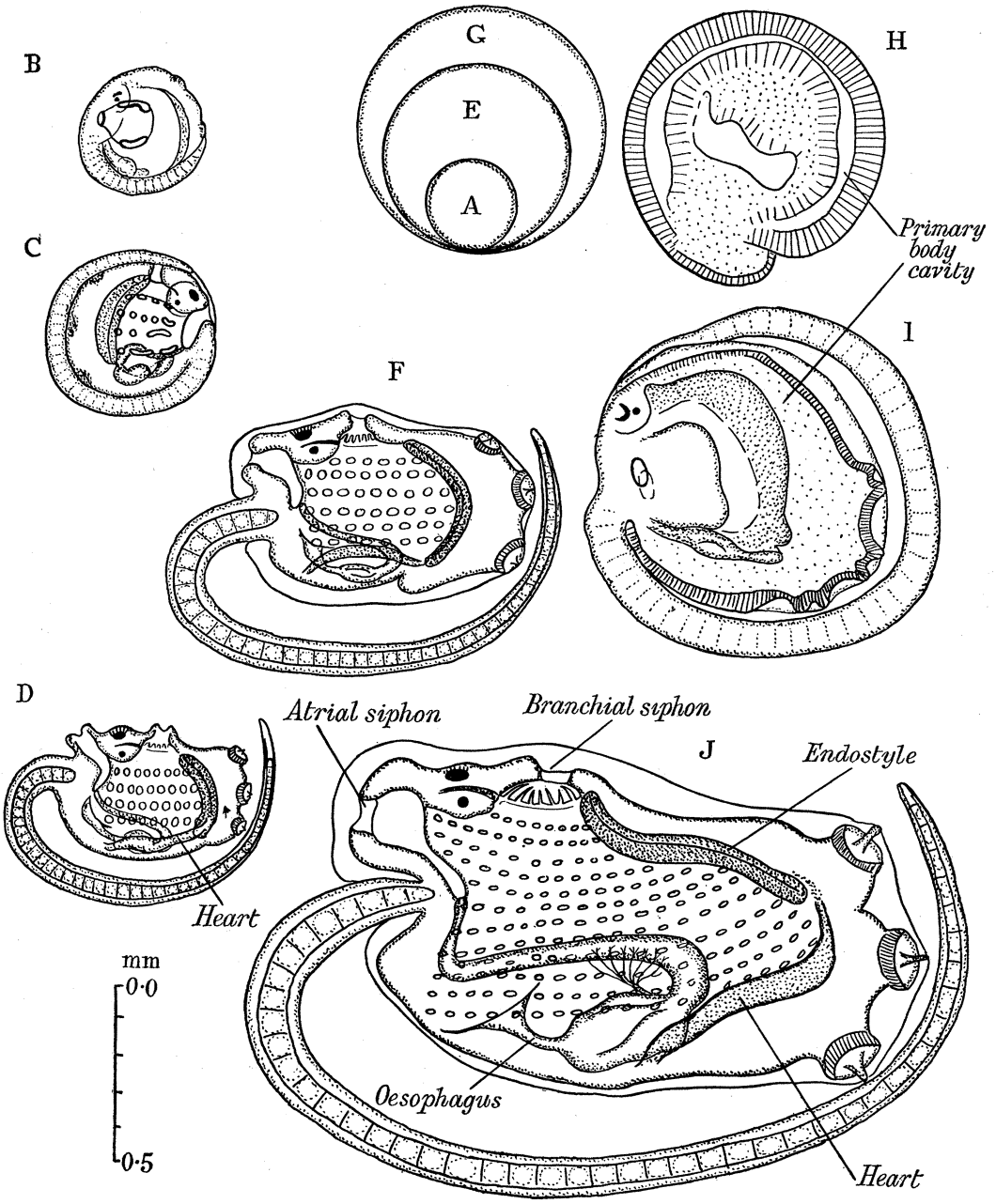

FIG. 12—A, B, C, and D, development of *Perophora listeri* up to the tadpole stage. A, egg ; B, early tadpole with trace of sensory pigment and with two perforate but non-functional protostigmata ; C, later stage showing subdivision of protostigmata into rows of definitive stigmata ; D, active tadpole with beating heart ; E, egg ; F, active tadpole of *Ecteinascidia conklini* ; G, egg ; H, embryo ; I, early tadpole stage with trace of sensory pigment but with no sign of protostigmata ; J, active tadpole of *Ecteinascidia turbinata*

have grown out from the anterior region and firmly fixed the oozoid to the substratum. Sixty hours after settling, a vascular septum forms in one of the ampullae; this ampullae thus possesses an afferent and efferent blood stream and becomes the main stolon. After a further 85 hours the post-branchial gut functions completely.

Throughout the latter part of the embryonic period there is considerable growth in size, and during that time the trunk region develops to a much more advanced stage even than that of embryos of *Botryllus*, *i.e.*, there is a great "telescoping." In the development of the stigmata, however, there is also acceleration and abbreviation.

In all members of the Phlebobranchia and Aplousobranchia, whether development is primitive or modified, the atrium develops not as in the Stolidobranchia by a single bifurcating invagination over the nerve cord, but by a right and a left invagination that later fuse together in the mid-dorsal line.

In *Perophora* the paired invaginations occur during the early embryonic period, and fuse to form the single atrial siphon long before hatching, in contrast to young *Ciona* and *Ascidia* in which fusion occurs after a month or two of post-larval life.

The stigmata themselves develop in a manner intermediate between those of the oozoid and the blastozooid of *Botryllus*. The final condition in the tadpole is four rows of definitive stigmata on each side.

In young embryos a single protostigma is formed on each side as the peribranchial sacs come into contact with the branchial wall. It divides rapidly into two and then four, and at the time the peribranchial apertures fuse to form the atrial siphon, 4 rows of definitive stigmata develop in line with the 4 protostigmata just described. There is therefore some abbreviation and much acceleration of the primitive process of stigmata development. It should be noted, however, that both in *Perophora* and in the more primitive development of *Ciona* and *Ascidia*, the formation of rows of definitive stigmata coincides with the fusion of the peribranchial sacs to form the atrium, although in the first case both occur before hatching, in the second many weeks afterward.

b—*Ecteinascidia turbinata* (Herdman), egg 0·72 mm diameter. The intercleavage interval is about 10 hours at 16° C, while at 16° C gastrulation commences 110 hours, sensory pigment appears 340 hours, and hatching, by rupture of the distended egg membrane, occurs 420 hours after fertilization.

The whole course of development resembles that of *Perophora*, except that in view of the greater size of the egg it is correspondingly longer. The free-swimming period lasts from three to six hours.

Instead of 4 rows of definitive stigmata, however, 12 rows are formed. These first function 2 or 3 days after settling. The heart, as in *Perophora*, commences its reversing beat often before liberation of the tadpole. In the adult there are about 30 rows of stigmata.

c—*Ecteinascidia conklini* (Berrill), egg 0·58 mm diameter. The adult of this form differs from that of *E. turbinata* chiefly in having only 20 rows of stigmata. At 16° C the tadpole hatches by rupture about 330 hours after fertilization.

Except for the size of egg and length of embryonic period, the development of this species is almost identical with that of *E. turbinata*. The only important difference is that 6 rows of definitive stigmata are formed in place of 12. Thus this species, both in its development and adult structure, bridges the gap between *Ecteinascidia turbinata* and *Perophora*.

d—Ecteinascidia conklini var. minuta (BERRILL). The eggs within one and the same individual tend to be of two sizes, 0·48 and 0·52 mm diameter. The embryonic

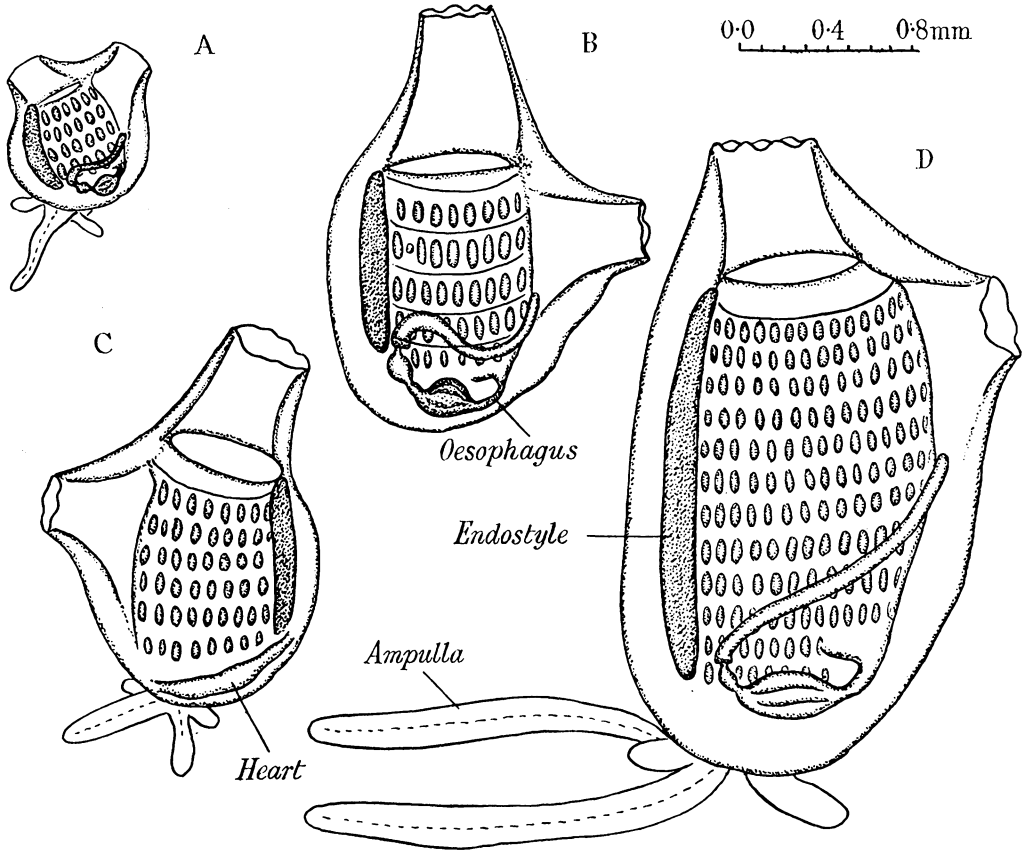

FIG. 13—Oozoids with hearts and stigmata both functional of the forms shown in fig. 12. A, *Perophora*; B, C, left and right side of *Ecteinascidia conklini*; D, *Ecteinascidia turbinata*

period is somewhat shorter than that of the type species, but otherwise the development is identical. Six rows of stigmata are formed in both. In the adult of this variety, however, the number of rows of stigmata rarely exceeds 15.

6—*Family Clavelinidae*—Fig. 14

The Clavelinidae is a family more heterogeneous than most and in modern classifications is a combination of the old families Clavelinidae and Polycitoridae. In the most recent publication (MICHAELSEN, 1931) they are retained as sub-families. In the present connection the two families will be treated as distinct.

a—*Clavelina lepadiformis* (MÜLLER), egg 0·26 mm diameter. At 16° C gastrulation occurs at about the same time after fertilization as in *Perophora* (from 40 to 50 hours). There is a trace of sensory pigment after 145 hours, although hatching by

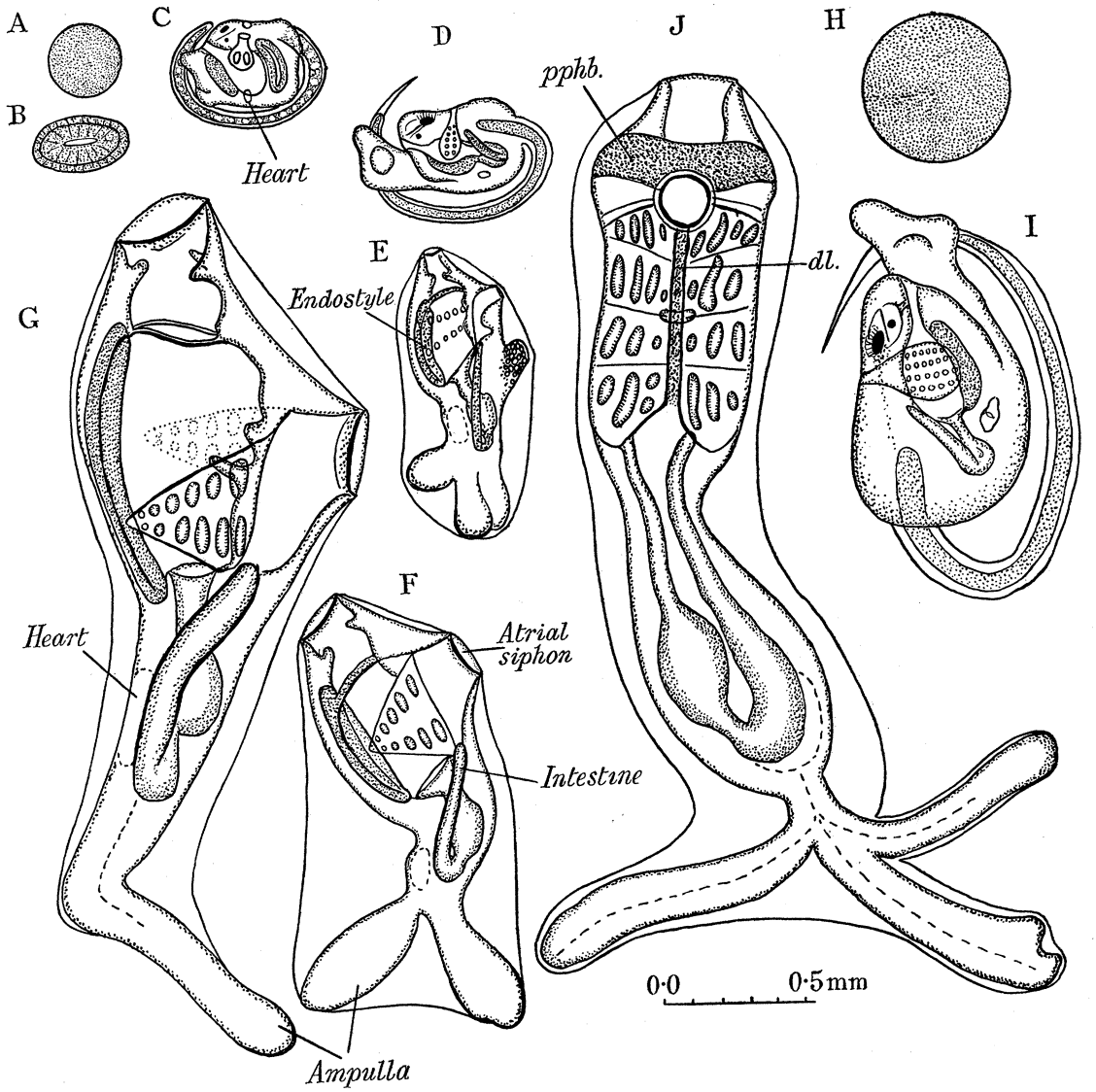

FIG. 14—Development of *Clavelina*. A–G, *Clavelina lepadiformis*; H, J, *Clavelina picta*. A, egg; B, embryo; C, early tadpole with trace of sensory pigment and with two protostigmata; D, active tadpole with two rows of stigmata; E, metamorphosed form with tail incompletely absorbed; F, G, two stages in growth of oozoid (the heart begins to beat at stage E); H, egg; I, tadpole showing four rows of stigmata; J, fully metamorphosed form viewed from dorsal side and corresponding to stage F of *Cl. lepadiformis*. *dl*, dorsal lamina; *pphb.*, peripharyngeal band

rupture of the distended egg membrane does not occur until 220 hours have elapsed. While the egg is of the same size as that of *Tethyum pyriforme* the embryonic period is therefore nearly four times as long.

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When the gut attains approximately that stage of development to be found in the swimming tadpoles of *Ciona* or *Ascidia*, the pair of peribranchial invaginations appear. On reaching and fusing with the branchial wall 2 protostigmata form on each side; as the area of fusion extends the 2 protostigmata develop into 2 rows of definitive stigmata. At the same time the peribranchial apertures fuse to form the single atrial siphon. Accordingly there are the same phenomena of telescoping, acceleration, and abbreviation of development as in *Perophora*, although the two genera are widely separated.

The free-swimming period lasts about 3 hours.

The heart starts beating 40 hours after settling, while the stigmata function about the same time as the post-branchial gut, 60 hours after settling.

As in *Perophora*, many tadpoles settle and grow normally while still in possession of a mobile tail, as though there is a relative independence in the development and function of tail and trunk.

The peculiar feature in the development of *Clavelina lepadiformis*, as also in *Clavelina rissoana*, and *Clavelina oblonga* (egg 0·31 mm diameter), is that only 2 rows of stigmata are formed in the post larva. This will be discussed later, although it may be stated here that this fact is not evidence that the primitive number of protostigmata in ascidians is two and not three, as some authors have suggested.

b—*Clavelina picta* (Verrill), egg 0·48 mm diameter. The development of this form is almost identical with that of *Clavelina lepadiformis*. As the egg is larger so development is slower and the tadpole hatches by rupture, not 220 hours after fertilization but after about 335 hours at 16° C. One striking feature is that some time before hatching numerous purple cells appear along the endostyle and as a peripharyngeal band. These eventually extend throughout the body and give rise to the typical purple colour of the adult colonies.

The tadpoles have a free-swimming period of about 2 hours. The heart beats within 24 hours, reversing from the very first, while the stigmata function 36 hours later.

The important difference between this species and the last is that 4 rows of stigmata develop in place of 2, although in other respects the tadpoles and post-larval forms are remarkably alike in the 2 species.

c—*Pycnoclavella aurilucens* (Garstang), egg 0·28 mm diameter. This is a form included by Hartmeyer within the genus *Clavelina*, but it is one which is in some ways intermediate between the Clavelinidae and Synoicidae. It produces tadpoles that have undergone an enormous amount of growth in size as embryos. Their free-swimming period lasts usually but a few minutes, and they possess a single complex sense organ somewhat like that of Botryllid tadpoles. Four rows of stigmata are formed. Altogether, the tadpole larvae resemble those of *Colella* much more than they do those of *Clavelina*.

7—*Family Polycitoridae*—Fig. 15

This family in some respects links the Clavelinidae with the Synoicidae. The mode of budding alone tends to separate this group from the Clavelinids.

Three genera have been investigated, *Distaplia*, highly specialized as adult and in development; *Archidistoma*, adult apparently primitive but with development similar to that of *Distaplia*; and *Eudistoma*, adult intermediate between *Distaplia* and *Archidistoma*, but with more primitive development resembling that of Synoicids.

a—*Distaplia rosea* (Delle Valle), egg 0·42 mm diameter.

D. clavata, *D. magnilarva* (Delle Valle), and *D. bermudensis* (Van Name) in their development show no significant differences from that of *D. rosea*.

At 16° C the tadpoles of *Distaplia rosea* hatch by rupture of the distended egg membrane about 370 hours after fertilization. Sensory pigment first appeared after 220 hours.

Before there is any appreciable tail outgrowth, the archenteric cavity expands, and before the tail has half circled the trunk ectodermal knobs foreshadow the formation of the three large adhesive papillae. At about the time sensory pigment appears two peribranchial invaginations grow down to meet and fuse with the lateral walls of the pharynx and on each side two protostigmata appear. These rapidly divide to form four, which in turn extend to form four rows of definitive stigmata as in *Perophora*. Moreover, as the rows appear the peribranchial apertures fuse to form the atrial siphon.

The free-swimming period lasts somewhat over one hour, during which time the heart starts beating, exhibiting its regular reversal from the first, while the branchial region becomes contractile. The stigmata do not function until the moment of settling. As in *Perophora* and *Clavelina* there is a marked tendency for the tail to remain unabsorbed.

The post-branchial gut functions about 48 hours after settling. The vascular septum in the ampulla appears after another 150 hours, and then, 300–330 hours, at 16° C, after settling, the oozoid completely degenerates.

The first and second blastozooids, visible in some species even in the tadpole, start growing and differentiating *after* degeneration of the oozoid and become functional a week or ten days later.

Distaplia magnilarva is said to differ from *D. rosea* in that the oozoid degenerates a few hours after settling, but in a colony taken at Plymouth no such difference was found. The gut became completely functional 30 hours after settling while degeneration of the oozoid occurred 11 days later. Conceivably there are several varieties of this species.

Distaplia bermudensis behaves exactly like *D. rosea*, except that only one blastozooid grows up in place of the oozoid instead of two.

b—*Archidistoma aggregata* (Garstang). Egg 0·23 mm diameter. At 16° C the tadpole hatches by rupture of the egg membrane about 280 hours after fertilization.

While in type of adult colony this form represents a much simpler association than *Distaplia*, the development is at least as specialized. The tadpole possesses 8 ampullae anteriorly in addition to the 3 adhesive papillae, and in general has much the same structure developed as in the tadpole of *Distaplia*. There is a free-swimming period of 2 or 3 hours.

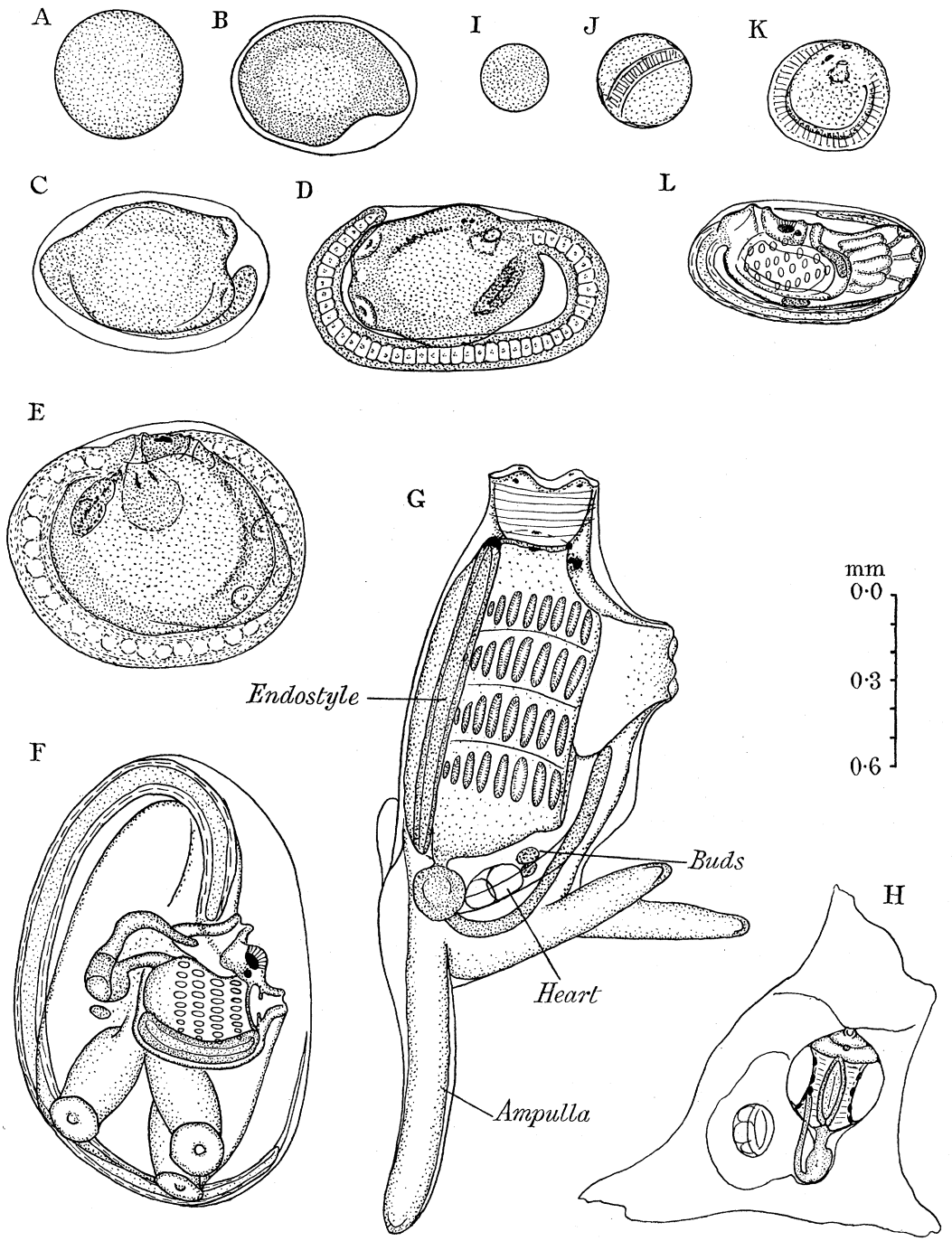

FIG. 15—A–H, development of *Distaplia rosea*; I–L, development of *Archidistoma aggregata*. A, egg; B, C, very early tadpoles showing precocious increase in size of trunk; D, E, young tadpoles with trace of sensory pigment and commencing peribranchial invaginations; F, active tadpole with heart and stigmata about to function; G, fully developed oozoid with two rudimentary buds; H, growth of buds after autolysis of oozoid; I, egg; J, young tadpole of *Archidistoma*; K, young tadpole with trace of sensory pigment; L, active tadpole (on point of hatching)

The peribranchial sacs invaginate separately and later fuse dorsally to form a single atrial siphon, and as in *Distaplia* 4 rows of stigmata are formed. No protostigmata were recognized and the 4 rows probably appear as independent perforations from the beginning.

8—*Family Synoicidae*—Fig. 16

This family is fairly uniform and well defined and in many respects more closely related to the *Cionid* and *Diazonid* stock than is any other group. All genera have fairly large eggs, although there is considerable variation. Of these, three have been studied.

Morchellium argus (Milne–Edwards), egg 0·34 mm diameter. The embryonic time was not determined with any degree of accuracy, but at 16° C is probably 260 plus or minus 40 hours.

As in the development of Perophorids, Clavelinids, and Polycitorids, there is considerable growth in size, the tadpole hatching by rupture of the distended egg membrane. During this period of growth separate peribranchial invaginations extend and fuse with the pharynx, 4 rows of definitive stigmata develop as independent perforations, and at the same time the atrial siphon forms through the fusion of the peribranchial apertures.

Four anterior ampullae are formed in addition to the 3 adhesive ampullae. In other forms the ampullae often break down into numerous small test vesicles.

In *Morchellium* the free-swimming period lasts usually 2 to 3 hours, but occasionally may last through 24. After about 30 hours from settling, the pharynx elongates and the heart starts beating, the siphons open and the stigmata function after another 5 to 10 hours, while the post-branchial gut becomes active only after 8 or 9 days.

In the tadpole and in much later stages there is a mass of yolky cells which comes to lie alongside the intestine, and by which they seem eventually to be absorbed. A similar mass is to be found in the tadpoles of *Eudistoma*, and to a lesser extent in those of *Didemnum*.

In both *Clavelina* and *Morchellium* the heart and gut descend into the stalk of fixation as soon as it appears. In *Diazona* such descent occurs many weeks after settling.

Two other Synoicids are figured, both possessing smaller eggs than *Morchellium*.

Sidnyum turbinatum (Savigny), egg 0·29 mm diameter.

It develops to form tadpole and post-larval stages identical with those of *Morchellium*, except in size.

Polyclinum sabulosum (Giard), egg 0·26 mm diameter.

The development is fundamentally the same as in the other two genera, except that the amount of excess yolk cells is much reduced and there is no elongation of the anterior end to form a stalk.

A fourth form, not figured, is *Amaroucium nordmanni* (Milne–Edwards). This has eggs larger than those of *Morchellium* but with a development identical except for size.

Amaroucium constellatum (Verrill), differs from the above in that the typical number of 4 stigmata rows in the oozoid is reduced to 3 (Grave, 1921).

The adult zooids of all these genera possess 7 or more rows of stigmata.

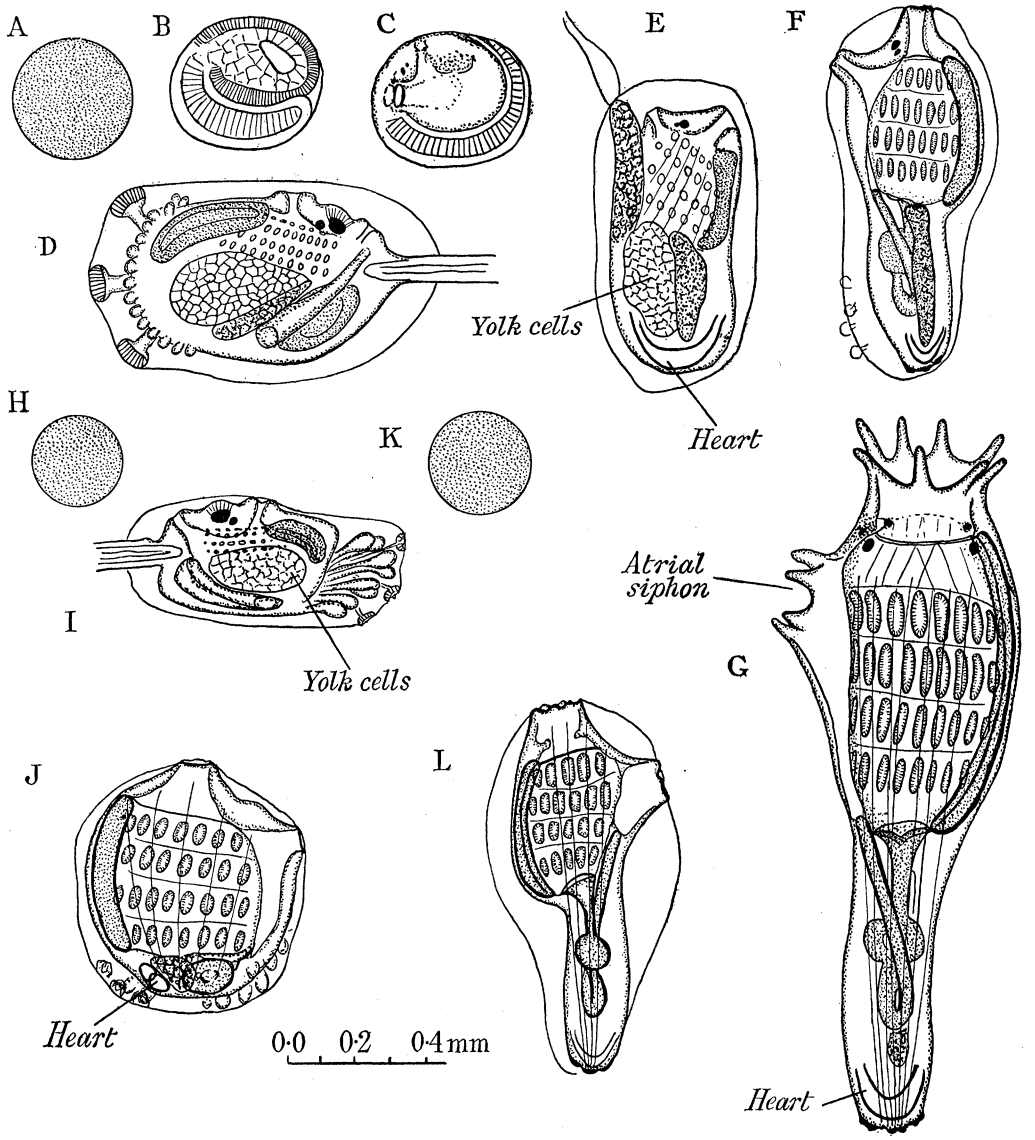

Fig. 16—A–G, development of *Morchellium argus*; A, egg; B, early tadpole stage; C, somewhat later stage with trace of sensory pigment and invaginating peribranchial sacs; D, anterior end of active tadpole showing residual endodermal mass; E, metamorphosing stage at which heart-beat commences; F, fully metamorphosed individual with functional stigmata; G, older individual with rudimentary post-abdomen and with endodermal mass absorbed; K, egg; L, stage corresponding to G, of *Sidnyum turbinatum*; H, egg; I, active tadpole; J, stage corresponding to G, of *Polyclinum sabulosum* showing that early development of an abdominal stalk depends upon a critical egg-size

9—*Family Didemnidae*—Fig. 17

This is also a well-defined family, in which there is much more uniformity with regard to adult organization than there is in mode of development.

The several types examined are arranged in order of increasing developmental specialization, although the embryonic times are known most accurately for the last of these.

Didemnum gelatinosum (Milne–Edwards), egg 0·49 mm. diameter.

While the development of this species is less specialized than that of the other Didemnids here described, the egg is by far the largest. At 16° C the tadpole hatches by rupture of the distended membrane about 320 hours from fertilization. This value, however, may have as much as 10% error.

There is a free-swimming period of about two hours. The tadpole possesses 8 ampullae, 4 rows of stigmata that developed as independent perforations, and a mass of yolky cells near the stomach. The heart, siphons, stigmata, function about one week after settling, budding not taking place until some time later.

Tridemnum cereum (Giard), egg 0·28 mm. diameter.

At 16° C the tadpole hatches by rupture of the distended egg membrane about 270 hours after fertilization (possible error 10%).

The tadpole resembles that of *Tridemnum* except in that the four rows of stigmata are reduced to 3 (as in the adult zooids) while the 3 adhesive papillae are reduced to 2. The stigmata reduction implies a reduction in adult body-size, the reduction in number of papillae implies a secondary reduction in tadpole-size and therefore of the egg.

The free-swimming period lasts about one hour. After settling, instead of the oozoid becoming functional within a week, and without any sign of budding, it remains relatively compact and apparently inactive for about three weeks (at 16° C). At the end of this time the heart, siphons, and stigmata of the oozoid become active, but at the same moment so do those structures in the blastozooid. The first bud is accordingly completely formed before the oozoid functions.

Polysyncraton (*Didemnum*) *amethysteum* (Van Name), egg 0·37 mm diameter.

The duration of the embryonic period was about 310 hours. The tadpole resembles that of *Didemnum gelatinosum* in having 4 rows of stigmata, and 3 adhesive papillae. It differs in having 16 ampullae. The free-swimming period lasts from 1 to 2 hours.

The post-larval development, however, is identical with that of *Tridemnum*, the oozoid remaining compact and apparently inactive for about 3 weeks after settling, after which time both oozoid and the blastozooid start functioning together. The post-larval development is similar, therefore, in spite of difference in size.

Diplosoma listerianum gelatinosum (Milne–Edwards), egg 0·37 mm diameter. Gastrulation commences about 100 hours, sensory pigment appears 330 hours after fertilization.

At 16° C the tadpole hatches by rupture of the distended egg membrane about 420 hours from fertilization.

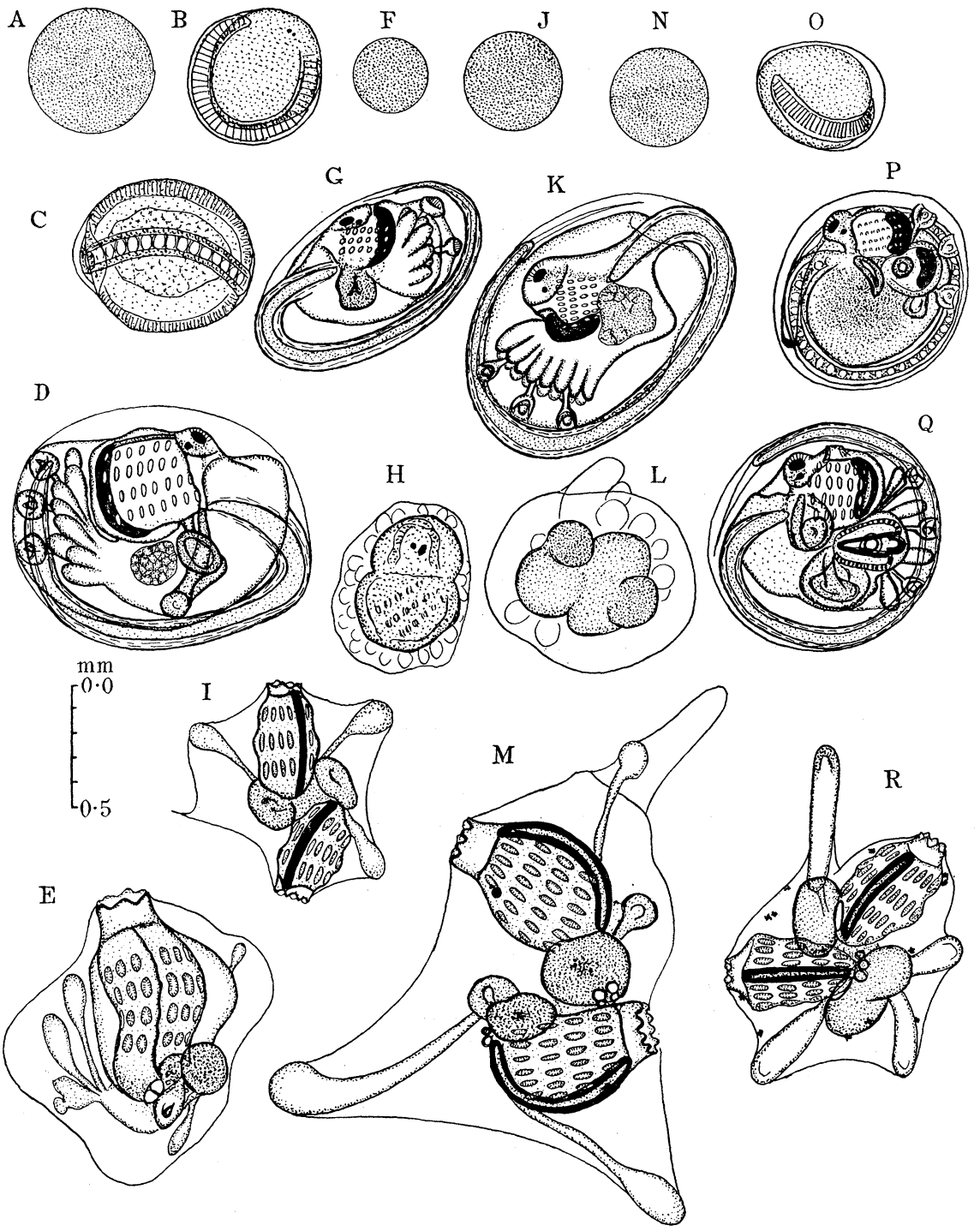

FIG. 17—Development of didemnids. A, egg; B, early tadpole with trace of sensory pigment; C, later stage showing primary body cavity and vacuolization of notochord; D, active tadpole; and E, fully metamorphosed individual with active heart and stigmata, of *Didemnum gelantinosum*; F, egg; G, active tadpole; H, prefunctional but budding phase of oozoid; I, fully functional oozoid and first blastozooid, of *Trididemnum cereum*; J, egg; K, active tadpole; L, prefunctional but budding phase; M, completely functional oozoid and first blastozooid of *Polysyncraton amethysteum*; N, egg; O, early tadpole; P, later tadpole stage with well-formed first blastozooid; Q, active tadpole with oozoid and blastozooid equally well developed; R, individual shortly after metamorphosis with fully active oozoid and first blastozooid, of *Diplosoma listerianum*. *Note*—the endostyle is shown in black throughout

The tadpole possesses 4 ampullae and 3 adhesive papillae, while 4 rows of stigmata appear as independent perforations of the branchial wall.

In this form, as in the other Didemnids, the peribranchial sacs invaginate separately, but at an early period in embryonic development fuse dorsally to form the atrial siphon.

The tadpole differs fundamentally from those of *Didemnum* and *Tridemnum* in possessing a blastozooid as well developed as the oozoid.

After a free-swimming period of from 5 to 6 hours this double-headed tadpole settles ; 6 hours later, at 16° C, the heart, siphons, and stigmata of both oozoid and blastozooid become active together. A colony of four zooids is formed within another 10 days. The development accordingly resembles that of *Tridemnum* and *Polysyncraton*, differing in that the tailed tadpole organization is retained well into the post-larval stages of these forms.

Diplosomoides lacazii (LAHILLE). This species was described by LAHILLE but has not been rediscovered.

Its interest is that its development apparently bears the same relationship to that of *Diplosoma* as does this last to that of *Polysyncraton*. The tadpole larva hatches with not merely one but three blastozooids all as well developed as the oozoid, a stage reached by *Diplosoma* a week or ten days after the tail of the tadpole has been absorbed.

VII—GENERAL

Taken as a whole, the facts presented in the preceding sections form a somewhat confusing picture, and it is only on analysis that certain significant correlations appear.

Eggs of the same size may develop at the same rate or at different rates ; eggs of different sizes may develop in the same time or may be widely different. Gastrulation may be embolic, epibolic, or intermediate between these two types. Tadpoles may have beating hearts, and functional stigmata, and may form asexual buds, or there may be no sign of any such structures. They may have a free-swimming period lasting but a few minutes or some days.

The tadpole stage is by definition that stage wherein the transitory larval organs have attained their maximum development and are functional. The notochord is fully extended and rigid. The lateral muscles of the tail are elongated and functional. The larval sense organs and adhesive papillae are fully developed, and there is a well-formed cuticular fin along the upper and lower side of the tail.

VIII—EGG-SIZE AND RATE OF DEVELOPMENT

In order to gain a true conception of the relationship between the size of an egg and the rate of development, the comparison must needs be limited to eggs that develop in the same way, and that form the same kind of tadpole. For this purpose

the ascidians may be divided into several groups: those whose eggs gastrulate embolically and form tadpoles with but a poorly differentiated trunk region, those in which gastrulation is embolic but which produce highly differentiated tadpoles, and those producing highly differentiated tadpoles but have gastrulated more or less epibolically.

The first type may be considered to be primitive, since it is typical of the oviparous small-egged species of every family. Gastrulation is truly embolic and the tadpole has but a simple pharyngeal sac with no gill slits, no heart, and with non-functional

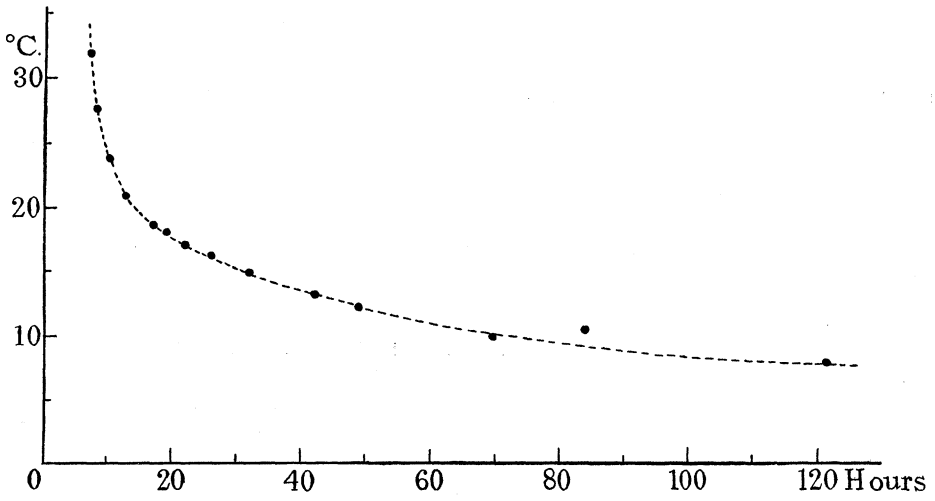

FIG. 18—Relationship between temperature and duration of embryonic development (to completion of tadpole). The values above 20° C are based upon *Ascidia atra* and *A. curvata*; between 20° and 13° C upon *Ascidia mentula*, *A. aspersa*, and *A. mammillata*; while below 13° the values relate to *Ascidia prunum*. The development of these various species is virtually identical and the eggs of all are about 0·17 mm diameter

mouth and intestine. The group is composed of the Ascidiidae, Cionidae, Molgulidae (part), Pyuridae, and Styelidae (exclusive of the Botryllids).

If the duration of development from fertilization to the formation of an active tadpole (at a given temperature, 16° C) is plotted against the diameter or radius of the egg, a relationship is obtained that is shown in fig. 19. For egg dimensions less than 0·2 mm diameter, a curve is formed; for dimensions exceeding 0·2 mm diameter, the shallow curvature disappears and the relationship is expressed as a straight line.

In other words, the rate of development (taken as a whole up to the formation of an active tadpole) of eggs exceeding 0·2 mm in diameter is inversely proportional to the diameter or radius of the egg. While the points plotted represent the total time from fertilization to tadpole activity, the same relationship holds for the rate of cleavage, the duration of gastrulation, the rate of tail outgrowth, etc. Development as a whole is retarded with increasing egg-size, and the retardation applies equally to

such diverse phenomena as cleavage rate, gastrulation rate, and the rate of notochordal extension. The absence of differential retardation is shown in Table II.

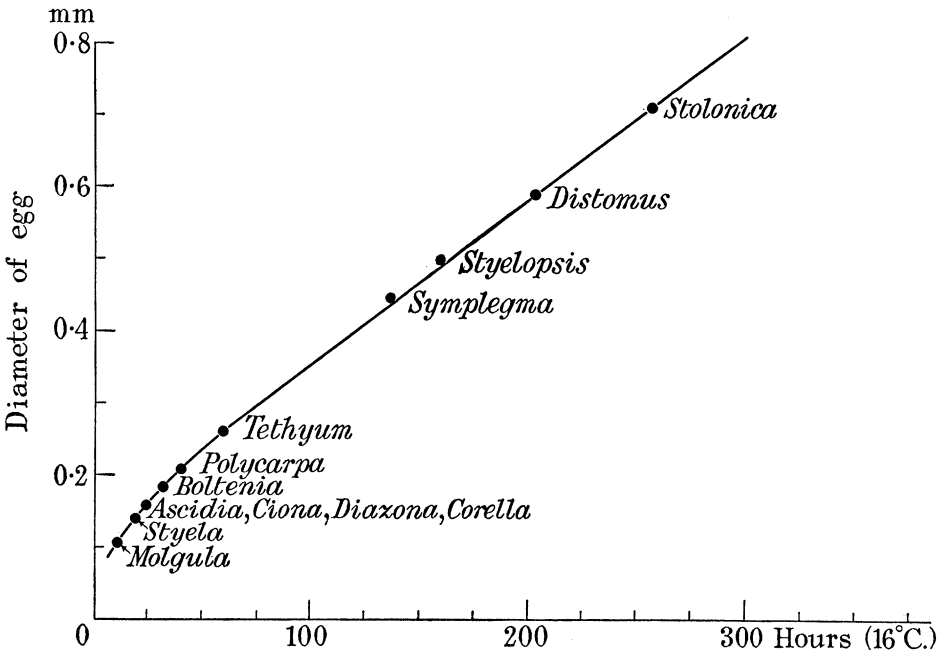

FIG. 19—Relationship between egg-size and duration of embryonic development (up to completion of tadpole) at 16° C, for all oviparous forms and for the large-egged and viviparous styelids

TABLE II

Species	*Phallusia mammilata*, *Ascidia aspersa*, *Ciona intestinalis*, *Diazona violacea*		*Tethyum pyriforme*		*Styelopsis grossularia*		*Stolonica socialis*	
Egg diameter in mm . .	0·17		0·26		0·48		0·70	
	Time		Time		Time		Time	
Period of Development at 16° C	Hours	% of Total	Hours	% of Total	Hours	% of Total	Hours	% of Total
2-cell to 4-cell	0·75	3	2	3·3	5	3·1	7½	3·1
Fertilization to onset of gastrulation	7	27	16	26·7	42	26	63	26
Onset of gastrulation to closure of blastopore .	4	15	9	15	25	16	35	14·5
Closure of blastopore to appearance of sensory pigment	8	30	33	31	48	30	72	30
First appearance of sensory pigment to completion of tail extension	7	27	17	28	40	25	64	26·5
Total duration from fertilization to hatching . .	26	100	60	100	160	100	240	100

The retardation with increase in size is thus general and without differential effect, and is the same whether applied to the whole (up to the tadpole stage) or merely to the early cleavage rate.

The rate of development is inversely proportional to the radius or diameter of the egg, and not to the volume or to the surface-area. If the rate were determined by the amount of work involved in rearranging the mass of material during cell division, it should be proportional to the egg-volume. If it were proportional to internal surface changes involved in phase alterations during cleavage or to tensions developed at the whole surface, the rate should vary inversely with the surface-area of the egg. It does so in neither case.

The rate varies with the egg radius. If this were to mean that the controlling factor is the centripetal or centrifugal flow in aster formation, the rate of cleavage should increase progressively as successive divisions reduce the size of blastomeres. On the other hand, the radius is a function of the ratio (volume)/(surface-area), not only of the undivided egg but also of the developing embryo as a whole. Since the changes in form of the embryo during development are virtually the same for all ascidians, they may be ignored.

Intercleavage intervals, at a constant temperature, were determined for *Ascidiella aspersa*. The results are shown in Table III, together with those of Conklin for *Styela*.

Table III

Interval	*Ascidiella*	*Styela*
	Min.	Min.
Fertilization to 1st cleavage	65	40
1st–2nd ,, ...	38	30
2nd–3rd ,, ...	39	20
3rd–4th ,, ...	37	20
4th–5th ,, ...	38	20
5th–6th ,, ...	—	20
6th–7th ,, ...	—	20
7th–8th ,, ...	—	20

The relatively long period from fertilization to the first cleavage is due to the extra time needed for the completion of maturation and the process of fertilization itself, in addition to the first cleavage proper. The comparison of successive intercleavage intervals is thus valid only from the first cleavage onwards. It is accordingly seen that there is no significant progressive decrease in the intercleavage interval. The rate of cleavage and development, being inversely proportional to the egg radius, must therefore vary with the ratio of the volume to the external surface of the whole and not with the ratio of volume to combined surfaces of the individual cells.

Since the ratio (volume)/(external surface area) seems to determine developmental rate, the controlling factor is probably the rate of gaseous exchange between the total protoplasmic mass and the total external surface of the egg or embryo.

If this be so, then in spite of the fact that the intercleavage intervals remain virtually constant as the blastomeres decrease in size, there should be an increase in

cleavage rate if the blastomeres are isolated from one another, since their originally mutual surfaces become part of the external exposed surface of each.

Reverberi (1932) has found that fertilized fragments of *Ciona* eggs divide more rapidly than the intact eggs. In *Ascidiella*, however, more exact data have been obtained. The perivitelline membranes and follicle cells were removed from unfertilized eggs by the digestion method (Berrill, 1933). The naked eggs were subsequently fertilized and were shaken during the first cleavage so that many fell apart as isolated right and left blastomeres. After two hours' continuation of development, the isolated blastomeres of the 2-cell stage had progressed to 12- and 16-cell stage in about equal proportions, while the normal 2-cell stage had reached the 16-cell stage. The control had therefore undergone three divisions in two hours (an intercleavage interval of 40 minutes), while each pair of the separated blastomeres had together undergone between three and a half and four divisions in the same time (an intercleavage period of 32 minutes).

The cleavage rate of the non-separated blastomeres is thus slower than those separated from one another, the ratio being 32/40, or 0·80 : 1.

The radius of a sphere may be expressed as $r = k \sqrt[3]{V}$, k being a constant and V the volume of the sphere. If $V = 1$, $r = l \times k$. If $V = \frac{1}{2}$ (the volume of an isolated blastomere of the 2-cell stage compared with the 2-cell stage as a whole), then $r = k \sqrt[3]{\frac{1}{2}}$, or $0{\cdot}79 \times k$.

The new cleavage rate is accordingly proportional to the radius of the new whole, or to the new (volume)/(external surface-area).

The general relationship may therefore be expressed as follows : the rate of cleavage and of development as a whole is determined by the extent of the external surface-area per unit mass. This suggests forcibly that the controlling factor is the diffusion of oxygen or carbon dioxide, or both, across the external surfaces of the membranes. Amberson (1928), working with Arbacia eggs, found that there was no change in the rate of oxygen consumption until the oxygen partial pressure was reduced below 20 mm Hg. Godlewski (1901) found that in an atmosphere of oxygen the developmental rate of frog embryos was but slightly accelerated, in an atmosphere of hydrogen development proceeded as far as gastrulation but no farther, while carbon dioxide killed the eggs immediately. These results suggest that development during early stages is more or less anaerobic and that in any case there is a superabundant oxygen supply. The very toxic effect of carbon dioxide, on the other hand, indicates the need for its removal from the egg, and this may be the pacemaker for development.

Some experiments were carried out with the eggs and embryos of *Ascidiella* in this connection, the results of which are summarized below.

1. *Effect of increased oxygen tension*—Passing pure oxygen through the water in which the eggs were developing had no effect whatever upon the developmental rate.

2. *Effect of decreased oxygen tensions*—Eggs were allowed to develop in water that has been brought into equilibrium with the normal atmosphere at reduced pressures.

(*a*) *Total atmospheric pressure* 15 *cm Hg* (18°–19° *C*)—Development proceeded relatively slowly to the gastrula stage, when it was completely arrested.

(*b*) *Total pressure* 8 *cm Hg*—Complete arrest at gastrula stage.

(*c*) *Total pressure* 25 *cm Hg*—After 8 hours both control and reduced pressure embryos had developed to elongated gastrulae.

After 21 hours, the great majority of the eggs developing at reduced pressure had attained the closed blastopore stage only. A minority were at various stages between that and the tadpole stage. The control embryos were hatching.

This pressure is accordingly the critical value with regard to the oxygen tension. The oxygen partial pressure of the normal atmosphere is accordingly about three times the partial pressure at which the embryos of *Ascidiella* begin to suffer from oxygen lack. Moreover, development up to the gastrula stage is virtually anaerobic.

3. *Effect of urethane*—Eggs (at the 2-cell stage) were placed in solutions of urethane in seawater at 18° C.

After 8 hours the controls and those in N/200 and N/100 solutions were elongating gastrulae, those in N/50 were early gastrulae, while those in N/25 had proceeded irregularly and were arrested short of gastrulation (64-cell stage).

After 20 hours, the controls were hatching, those in N/200 were arrested at a very advanced stage, while in N/100 and N/50 there was only a slight extension of the notochord to form a tail.

Parnas and Krasinska (1921) found with amphibian embryos that development would proceed up to or after gastrulation, but not through, in concentrations of urethane N/10 and N/20. Urethane N/50 caused a retardation of seven times.

In *Ascidiella*, therefore, inhibition by urethane is most marked with regard to cleavage and gastrulation, but it also has a marked effect upon the swelling and interdigitation of notochord cells.

4. *Effect of carbon-dioxide tensions*—The actual CO_2 tensions were not determined owing to the complexity of the carbonate buffer system of seawater, but CO_2 was bubbled through the water until the hydrogen ion concentration was reduced from p_H 8·0 to various values. Eggs were placed in water of various CO_2 tensions, at the 2-cell stage, with the following results.

p_H 8·0 (control), normal development.

p_H 7·0, cleavage and development retarded but not arrested.

p_H 6·8, cleavage retarded—development arrested at gastrula stage.

p_H 6·5, cleavage and development arrested.

p_H 6·2, cleavage and development arrested.

(Blowing out the CO_2 with air raised the p_H in one experiment from 6·5 to 7·9, but development only proceeded from the 2-cell stage to the 4-cell stage.)

Accordingly, increased CO_2 tensions have a much greater effect upon developing *Ascidiella* eggs than have decreased O_2 tensions. Also cleavage stages are almost as susceptible as later stages.

The above experiments, while not conclusive, indicate that the rate of development is controlled primarily by the CO_2 tension produced within the egg, rather than the

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oxygen tension. In other words, the rate of development varies with the internal CO_2 tension, which in turn is controlled by the CO_2 tension at the external surfaces. This last is determined by the mass or volume of protoplasm producing the CO_2 divided by the total external surface available for its escape ; *i.e.*, (volume)/(surface-area) = radius $\times$ k.

Ascidiella eggs have a diameter of 0·17 mm, which is an index of the ratio (volume)/(surface-area). The oxygen tension necessary for normal development is about one-third that of the partial pressure of oxygen in the atmosphere. One might, therefore, expect the oxygen tension to become of equal importance in controlling the developmental rate when the (volume)/(surface-area) ratio is increased to three times its value for *Ascidiella* eggs, *i.e.*, for eggs of diameters exceeding 0·17 $\times$ 3 or 0·51 mm. In any case, increase in egg-size increases the (volume)/(surface area) ratio and progressively renders more difficult the elimination of CO_2 from the egg and embryo surfaces. In consequence, it is easy to understand that the larger the egg the more susceptible it becomes to any external accumulation of CO_2. The eggs of *Polycarpa*, diameter 0·22 mm, or of *Styelopsis*, diameter 0·48 mm, will develop only while bathed all round by a continuous flow of water removing the carbon dioxide and supplying oxygen. This condition is met in the atrial cavities of the parents, in which the eggs of these forms develop. Outside the parent it is very difficult to induce the continuation of development, and especially for it to proceed through gastrulation.

In *Distomus* the eggs are larger, 0·59 mm, and they not only tend to stop developing on extraction from the atrial cavity but they readily cytolyse, while those of *Stolonica*, diameter 0·70 mm, will cytolyse even within the parent if the siphons remain closed for any length of time. It is of interest to note that the stages that cytolyse first are the gastrulae, then cleavage stages, and lastly the post-gastrular stages.

The foregoing account is based entirely upon the development of those forms included in group one, *i.e.*, *Styela*, certain Molgulae, *Ciona*, *Corella*, the family Ascidiidae, *Polycarpa*, and *Stolonica*. The eggs of all these divide, gastrulate, and hatch in the same way, and produce tadpoles with one and the same degree of differentiation. Within this group the developmental rate varies directly with the radius or diameter of the egg, and this has been shown to mean that the controlling factor is the ratio (volume)/(surface-area) and that this in turn controls the CO_2 tension at the surface and through the interior. It is probable that for eggs of this type the maximum size has been attained in the case of *Stolonica*, and that larger eggs than those would be too susceptible to any slight environmental fluctuations in the CO_2 or O_2 tension.

The rate of development of ascidian eggs of group one is determined by the combined influence of the (volume)/(surface-area) ratio and the temperature. The relationship between temperature and developmental rate is shown in fig. 18.

With other ascidians another factor enters, and that is the proportion of yolk to cytoplasm. This is practically the same throughout group one, whatever the total volume of the egg, but in others there is a marked tendency for the proportion of

yolk to become greater irrespective of the volume of the egg as a whole. The yolk droplets become more closely packed together, so that the cytoplasm is in effect diluted and the metabolism per unit volume decreased.

The eggs of *Molgula citrina* and *M. complanata* are of the same size as those of *Polycarpa rustica*, but they contain relatively more yolk and develop much more slowly. Similarly *Botryllus* and *Symplegma* eggs are much the same size, but those of *Botryllus* contain more yolk and develop more slowly. The relative retardations compared with group one development is to be seen in fig. 20; for *Molgula* the retardation is 3·5, for *Botryllus* 1·5 compared with the group one forms with the same egg-size, viz., *Polycarpa* and *Symplegma*.

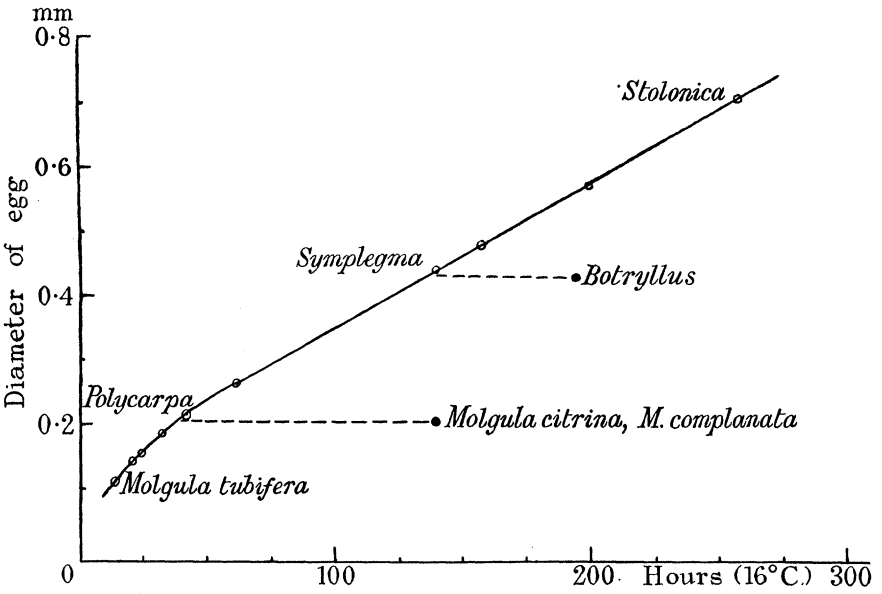

FIG. 20—Relationship between egg-size and duration of embryonic development, to show the influence of an increase in proportion of yolk to cytoplasm in the case of certain Molgulae, and of *Botryllus* (closely related to *Symplegma*)

The ratio (volume)/(surface-area) is thus an index of the development rate, for a given temperature, only while the yolk/cytoplasm ratio remains constant. The rate depends more exactly upon the product

$$\text{(volume)/(surface-area)} \times \text{(yolk)/(cytoplasm)}.$$

While the proportion of yolk to cytoplasm has not been accurately determined, there is no doubt that the series Polycarpid ——→ Perophorid ——→ Clavelinid ——→ Synoicid, represents an order of increasing yolk/cytoplasm ratios. With each successive increase there is a definite decrease in developmental rate. Within each group, however, there is variation in egg-volume independent of the proportion of yolk to cytoplasm, and the (volume)/(surface-area) ratio again becomes the controlling factor. These relationships are shown in fig. 21.

It is apparently only within the Didemnidae (and possibly the Molgulidae) that there are variations in both egg-volume and yolk proportion. In the remaining families the proportion of yolk to cytoplasm seems to be much the same whatever the size of the egg. The didemnids, *Tridemnum* and *Polysyncraton* have eggs differing in size but not in yolk proportion. *Diplosoma* has eggs the same size as those of *Polysyncraton*, but with relatively more yolk, while *Didemnum* has somewhat larger eggs but with rather less yolk. The developmental times for these forms is shown in fig. 29. It will be seen that the rate of development is a function of either the (volume)/(surface-area) or the (yolk)/(cytoplasm) ratio, or both together. An increase in proportion of yolk to cytoplasm effectively dilutes the cytoplasm per unit

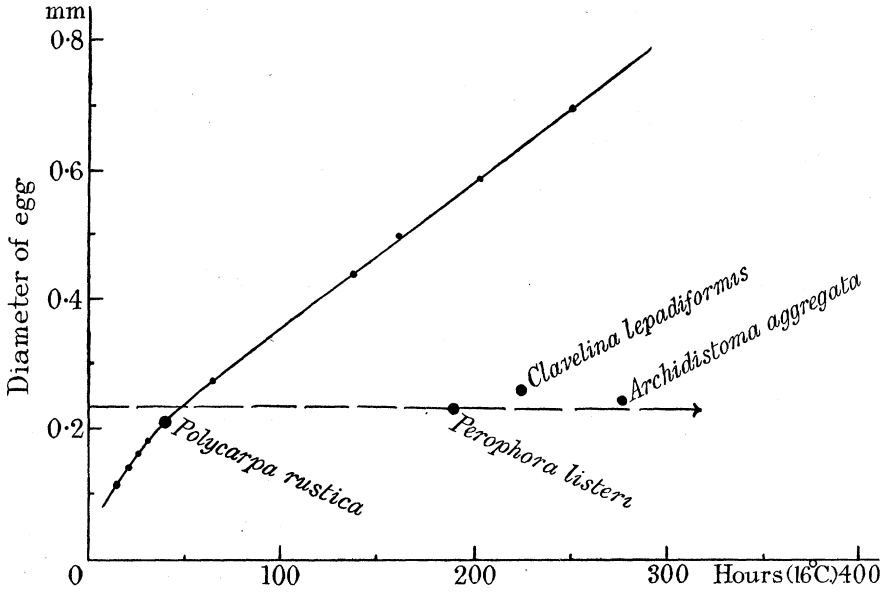

FIG. 21—Relationship between duration of embryonic development and proportion of yolk to cytoplasm for a given egg-volume. The series *Polycarpa–Perophora–Clavelina–Archidistoma* represents an order of increasing yolk/cytoplasm ratios

volume, and in consequence its metabolic rate. The rate of gaseous exchange across the external surface is thus lowered and eggs and embryos should be less susceptible to fluctuations in external oxygen or carbon-dioxide tensions than those with the maximum rate of metabolism per unit volume.

The eggs of *Distaplia* develop in brood pouches isolated in the test of the colony, after the degeneration of the parent zooid and without circulation of either blood or seawater. Eggs of *Diplosoma* or *Didemnum* develop isolated in test material, while the very large eggs of *Ecteinascidia* develop fairly readily in still water outside the parent. On the other hand, the large eggs of *Styelopsis* and *Distomus* (group one) will not develop in still water, while those of *Stolonica* (also group one) are killed by a temporary closure of the siphons of the parent. Large eggs can accordingly develop safely only when the rate of development and of metabolism per unit volume

has been reduced by an increase in proportion of yolk to cytoplasm, independently of the size of the egg.

Not only may there be variation in egg-volume and in the relative amount of yolk, but there may also be variation in yolk density, either absolutely or relatively to the cytoplasm. In the Perophorids, Botryllids, and large-egged Molgulids, the increase in relative amount of yolk apparently involves no change in yolk density since every part of the egg is equally affected.

In the Clavelinids, Distomids, Didemnids, and Synoicids, however, there has been an accompanying increase of density so that the vegetative region of the egg becomes more closely packed with yolk than the animal region. Consequently, the expression (volume)/(surface-area) $\times$ (yolk)/(cytoplasm) as an index of developmental rate applies to the parts of such eggs rather than to the whole.

IX—Gastrulation

The occurrence of the three variables, egg volume, proportion of yolk, and density of yolk, makes possible a more exact determination of the influence of yolk and egg-size upon the process of gastrulation.

Gastrulation may be embolic or epibolic, and may occur when there are but few cells or many. In ascidians, whatever the egg-size or the proportion and density of the yolk, the process of invagination commences between the sixth and seventh cleavage, *i.e.*, about the 76-cell stage. In other words, the rate of cleavage and the rate of differentiation of the inducing agent for gastrulation, whatever it may be, are affected to the same degree by changes in egg-volume or proportions of yolk. In all members of group one (in which the (yolk)/(cytoplasm) ratio is at its minimum) gastrulation is strictly embolic. In the Perophoridae, in *Botryllus*, and in large-egged Molgulae, in which increase in the proportion of yolk to cytoplasm applies equally to all parts of the egg, gastrulation is again embolic. In the eggs of *Clavelina* species the yolk is relatively denser and is confined to a greater extent to the vegetative hemisphere. In these gastrulation is of a type between embole and epibole. In the Distomids, Synoicids and Didemnids, both the proportion and density of the yolk are high, and gastrulation is epibolic. These types are shown in fig. 22.

It is accordingly obvious that egg-volume alone has no influence upon mode of gastrulation, nor upon the stage at which gastrulation commences. The (yolk)/(cytoplasm) ratio also has no influence if it affects equally all parts of the egg ; only when the ratio is changed in one part relative to another does it produce epibole, but even then there is no significant influence on the stage at which the process starts.

X—Analysis of Embryonic Development

There is reason to believe that the type of development characteristic of group one (all oviparous species and the viviparous Polystyelids) may be regarded as the least modified. The developmental rate is at its maximum and the larval tadpole is

produced in its simplest state. In this group the effect of increase in egg-volume is virtually non-differential. The cleavage sequence and form remains unmodified, gastrulation is always embolic, and occurs immediately after the sixth cleavage, while tadpoles (hatching through the digestion of the egg membrane) are active at a stage at which the permanent trunk organs are barely discernible.

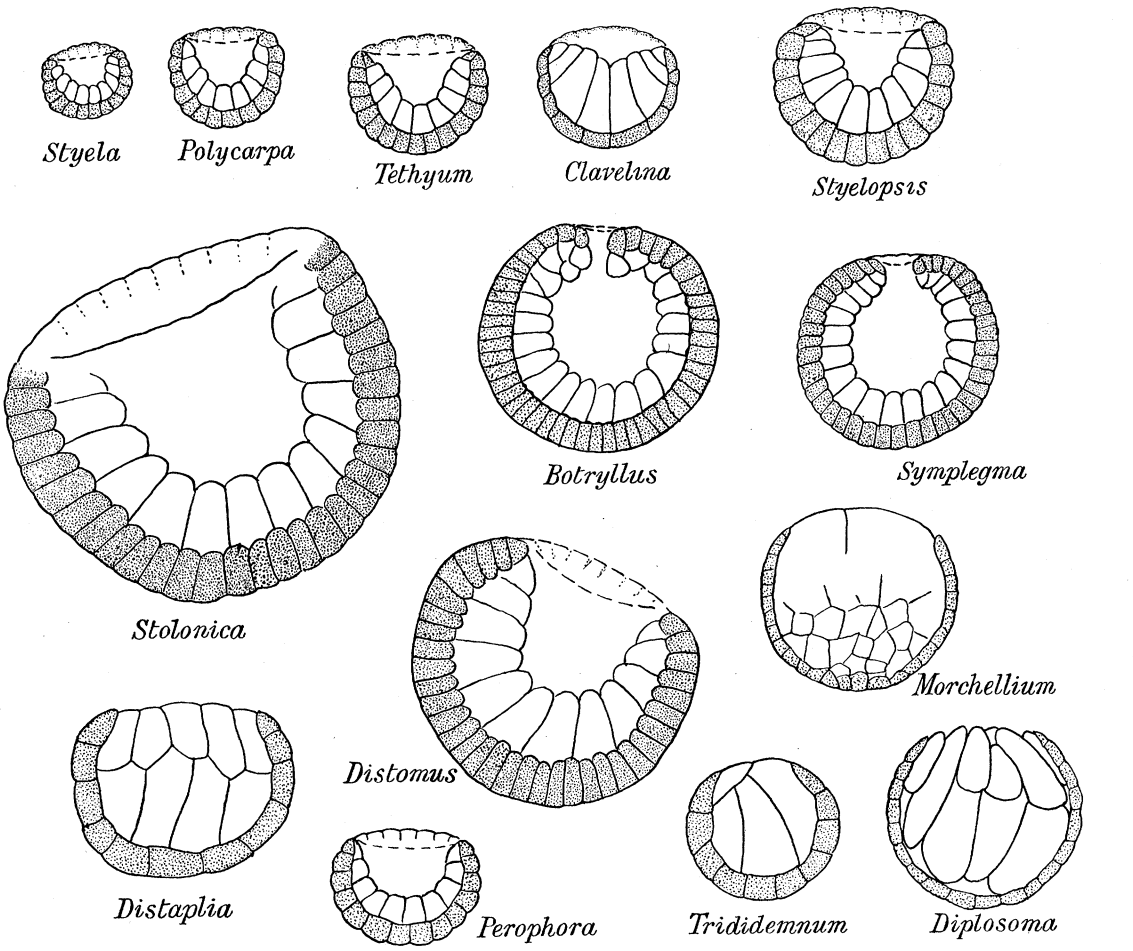

FIG. 22—Comparison of middle gastrular stages of various ascidians, all drawn to one and the same scale, showing the independence of type of gastrulation from size of egg

Development as a whole may be divided into various phases, cleavage stages, gastrulation and closure of the blastopore, tail outgrowth, etc., while cell division and differentiation continue throughout. From about the onset of gastrulation the different cell-types or tissues become segregated from one another. At this stage there are 8 chordal, 6 muscle, 12 mesenchyme, 14 neural, 10 endodermal, and 26 ectodermal cells. Each of these continues to divide until either a certain number or a certain cell-size is attained, when there follows a period of differentiation. Segregation is precocious. This condition holds not only for *Styela* and *Ciona* (CONKLIN, 1905) and *Boltenia* (BERRILL, 1929), but even for such large eggs as those

of *Styelopsis*, *Distomus*, and *Stolonica*. In all these there become apparent an obvious difference between the development of the tissues concerned with the formation of the temporary structures of the tadpole and the permanent organs of the adult.

Ectoderm, endoderm, and mesenchyme cells divide until a minimum cell-size is reached, after which differentiation occurs and further division depends upon subsequent growth. On the other hand, chordal, muscle, and neural cells divide, not to form a minimum cell-size but to produce a certain number of cells, after which differentiation occurs. In the first set the number of cells formed varies with the egg-volume, in the second set the number remains virtually unaffected, but the cell-size varies with the volume of the egg.

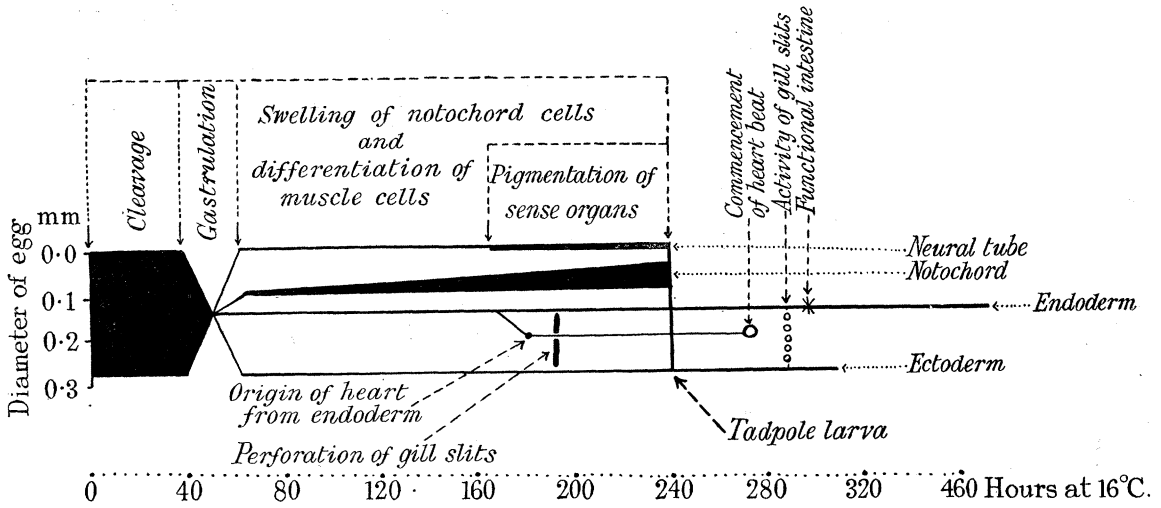

FIG. 23—Graphic or schematic representation of embryonic and post-larval development of *Clavelina lepadiformis*, at 16° C. This figure is intended to be used as a key to subsequent diagrams of the same type. A time scale is shown at the base, a scale at the side for the diameter of the egg and for the final width of the notochord cells. Cleavage is shown in black, as also is the swelling or differentiation of the notochord and sense organs. The vertical line denotes the attainment of the tadpole stage. The activity of the heart and stigmata is shown by hollow circles, their non-functional anlagen by solid. A row of circles denotes definitive stigmata, a single elongate ellipse a protostigma. Functioning of the post-branchial intestine as evidenced by faeces is marked with an asterisk. Differentiation of tail muscle coincides with the differentiation of the notochord and has been omitted, nor is there any indication of the extent of embryonic growth in size, if any, and the reader must therefore consult figures 5 to 17 for this information

Development may therefore be considered in three parts, a common phase up to and including gastrulation, a development of permanent organs consisting of ectoderm, endoderm, and mesenchyme, continuous and blending with the normal growth processes of the young adult, and the development of the larval structures of notochord, lateral tail muscle, neural tube and sense organs. Such development is shown graphically in fig. 23.

The graph, fig. 24, based partly on data determined by BALINSKY (1929), for *Ciona*, shows that all division ceases in the notochord and muscle cells after about one-third

of the embryonic period has passed. Since the attainment of the tadpole stage is determined by the completion of the functional differentiation of the tail, it is evident that the remaining two-thirds of the period represents the duration of that differentiation, *i.e.*, the swelling and consequent interdigitation of the chordal cells, the elongation of and fibril-formation in the muscle cells. The muscle cells are arranged in three rows on either side of the notochord so that six cells extend through the whole length of the tail. Cells of the neural tube cease dividing after about half to two-thirds of the embryonic period has passed.

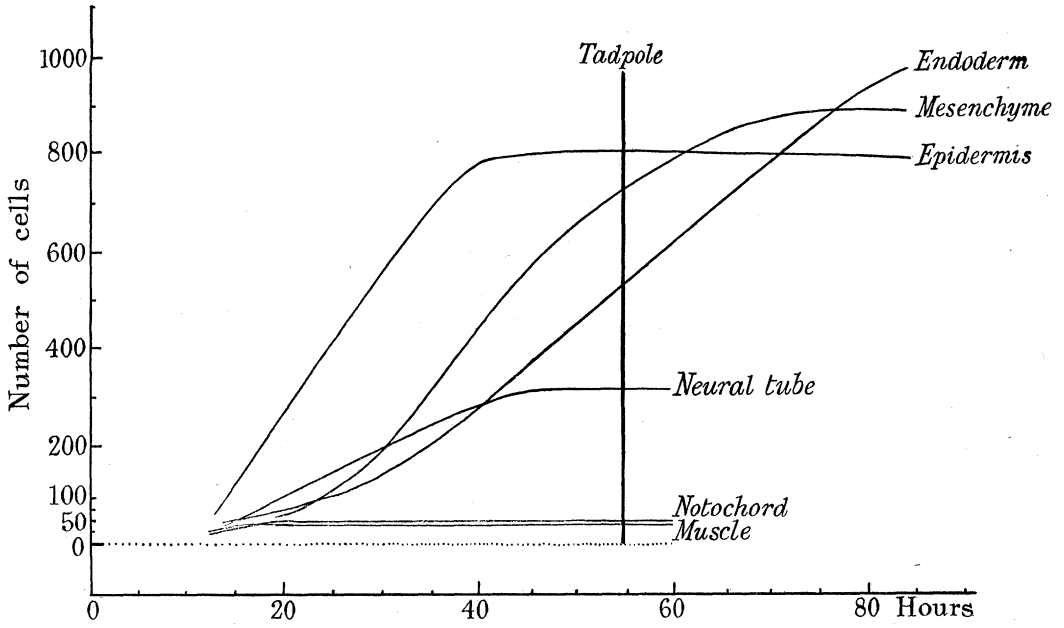

FIG. 24—Graph showing the relation between the time of development and number of cells formed in each tissue, temperature about 13° C, based partly upon data recorded by BALINSKY for *Ciona intestinalis* and partly upon confirmations made upon *Ascidiella aspersa*. The vertical line represents the attainment of the tadpole stage

The other tissues, especially the endoderm and mesenchyme, continue to divide throughout the whole embryonic period.

There is accordingly a sharp demarcation between the development of the temporary larval organs and the permanent organs of the adult.

XI—DEVELOPMENT OF LARVAL STRUCTURES

From the account given of the abbreviated development of certain Molgulae (BERRILL, 1931), it is obvious that the development of the whole larval complex of notochord, muscle, neural tube and sense organs may be suppressed without affecting the development of any of the permanent organs. This consideration, together with the fact that in the urodele development the chordal and muscle cells cease dividing and commence their final differentiation at a very early stage, whereas

the tissue cells of the permanent organs divide indefinitely, suggests that there are two independent developmental mechanisms proceeding side by side. It is conceivable, therefore, that changes in egg-size and relative amount of yolk may affect them differentially.

It has been argued that the developmental period from fertilization to formation of an active tadpole varies directly with the volume of

$$\text{(volume)/(surface-area)} \times \text{(yolk)/(cytoplasm)},$$

and that the rate of early cleavage and of gastrulation is inversely proportional to the same ratio. Shortly after gastrulation is complete, the full number of notochord and muscle cells have been attained, so that the period of differentiation must be equally proportionate to the same ratio, *i.e.*, the rate of differentiation as well as the rate of cleavage depends on the product of (volume)/(surface-area) × (yolk)/(cytoplasm), the surface-area always being the free surface available for metabolic exchanges and not the total surface-area of all cells.

The close correlation between the rate of chordal differentiation and the rate of early cleavage and of gastrulation is probably due to the fact that chordal cell division is virtually confined to these early phases. In other words, whatever the egg-volume, whatever the rate of development, the chordal cells, first segregated in the 76-cell stage as 8 cells, undergo merely two to three further divisions to form about 40 chordal cells. These are completed before the closure of the neural plate and before there is any sign of tail outgrowth, *i.e.*, while the embryo is still compact and with virtually the same (volume)/(surface-area) ratio as the undivided egg.

Thus the whole embryonic period may be divided into two parts—the cleavage-gastrulation-neural-plate phase, during which there is practically no change in size or proportions, and the period of tail outgrowth during which there is no further division of notochordal or muscle cells. Chordal cells, muscle cells, and neural cells are segregated at about the same time. Their completed functional differentiation coincides with the activity of the tadpole. Therefore their period of differentiation is much the same, and the question arises whether the differentiation rate is the same because that of each is controlled by the same factors, or because one is a pace-maker and the others are dependent.

In notochord cells differentiation consists of a prolonged and progressive swelling, presumably due to imbibition of water (Berrill, 1931, p. 329). As the swelling increases the individual 40 cells slide and interdigitate until in place of four rows each of 10 cells, a single row of 40 cells is formed, which, moreover, continues to elongate as a unit. Only 40 cells are formed and at an early stage, so that their individual and aggregate volumes must vary directly with the volume of the egg. Since swelling must be essentially a process of imbibition of water through free surfaces and of oxygen and carbon-dioxide exchange through the same surfaces, the ratio (volume)/(free surface-area) × (yolk/(cytoplasm) is as significant with regard to the rate of swelling as it is to the development as a whole.

The 36 muscle cells characteristic of many embryos differentiate during practically the same period as the chordal cells. The rate of differentiation again is probably

controlled by the same ratio, but at the same time their active stretching by the extending notochord must also be an important factor.

Somewhat the same condition is found for the neural tube, ganglion, and sense organs. Cell division continues approximately until the notochord has attained its full length. Again there is the possibility that the rate of extension of the notochord is the pace-maker. It is significant that the period from the appearance of the first trace of sensory pigment (marking approximately the beginning of neural differentiation) to the formation of the active tadpole is always a constant proportion of the whole embryonic period, and therefore of the duration of chordal extension. This relationship is shown in Table II, and may also be seen from figs. 25, 28, 30, and 31.

Since the cell-number of chordal and, with some exceptions, muscle tissues is practically constant for all ascidian tadpoles, it is understandable that the cell-size of each tissue varies directly with the size of the egg.

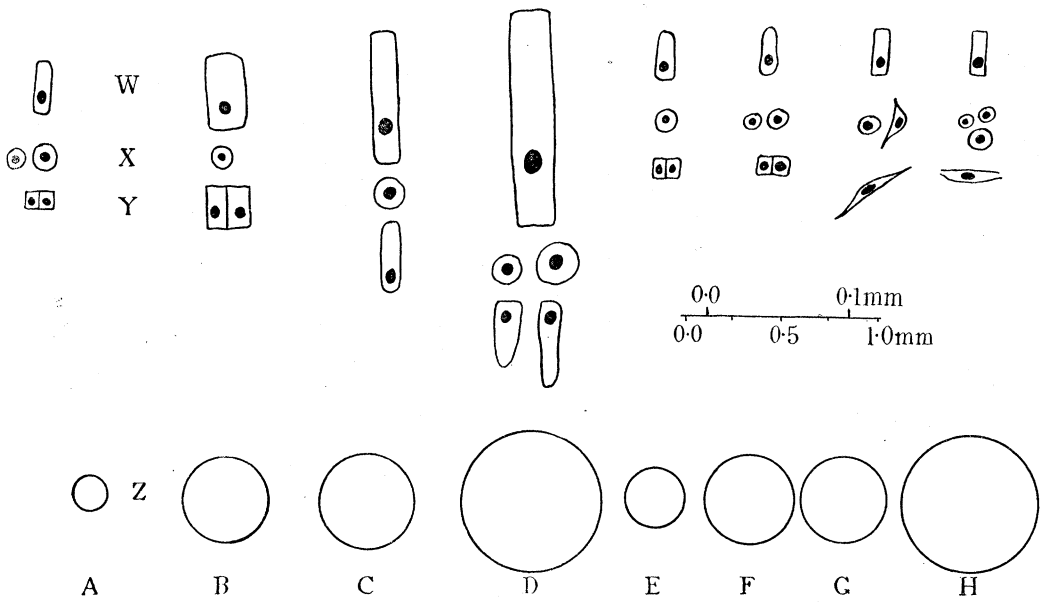

FIG. 25—Cell-sizes at the tadpole stage and egg-sizes of various ascidians. W, epithelial cell of intestine; X, mesenchyme cell; Y, epidermal cell; Z, egg-size, of A, *Phallusia mammillata*, B, *Symplegma viride*, C, *Styelopsis grossularia*, D, *Stolonica socialis*, E, *Clavelina lepadiformis*, F, *Botryllus gigas*, G, *Distaplia rosea*, H, *Ecteinascidia turbinata*. The eggs of E to H have a higher proportion of yolk to cytoplasm than have the others, and the cells of the three tissues reach the minimum cell size at the tadpole stage. Compare with fig. 3

XII—DEVELOPMENT OF PERMANENT STRUCTURES

From considerations of cell lineage and embryonic segregation, development may be expected to be dichotomous. During gastrulation the larval tissues, chordal, muscle, and neural, become completely segregated and proceed on their own course

practically unaffected by, and without influence upon, the development of the endodermal, epidermal, and mesenchymal tissues.

The tadpole larvae of group one have not only the same general organization of larval chordate structures, but they are also alike in having a trunk region that is poorly differentiated. The tadpole length varies from about 0·22 to 4·2 mm, but in each the mouth and atrial siphons are closed, the gut and pharynx are compact and functionless, there is no heart, the ampullae are barely discernible, the peribranchial invaginations are merely commencing their growth over the pharynx, while growth in size is insignificant.

The degree of development and differentiation of the permanent organs is therefore much the same. It might, in consequence, be expected that the cell-numbers of endodermal, mesenchymal, and epidermal tissues at the tadpole stage will be as constant as those of the larval tissues, and that the cell-size of each tissue might bear a definite relation to the size of the egg. In reality, however, the situation is not so, nor is it the opposite extreme of a constant cell-size and varying cell-number for each tissue. In the small newly hatched tadpoles of *Ciona* and *Ascidia* there are about 800 epidermal, 600 mesenchymal, and 500 endodermal cells. After hatching, and before metamorphosis, the mesenchymal and endodermal cells increase in number to about 900.

In *Symplegma*, with an egg 14 times the volume of that of *Ciona*, the newly hatched tadpole possesses about 3000 epidermal, 4000 mesenchymal, and 1400 endodermal cells. In *Stolonica*, with an egg 70 times the volume of that of *Ciona*, there are roughly 12,000 epidermal, 30,000 mesenchyme, and 3000 endodermal cells.

From the cell-numbers mentioned above, or from Table IV, it is clear that whereas the rate of differentiation of the trunk region is as rigidly controlled by the ratio (volume)/(surface-area) as is the differentiation rate of the tail organs, and while the cleavage rate becomes accelerated in later development, the acceleration is not the same for all three tissues. The division rate for the endoderm is accelerated least and that of the mesenchyme most.

TABLE IV

Egg volume	Endoderm		Epiderm		Mesenchyme		Species of tadpole
	Cell number	Cell volume	Cell number	Cell volume	Cell number	Cell volume	
1	1 (700)	2·5	1 (800)	1	1 (600)	3·5	*Ciona*
14	2 (1400)	12	4 (3000)	5	7 (4000)	4	*Symplegma*
70	3 (3000)	60	16 (12,000)	12	60 (30,000)	5	*Stolonica*

Epidermal cells have a greater free surface compared with their volume than have the endodermal cells, and are, moreover, in a better position for gaseous exchange with the environment. It is accordingly understandable that their cleavage rate should accelerate the more rapidly. In the same way, mesenchyme tissue consists of free cells and not cell-sheets like the epidermis or a compact mass like the endoderm.

For each mesenchyme cell the ratio (volume)/(free surface-area) is thus lower than for either the epidermis or the endodermis. The cleavage rate accelerates most rapidly of all. In *Ciona*, *Symplegma*, and *Stolonica*, and other members of group one, the yolk granules are fairly evenly scattered and the epidermal and mesenchymal cells are almost as yolk-laden as the endodermal. The differential acceleration can accordingly hardly be due to changes in the ratio (yolk)/(cytoplasm) following segregation.

To sum up, the larval tissues of the tadpole stage are alike, with constant cell-numbers and with all sizes proportional to the volume of the egg; while of the permanent tissues the endoderm alone has a relatively constant cell-number and has a cell-size varying with the egg-volume, the epidermal and mesenchymal cells become comparatively smaller and more numerous with increasing egg-volume.

XIII—Differentiation of the Trunk

The *organization* of the permanent organs of the tadpole trunk is the same in all, in spite of the differential acceleration of division rates. This organization consists of a relatively simple endodermal vesicle divisible into a pharyngeal region with a rudimentary endostyle and an intestinal part; the ectoderm is invaginated in front to form the future mouth and behind to form the future pair of peribranchial sacs. In other words, the invaginations occur at a time determined more by the degree of development of the endoderm than by that of the ectoderm. Accordingly, the invaginating regions must either be self-determining and self-differentiating, and their rate of differentiation independent of rate of cell-division, or the time at which invagination commences must be determined by the attainment of a critical degree of development by the endoderm. The last is the more probable, though the regions invaginating may be locally specialized and alone be able to respond to the endodermal (pharyngeal) influence.

In the Stolidobranchia a single median invagination that forks over the nerve cord replaces the pair of peribranchial invaginations occurring in the other two orders. This would be difficult to explain except as the fusion or combination as a single median rudiment of a pair of lateral regions. Moreover, the bifurcation over the nerve cord could be due to an attraction toward the endoderm of either side.

XIV—Post-Larval Development

Throughout group one the organization of the tadpole is constant, both with regard to larval and to permanent organs, except for a variation in number of epidermal and mesenchymal cells. The chordate structures of the tadpole, notochord, muscle, nerve tube, etc., are absorbed after a certain period of activity. The epidermis, mesenchyme, and endoderm, however, undergo further cell-division until a maximum number and minimum size of cells is reached in each case, after which an increase in number depends upon food obtained by a fully functional organism.

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With regard to the continuous cell-division of the trunk tissues, it is evident that the active tadpole represents merely an arbitrary stage in their development, a stage that happens to be the same throughout group one, because the rate of development of the endoderm was the pace-maker and was determined by the primary (volume)/(free-surface area) ratio. The development of the permanent tissues is continuous and progresses long after the loss of the tadpole structures to blend finally with the processes of normal somatic growth. This post-larval development proceeds in a very definite order in all forms. The final functional and structural differentiation of cells apparently does not take place until the minimum cell-sizes have been attained and cell-division virtually ended. After complete differentiation cell-division obviously becomes a slower and more difficult process.

Post-larval development always occurs in the following order :

a. outgrowth and functioning of epidermal ampullae ;
b. rotation of the body through growth of the anterior lip ;
c. formation and functioning of the heart ;
d. spontaneous contractions of siphons and branchial sac ;
e. opening and functioning of the stigmata ;
f. functioning of the post-branchial intestine.

In no case, for example, do the cilia of the gill slits (stigmata) beat before the commencement of the heart-beat, however much retardation or acceleration of the whole development there may be. The relative times from fertilization to the tadpole stage and to the activity of the various systems is shown in Table V in hours, at 16° C.

TABLE V

Species	Egg diameter mm	Tadpole hours (16° C)	Ampullae hours	Heart hours	Stigmata hours	Intestine hours
Ciona, *Ascidiella*, *Diazona*	1·7	24	50	150	260	320
Polycarpa	2·1	40	60	200	280	360
Symplegma	4·4	140	150	200	240	380
Styelopsis	4·9	160	180	280	340	430
Distomus	5·8	190	230	360	440	500
Stolonica	7·0	240	290	390	460	540

Structural and functional differentiation of cells takes place after rapid division has come to an end and a minimum cell-size attained. In the oviparous small-egged *Molgula*, *Styela*, *Ascidia*, *Ciona*, and *Diazona*, this state is reached shortly after metamorphosis, and the greater part of the period from the tadpole stage to the functioning of the heart and stigmata must be assigned to the period of differentiation.

In the larger embryos of *Symplegma*, *Styelopsis* and *Stolonica*, while the organization of the tadpole is practically the same as that of the above-mentioned forms,

cell-division of the permanent trunk tissues becomes progressively accelerated. So that in spite of the infinitely larger number of cells eventually formed in each case, the maximum number and minimum size is reached probably within about 48 hours after the loss of the tadpole stage, leaving a period for differentiation and organization much the same as that of *Ascidia* or *Ciona*.

The result is that while the time necessary for the development of the tadpole of *Stolonica* is about ten times as long as that required for *Ciona*, the young functional adult takes less than twice as long.

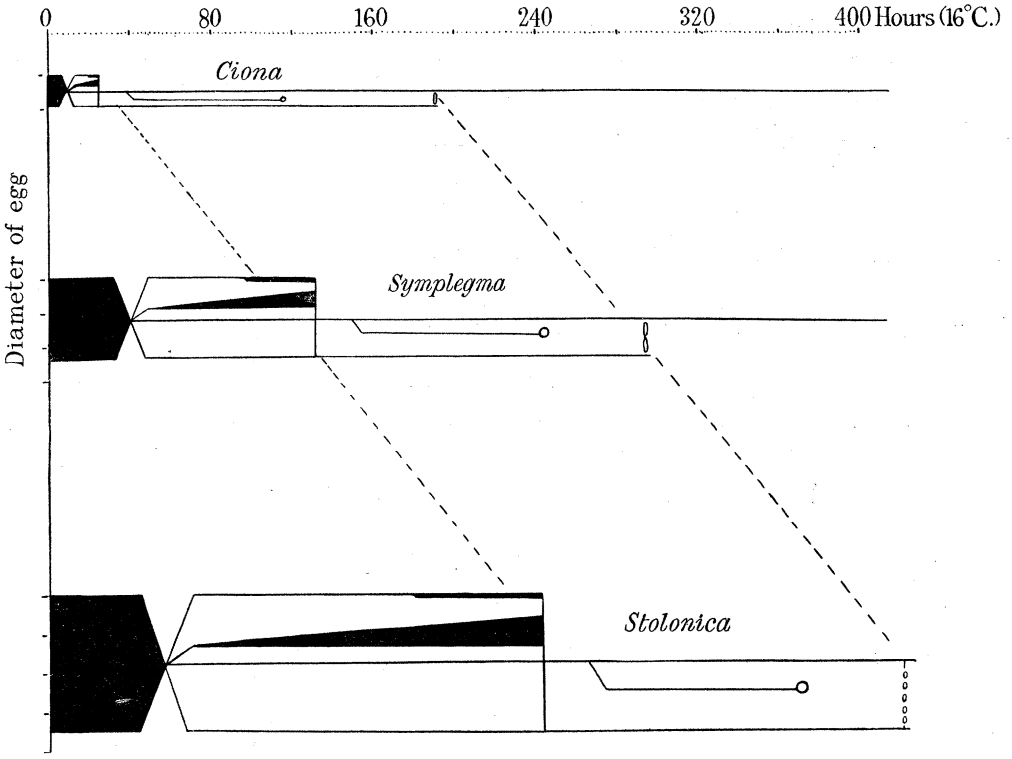

FIG. 26—A schematic comparison of the development of *Ciona*, *Symplegma*, and *Stolonica*. For key *see* fig. 23. It should be noted that whereas the embryonic period varies directly with diameter of the egg, the period of post-larval differentiation is practically the same in all three forms

A graphic comparison of the development of various members of group one is shown in fig. 26. In every case growth in size commences shortly after or shortly before the attainment of the tadpole stage. It seems to be due to the cell proliferation of the sheet-like epidermal and endodermal tissues that increases their area and diminishes their thickness. The more rapid extension of the epidermis results in the appearance of the primary body cavity (there is no coelom) between the two sheets of tissue. Such growth begins during or immediately after the tadpole stage, when the egg is less than 0·50 mm diameter, somewhat earlier when the egg exceeds that size.

XV—Development of the Stigmata

The only differences discussed so far in connection with the development of the small- and large-egged ascidians of group one have been the differential retardations and accelerations in the developmental rate of the larval and post-larval tissues. The organization of the tadpole larvae is practically indentical in all and the order in which various systems begin to function is constant.

There is, however, an important morphological feature of development that varies very considerably—the course of development of the stigmata. They always arise as perforations of the fused tissues of the pharynx (endoderm) and peribranchial lining (ectodermal). Fusion of these two layers must necessarily precede perforation, and since the peribranchial invaginations first appear during the active tadpole phase, the development of stigmata is always confined to the post-larval period. As in other tissues, the final structural and functional differentiation of the ciliated stigmata cells occurs after cell-division has ended and a minimum cell-size reached. Stigmata therefore appear as functional units only when a minimum cell-size has been attained. This cell-size, including cilium length, is virtually constant throughout the ascidians and all stages of growth, so that the number of cells in the functional branchial wall must vary directly with the size of the egg, or with the amount of active growth.

The course of development of the stigmata on each branchial wall varies, within group one, as shown in Table VI.

Table VI

Species	Egg diameter mm	First appearance of stigmata	Nature of later multiplication
Molgula, *Styela*, *Ascidia*, *Ascidiella*, *Corella*, *Ciona*, *Diazona*, *Polycarpa*	0·11→0·21	2 protostigmata appear and function	Increases slowly to 6 protostigmata with growth of organism; eventually each of 6 subdivides to form 6 rows of definitive stigmata; number of rows increases with further growth.
Tethyum	0·26	4 ,, ,,	,, ,,
Symplegma	0·44	4–(9) ,, ,,	Rapidly subdivide to form 4 rows.
Styelopsis	0·48	6 ,, ,,	Subdivision from rows is rapid, depending on length of protostigmata.
Distomus	0·59	4 rows of definitive stigmata arise as independent perforations	Number of rows increases only after true growth is well advanced.
Stolonica	0·70	4 ,, ,,	,, ,,

It may be stated with some confidence that the number of protostigmata that appears and function as independent perforations depends upon the antero-posterior length of the branchial wall. In the same way, their appearance as a protostigma or as a row of definitive stigmata depends on the dorso-ventral length of the wall. Since at the time of differentiation of the ciliated cells the cell-size is practically constant throughout the group and at its minimum value, the relative area of the branchial wall must be an index of the number of cells composing it. The number of perforations in a cellular sheet may thus be determined by the total number of cells present.

Whether a row of definitive stigmata appears as a row of independent perforations of the fused pharyngeal-peribranchial wall, or whether it is formed through the sub-division of a protostigmata by downgrowths, depends upon the developmental stage at which they are formed. If the cell-number and width of the branchial wall exceeds a certain critical value *before* cell-division slows down and differentiation takes place, a row of stigmata will be formed. If, as in the development of small-egged forms, the minimum size and differentiation is attained before the critical values are reached, a protostigma is formed. Once fully differentiated, the transformation of a protostigma into a row of definitive stigmata becomes a more complex process than the formation of a stigmata row from undifferentiated cells.

XVI—Increase in Relative Amount of Yolk

In the preceding section it was shown that increase in egg-volume alone had little or no influence on the organization at the tadpole stage, and in later stages resulted merely in an increase in rate of cell-division in the permanent tissues, and in the number and method of formation of the stigmata. Increase in volume that does not affect the proportion of yolk to cytoplasm has but little differential influence.

Whereas the volume of an egg may vary without affecting the yolk-cytoplasm ratio, the proportion of yolk to cytoplasm may vary without involving changes in egg-volume. The general effect is the same, a retardation of developmental rate.

At a given temperature, however, the retardation due to an increase in relative amount of yolk is absolute and is not proportional to the egg-volume. The eggs of two perophorids, *Perophora listeri* and *Ecteinascidia turbinata*, differ from one another only in volume, but they both differ from the group one type by the same increase in relative amount of yolk. *Perophora*, at 16° C, reaches the active tadpole stage in 220 hours. Of group one species—with lower yolk/cytoplasm ratio—the somewhat smaller egg of *Polycarpa* develops in 40 hours, the slightly larger one of *Tethyum* in 60 hours. The relative retardation is thus four. The absolute is 165 hours. *Ecteinascidia* develops to the tadpole stage, at 16° C, in 420 hours, *Stolonica* (group one), with eggs of the same size, in 260 hours. The relative retardation is of 1·6, but there is much the same absolute retardation (180 hours). These relationships must mean that the presence of a greater amount of yolk per unit mass lowers the general metabolic rate by a definite amount, but as long as the amount per unit

mass remains unchanged, variations in egg-volume affect the developmental rate according to the ratio (volume)/(free surface-area).

According to fig. 27, the lines joining the developmental times for perophorids, clavelinids, and distomids are parallel to that of the styelids, the distance apart of these lines being an indication of the relative amount of yolk per unit mass of ooplasm in each group.

Increase in relative amount of yolk may or may not involve an increase in the relative yolk density. In *Botryllus*, large-egged Molgulae, and in Perophorids the increase in proportion of yolk apparently affects all parts of the egg equally. In the Distomids, Didemnids, and Synoicids, the yolk is also relatively heavy and crowds

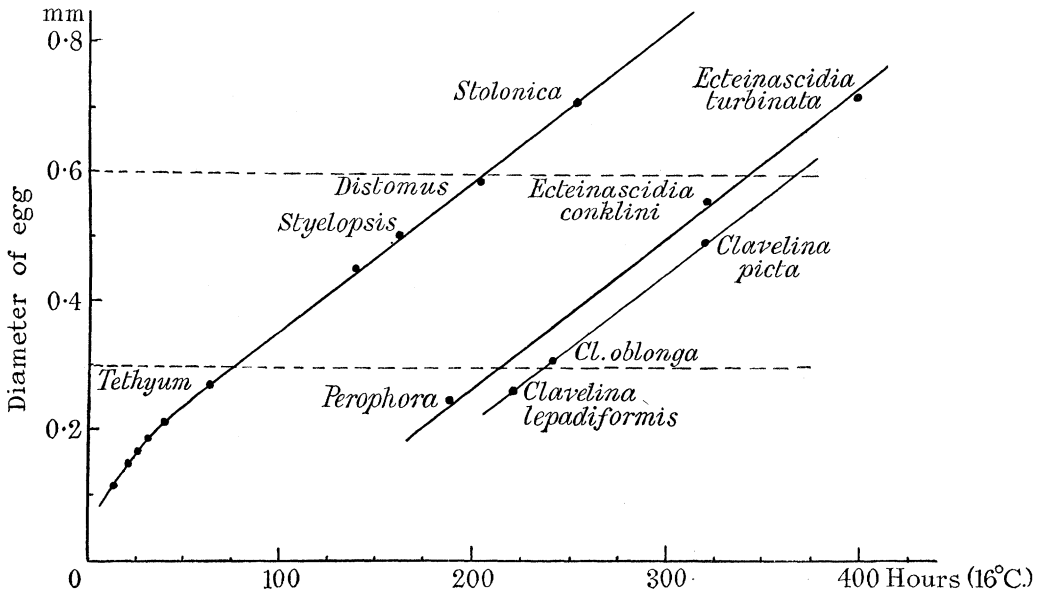

FIG. 27—The relationship between egg-size and duration of embryonic development, showing that where the yolk/cytoplasm ratio remains constant, the duration is proportional to the radius or diameter of the egg. Each line represents a certain yolk/cytoplasmic ratio

the vegetative much more than the animal hemisphere. In the former, therefore, the cleavage and gastrulation remain unaffected, in the latter cleavage becomes unequal and gastrulation more or less epibolic. The main difference between the post-gastrular embryo in the two cases, however, is in the relatively larger size and smaller number of endodermal cells in those with the heavier yolk. The various species exhibiting relative yolk increases may be discussed in order of their respective yolk/cytoplasm ratios.

The two forms with least excess yolk are *Botryllus* and *Molgula citrina* (and *M. complanata*). They have already been discussed to some extent (p. 299). The two Molgulae develop to the tadpole stage in 140 hours, at 16° C, whereas *Polycarpa*, with the same egg-diameter of 0·21 mm develops in 40 hours. The retardation is 100 hours, at 16° C, or 3·5 times. *Botryllus*, with an egg-diameter of 0·43 mm, reaches

the tadpole stage after 200 hours, while *Symplegma*, with the same egg-size and at the same temperature, does so in 140 hours, a retardation of 60 hours, at 16° C, or 1·4 times.

The tadpole stage is by definition the stage wherein the tadpole locomotor organs are perfected and the tadpole becomes active. The retardation of development thus refers specifically to the development of the tadpole organization. It is found that the early cleavage rate, the rate of gastrulation, are proportional to the developmental rate as a whole, and also that the cell-numbers of the notochord, muscle, neutral tube and sense organs are the same as in tadpoles of group one. So that the increase in proportion of yolk affects equally the cleavage and gastrulation rate, and the rate of differentiation of the chordate larval structures. The rate of all these is retarded by a uniform increase in yolk/cytoplasm ratio of the egg. The yolk is merely a hindrance to the development of the larval structure.

The developing trunk or permanent tissues are, on the other hand, very differently affected. Their early development up to and including gastrulation is retarded equally with the larval tissues, but the tendency for cell-division in the larger embryos of group one to accelerate progressively thereafter becomes much more striking. Two factors enter, the increase in relative amount of yolk allows a larger number of cells to be formed before functional and structural differentiation is necessitated, and the retardation or prolongation of the development of the larval structures enables the permanent tissues to attain a relatively advanced developmental stage by the time the tadpole is fully formed. There is, in fact, a telescoping of the development of the larval and adult organization owing to the retardation of the rate of development of the former. Compared with the tadpole of *Polycarpa*, those of *Molgula complanata* and *M. citrina* have identical larval structures but have trunk regions exhibiting growth in size and possessing a far greater number of cells. It is of interest that in spite of the great difference in duration of embryonic development between *Polycarpa* and the above-mentioned species of *Molgula*, the time from fertilization to the formation of a miniature adult with functional heart and stigmata is practically the same. The tadpole stage of *Molgula* is in effect prolonged into the post-larval phase. The morphological differences between the tadpoles of *Polycarpa* and these particular species of *Molgula* are, however, not very striking. They consist mainly of differences in cell-number of the trunk tissues. This may be because the eggs are relatively small and the number of cells formed even in the *Molgula* embryo not very large.

In *Botryllus*, as compared with *Symplegma*, the developmental retardation is less, both relatively and absolutely, but the eggs are much larger, and it was seen even in *Symplegma* that at the time of liberation of the tadpole the trunk tissues were in rapid cell-division and were already accelerating free of the early retardation due to large egg-volume. The additional 60 hours, at 16° C, included within the embryonic period of *Botryllus* thus represents a more spectacular developmental phase than does the extra embryonic period of *Molgula citrina*. *Symplegma* tadpoles on liberation have virtually the primitive organization (of the trunk), the heart functioning about

120 hours, the stigmata 170 hours, and the intestine about 280 hours afterwards. In *Botryllus* the heart functions 24 hours, the stigmata 40 hours, and the intestine 100 hours after liberation. In *Botryllus* the first bud appears shortly after metamorphosis, in *Symplegma* not for many days. These differences are shown more clearly in fig. 31.

In other words, the increase in relative amount of yolk retards the rate of cleavage, of gastrulation, and of larval differentiation throughout, but the development of the permanent tissues and organs is retarded only at first, and by the time the heart or stigmata first functions the early retardation of the yolk has become an acceleration due to intracellular energy. There is thus a telescoping of larval and adult structures due to the retardation of the larval development on the one hand and to acceleration of adult development on the other.

In the Perophorids the retarding and differential effects of an increase in the yolk/cytoplasm ratio is even more pronounced. Compared with *Stolonica*, *Distomus*, and *Polycarpa*, the group one forms with eggs approximating in value to those of *Ecteinascidia turbinata*, *E. conklini*, and *Perophora listeri*, there is a prolongation of embryonic development, at 16° C, of about 170 hours. In *Perophora* the prolongation is based on a standard of 50 hours, whereas in the two species of *Ecteinascidia* the standard is 190 and 260 hours respectively, the total embryonic periods being 220, 360, and 420 hours. Again there is telescoping of larval and adult structures. A comparison of embryonic and larval developmental times for the six species is shown in Table VII, and graphically in fig. 28.

TABLE VII

Species	Egg-size in mm	Time, in hours, at 16° C, from fertilization to functional development			
		Tadpole	Heart	Stigmata	Intestine
Polycarpa	0·21	40	200	280	360
Perophora	0·24	170	170	180	320
Distomus	0·59	190	360	400	480
Ecteinascidia conklini	0·58	330	330	360	420
Stolonica	0·70	240	390	420	510
Ecteinascidia turbinata	0·72	420	420	460	490

In spite, therefore, of the difference of 170 hours, at 16° C, between the embryonic period of each pair, the heart and stigmata function sooner rather than later in the member with the longer period. The influence of additional yolk is accordingly a retardation of early development as a whole, a proportionate retardation of the later development of the larval structures, and an acceleration of the later development of the permanent structures. During late embryonic stages, the use of the yolk droplets and extensive cell-division results is marked growth in size of the trunk as a whole.

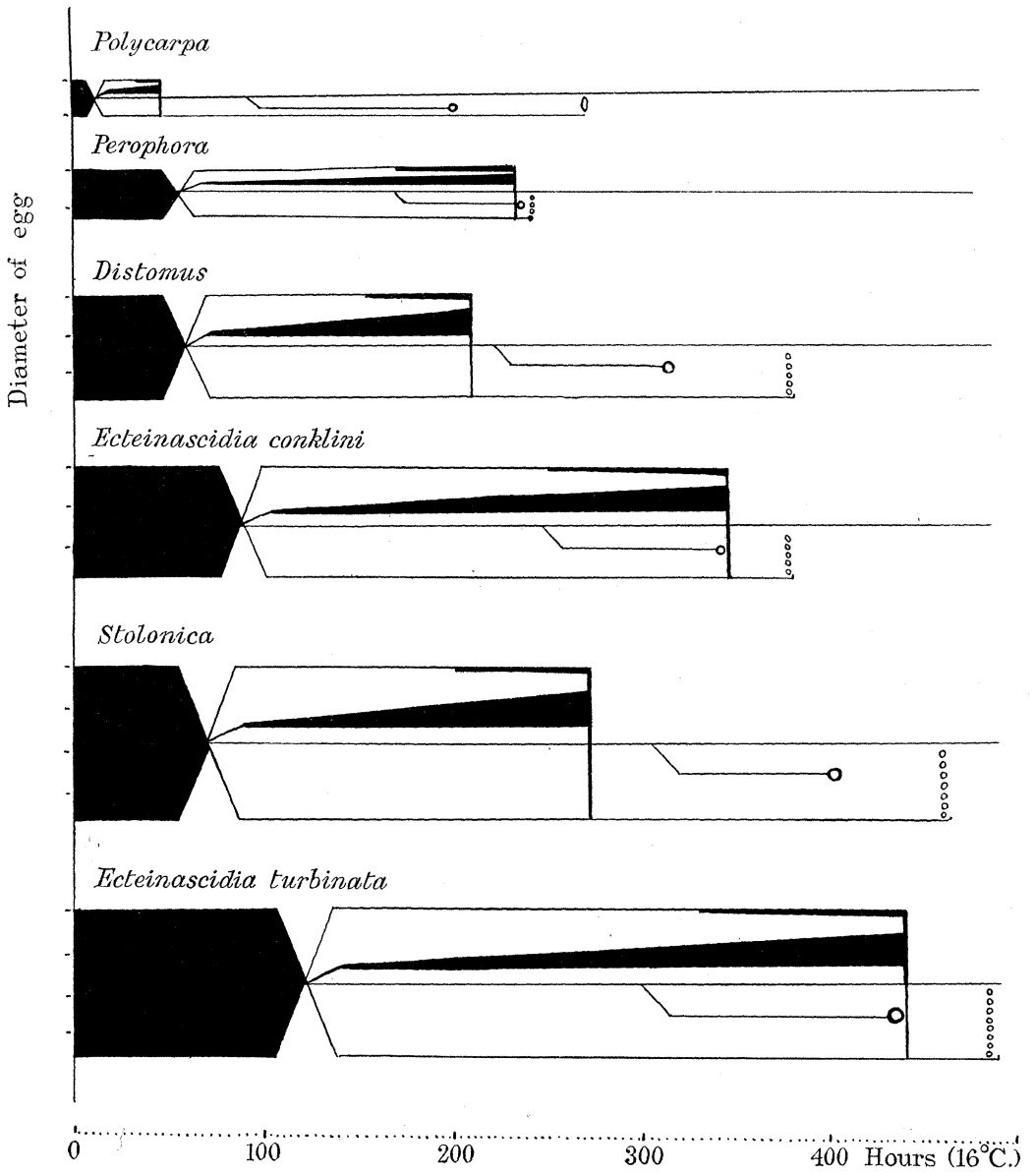

FIG. 28—A schematic comparison of the development of three Styelids with that of three Perophorids, representing three egg-sizes. The telescoping of development in each Perophorid when compared with the corresponding Styelid is very apparent. The development of the Perophorid tadpoles is relatively much retarded, but it should be noted that time of functioning of the heart and stigmata is approximately the same for each pair. Actually there is much more growth in the case of the Perophorids and the total number of stigmata greatly in excess of those of the Styelids (compare figs. 6 and 7 with figs. 12 and 13). It may be seen that only the rate of swelling of the notochord cells differs within each pair and not the final degree, moreover the period of differentiation of the sense organs bears a constant relation to the total embryonic period

Apart from the relative retardations and accelerations there are, just as in the development of the largest eggs of group one, certain morphological differences in development. In the Styelid series (group one) it was argued (p. 311) that when the number of cells in the branchial wall exceeded a critical number before the minimum cell-size and maximum number had been reached, that a short cut took place and rows of definitive stigmata appeared in place of a small number of protostigmata that had to subdivide much later by means of downgrowths.

In the perophorids, the additional yolk per unit mass enables the embryo to form many more cells before the yolk is all used and cell-division of the trunk tissues slows down. In *Perophora listeri* 4 rows of definitive stigmata are consequently formed from the beginning, whereas in the *Polycarpa* species only 2, and in *Tethyum* only 4 protostigmata appear. In *Ecteinascidia conklini* 6 rows are formed, in *Distomus* —with an egg the same size but with less yolk per unit volume—only 4 rows are formed. While in *Ecteinascidia turbinata* 12 rows appear as independent perforations from the beginning, although the Styelid *Stolonica* forms only 4.

Stigmata development in the perophorid series thus bears the same relationship to stigmata development in the styelid series as does that of the large-egged Styelids to that of the small-egged Styelids. In every case the controlling factor seems to be the number of cells present at the time cell-division slows down and differentiation (commencing with perforation) takes place.

In *Perophora* the condition is apparently at a somewhat critical stage when perforation commences. Perforation commences while the division process is still proceeding, and in *Perophora* there is a tendency for 2 protostigmata to develop, but before the cells involved become structurally differentiated, the first 2 perforations rapidly become 4 and each of the 4 a row of definitive stigmata.

Before structural differentiation of the ciliated cells, subdivision of a protostigmata thus occurs very rapidly. After differentiation it is slow an d diffiult, and depends on the formation of tongue bars.

XVII—Increase in Relative Density of Yolk

In the Clavelinidae, Polycitoridae, Synoicidae, and Didemnidae, not only is there a further increase in the proportion of yolk to cytoplasm, but the yolk also is dense and becomes closely packed towards the vegetative pole of the egg. The greater the increase in the proportion of yolk, the greater the difference in yolk content between the animal and vegetative poles.

The effect of the unequal distribution is to retard the cleavage rate of the vegetative to a greater degree than that of the animal hemisphere. In *Clavelina* species the inequality is not very great, the 4 animal cells of the 8-cell stage dividing only 1·2 times as rapidly as the 4 vegetative cells. At the time of gastrulation, however, the vegetative cells are relatively few and large, and gastrulation tends towards epibole. Of more importance is the fact that the rate of development is determined more by the endodermal than by the ectodermal region, and the

developmental rate is accordingly retarded to a greater degree than if the yolk had been evenly distributed. Moreover, the chordal cells partake of the yolk proportions of the endoderm and are usually as heavily yolk-laden as any cells in the body (*cf. Stolonica*). Since chordal cell-division ceases before the yolk can be in any way used, its presence retards the rate of chordal differentiation (swelling), and in consequence the whole development of larval structures up to the tadpole stage is greatly retarded by virtue of the greater concentration of yolk in the vegetative hemisphere.

Increase in yolk density and correlated uneven distribution has therefore a threefold result. The additional reserves produce an increase in the number of cells formed before minimum size and differentiation is attained. The relative concentration of yolk in the vegetative hemisphere enables the gut region to develop to a comparatively advanced stage before final differentiation occurs; while the same relative concentration not only retards the rate of cleavage and gastrulation but causes an additional retardation of the rate of chordal swelling and extension, so that the embryonic period as a whole is prolonged.

Three Clavelinids were investigated—*Clavelina lepadiformis*, egg diameter 0·26 mm, *Clavelina oblonga*, egg-diameter 0·31 mm, and *Clavelina picta*, egg-diameter 0·48 mm. The course of cleavage, gastrulation, development of tadpole, etc., is identical in all three, the developmental rate being proportional to the ratio (volume)/(free surface-area) of the egg. The developmental rate of them all is, however, slower than that of a perophorid of the same egg-size, and much slower than that of a corresponding styelid egg. In *Clavelina lepadiformis* rows of definitive stigmata develop in much the same way as in *Perophora listeri*, egg-diameter 0·24 mm, *i.e.*, following a transient appearance of protostigmata. In this species and in *Cl. oblonga* only 2 rows are formed, but in *Cl. picta* 4 rows develop without showing protostigmata temporarily. The formation of 2 and 4 rows in the species of *Clavelina* as compared with 4 and 6 rows in *Perophora* and *Ecteinascidia conklini*—forms with comparable egg-volumes—needs some explanation. The pharyngeal area of *Cl. lepadiformis* is as large as that of *Perophora*, but the peribranchial ectodermal tissue only extends across its outer surface as a median band and only in that band, therefore, is fusion between the two layers possible and stigma formation feasible. The number of stigmata rows and stigmata per row is, however, just as proportional to the area and cell-number of the *fused* region as in other forms (*cf.* figs. 3, 5, 7, 8, 9, 12, 14, and 17).

Another form with an egg-volume approximating to that of *Polycarpa*, *Perophora*, and *Clavelina lepadiformis* is *Archidistoma*, egg-diameter 0·23 mm. The egg is even more densely packed with yolk than is that of *Clavelina*, and the embryonic period correspondingly prolonged—about 280 hours at 16° C. Gastrulation is truly epibolic, and even though the egg is somewhat smaller than that of *Perophora*, 4 rows of definitive stigmata are formed as independent perforations without any trace of protostigmata.

While even before liberation of the tadpole occurs the first abdominal bud can be seen.

It may be significant that yolk proportion, the uneven or even distribution of the yolk in the egg, can be correlated with the presence or absence of an abdominal extension of the body. With the exception of *Diazona*, which is oviparous and has small unmodified eggs, the only ascidians in which the abdomen and post-abdomen is a comparatively bulky region of the body occupying the post-branchial stalk are those forms in which the yolk is concentrated toward the vegetative pole of the egg and gastrulation tends to become epibolic.

As a single family, the Didemnids are perhaps the most interesting, for, unlike other families in which variations concern primarily differences in egg-volume alone, in this there is considerable variation both with regard to egg-volume and concentration and distribution of yolk within the egg.

Four genera were investigated, namely, *Didemnum*, *Polysyncraton*, *Trididemnum*, and *Diplosoma*. In all the yolk is sufficiently concentrated to produce a very slow rate of cleavage and an extremely epibolic type of gastrulation. Concerning the relative amount of yolk, all that can be said with confidence is that it is greater in the egg of *Diplosoma* than in the others. At the same time the fact that epibolic gastrulation is less marked in *Didemnum* than in the rest suggests that the yolk is less concentrated toward the vegetative pole. On this basis, namely, that there is proportionately more yolk in the egg of *Diplosoma* and a somewhat even distribution of yolk in the *Didemnum* egg, the differences in course of development may be explained. *Trididemnum* and *Polysyncraton* eggs differ apparently merely in size. The eggs of *Didemnum*, *Polysyncraton*, and *Diplosoma* have approximately the same volume.

Didemnum, in spite of its having the largest egg, develops to the tadpole stage in the shortest time. The oozoid becomes, at 16° C, a functional miniature adult about a week after liberation of the tadpole. At the tadpole stage, as there is also at the same stage of Synoicids and *Eudistoma*, a mass of yolky cells are to be seen beneath the branchial sac which are incorporated in the developing abdominal region before the gut becomes active. A miniature adult thus becomes functional before there is any sign of the oesophageal budding typical of the family.

In *Polysyncraton* the yolk is heavier, or at least is relatively more concentrated toward the vegetative pole. Gastrulation is therefore even more epibolic and the chordal cells more heavily laden and more slowly swelling.

The structure of the tadpole shows a close resemblance to that of *Didemnum*, but the course of post-larval development differs markedly. As the mass of yolky cells becomes incorporated in the oesophageal and abdominal region, oesophageal buds develop to form the first blastozooid. This occurs after the loss of the tadpole organization and before the oozoid becomes functional, and after about 18 days at 16° C both oozoid and blastozooid become active together. The activity involved in the process of budding must accordingly interfere with the final differentiation of the oozoid tissues, otherwise the oozoid should become active before the blastozooid. The course of development of *Trididemnum* is identical with that of *Polysyncraton*, except that the rate is faster since the egg is smaller. There is the same long post-larval period at the end of which both oozoid and blastozooid become active together. The

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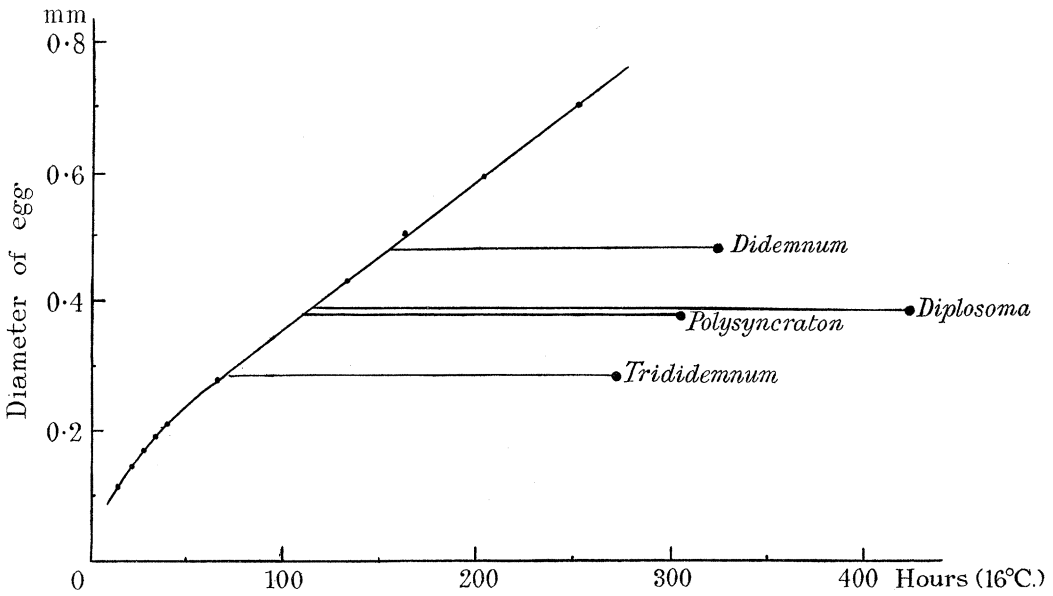

FIG. 29—Graph showing the extreme retardation of development of various Didemnids, the Styelid series being used as a base-line

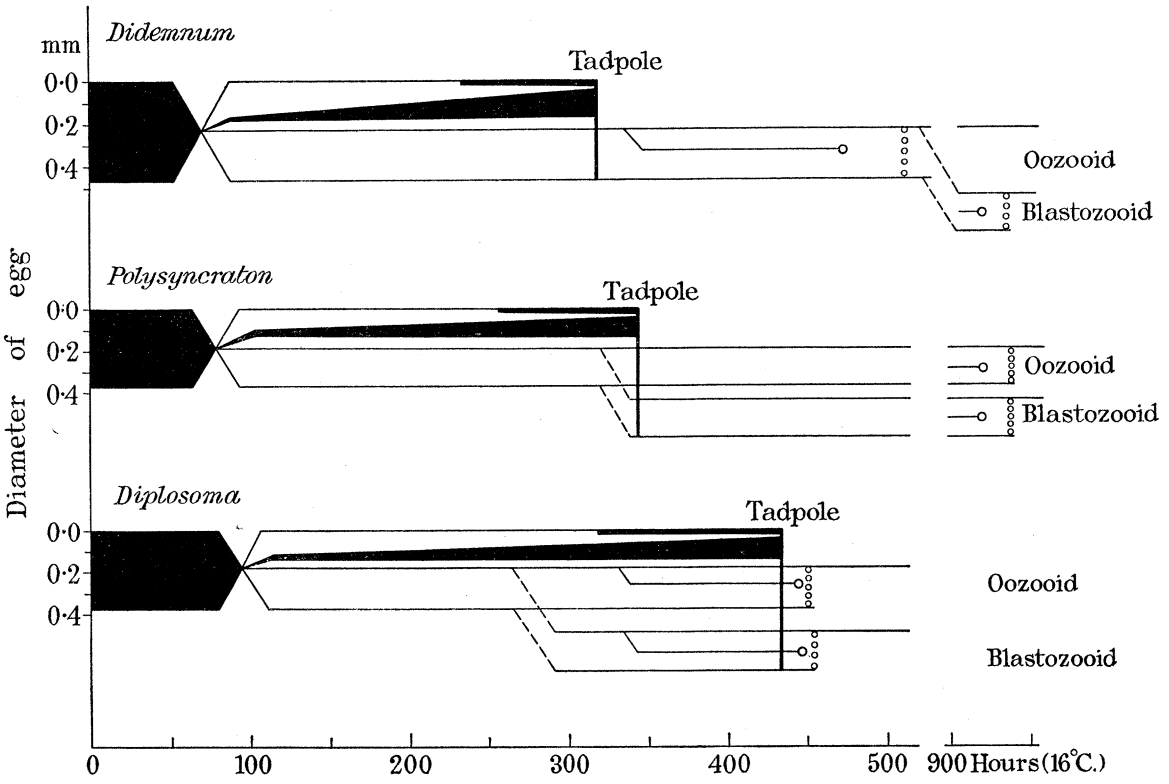

FIG. 30—A schematic comparison of the development of three of the four didemnids shown in figs. 29 and 17. Various degrees of telescoping are shown, and in particular with regard to the formation of the first bud. The origin of the bud is shown by the two broken lines arising from lines representing the endoderm and epiderm (for key consult fig. 23)

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reduction in number of adhesive organs of the tadpole from three to two is evidence that the difference in egg-size between *Polysyncraton* and *Tridemnum* is due to size reduction in *Tridemnum* rather than the converse. Adult differences support this contention.

The egg of *Diplosoma* is more heavily yolked than those of the other three genera. The rate of cleavage, of gastrulation, and of chordal swelling and extension is consequently more retarded, the embryonic period being, at 16° C, 420 hours. At the same time, the developmental stages associated with the post-larval development of *Polysyncraton* and *Tridemnum* in *Diplosoma* fell within the embryonic period and that in which the tadpole is liberated, in which the oozoid and blastozooid are both within 24 hours of complete activity. Associated with the additional yolk, the young colony is able to form 2 more blastozooids within 10 days without needing food from external sources.

The development of the four genera just described is shown in figs. 17, 29, and 30.

Diplosoma thus represents the extreme example of telescoped development, a telescoping resulting from retardation of the developmental rate of the larval structures, and of acceleration of the permanent structures.

XVIII—Reduction in Egg-Size

The outstanding example of *reduction* in egg-size is to be found in *Botrylloides*, the egg of *Botryllus* being recognized as the normal type for the sub-family. The egg of *Botryllus* has a diameter the same as that of *Symplegma*—0·45 mm—the egg of *Botrylloides* one of 0·26 mm, *i.e.*, one-sixth the volume. There is little doubt that the small size of the *Botrylloides* egg is due to secondary reduction and is not primitive.

The reduction is correlated with an apparent placentation of the egg. In *Botryllus* the eggs and embryos are born by cup-like folds of the peribranchial wall (Arnbäck, 1923), the tadpoles escaping eventually into the cloaca of the colony. In *Botrylloides* the eggs do not pass up the oviduct to the peribranchial region at all, but remain as lateral bulges of the body in the abdominal region. A special fertilization duct (according to Garstang and Garstang, 1928) passes direct from the testis to the single-egged ovaries, while the tadpole eventually escapes into the cloaca by rupture of the thin body wall of the parent. These authors further suggest that nutriment is supplied to the developing embryo by means of a narrow duct penetrating the egg membranes.

In spite of the smaller egg-size, the tadpole of *Botrylloides* is as large and is quite definitely somewhat more highly organized than that of *Botryllus*, while the post-larval development is almost identical in the two cases. Their graphical comparison is shown in fig. 31.

That there is true placentation in *Botrylloides* is merely an assumption based upon the reduced size of the egg and its close relationship with the parent. On analysis, however, parental nutrition of the embryo during development seems to be very

doubtful. In the first place, the tadpoles of *Botrylloides* and *Botryllus* are not only very similar, but the post-larval development is remarkably alike for at least three weeks. The embryonic period from fertilization to the liberation of the tadpoles, moreover, is practically the same, so that since both the morphological and time sequence of embryonic, larval, and post-larval development is virtually identical, it follows that the eggs, either before or after fertilization, must receive practically the the same amount of nutrition for the present.

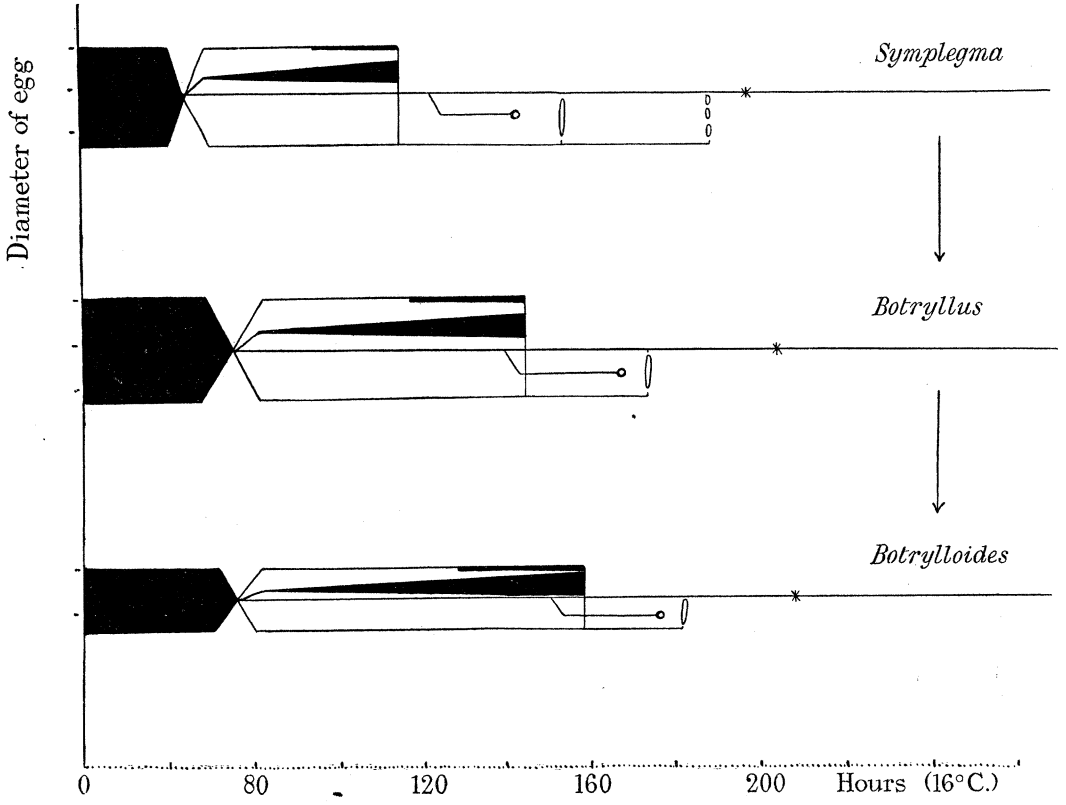

FIG. 31—A schematic comparison of the development of *Symplegma*, *Botryllus*, and *Botrylloides*, showing the telescoping of development of *Botryllus* as compared with that of *Symplegma*, and the great similarity of the development of *Botrylloides* and of *Botryllus* in spite of the greatly reduced size of the egg of *Botrylloides*. There is much evidence that the three genera actually represent a true phyletic series in the direction shown by the arrows

Secondly, the peculiar structure of the *Botryllus* tadpole as compared with that developing from the more primitive *Symplegma* egg can be explained only as the result of an increase in the relative amount of yolk, an increase showing its effect chiefly through the retardation of cleavage and in particular in rate of extension of the notochord, thus allowing the endoderm of the trunk to attain a relatively advanced state of organization by the time of liberation of the tadpole. In other words, the peculiarities of the tadpole are due chiefly to an incidental inclusion of yolk in the chordal cells, thus retarding their rate of differentiation.

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Any extra-embryonic nutrition replacing a loss of intra-cellular yolk must therefore be fully supplied before the chordal cells are eliminated. It must also be of such a nature as to retard their rate of differentiation to almost exactly the same extent as the presence of the inactive yolk in the chordal cells of the *Botryllus* embryo, which is hardly conceivable. Furthermore, if there is extra-embryonic nutrition sufficient to overcome a diminution of egg content by six volumes, it should show its effect as a prolongation of the whole developmental period without inducing telescoping, and, in consequence of the small initial volume of the egg, the early development should be very rapid. Compared with *Botryllus*, the cleavage rate and gastrulation rate should be accelerated.

If the embryonic times are analysed, however, conditions are found to be quite different. Not only is the embryonic period as a whole much the same length as that of *Botryllus*, but the inter-cleavage interval, the time from fertilization to gastrulation, and from the onset of gastrulation to the beginning of tail outgrowth are practically the same. In other words, the course and times of development are almost identical and the only difference is in the size of the egg. It seems impossible to conceive any means by which such identity can be maintained when extracellular parental nutrition is substituted for intracellular yolk.

On the other hand, if the difference in egg-size of these two genera is due, not to quantitative reduction merely in egg-volume, but to a diminution in the water content or cytoplasmic content of the egg, the unchanged course of development can be explained.

The above suggestion implies that the amount of yolk per egg remains unchanged, so that development can continue to the same stage before an external source of food becomes necessary. The yolk content per unit volume is greatly increased, however, and this explains the fact that the cleavage and gastrulation rate is not accelerated by the reduction in egg-volume. The embryonic period as a whole remains unchanged because the chordal cells receive their full load of yolk, so that in spite of their smaller size they swell more slowly rather than less. The endodermal cells receive their full yolk complement also and are therefore able to develop at least as far as do those of *Botryllus* during the embryonic period. It is suggested, therefore, that instead of there being placentation of the *Botrylloid* embryo, the reduction in egg-size has left the total amount of yolk per egg unchanged. The appearance of the eggs of *Botryllus* and *Botrylloides* is in harmony with the above conclusion.

XIX—Conclusions and Summary

The development of 44 species representing 35 genera of ascidians has been investigated, 11 species being oviparous, 25 viviparous.

The relationship between viviparity and increase in egg-volume or in proportion of yolk in the egg is discussed. It is concluded that viviparity must arise before increase in egg-size can be anything but disadvantageous. Increase in egg-size or accumulation of yolk is the result of long-established viviparity.

Viviparity itself may be produced by a variety of causes—by excessive reduction in mature size in the Molgulidae, by the subdivision of the gonads into Polycarps at some distance from the atrial siphon in the Styelidae and Botryllidae, and by a relative shortening of the oviduct so that the eggs are laid at the base of the atrial chamber in the Perophoridae and the order Aplousobranchia (Krikobranchia).

The cell-size of different tissues of various adult ascidians has been determined. In epidermal and mesenchymal tissue the cell-size is found to be almost constant in spite of differences in adult volume of as much as 1 : 18,000. Intestinal epithelial cells varied in volume no more than 1 : 8. Adult body-size is thus an expression of the cell-number. The number of stigmata and of rows of stigmata in the branchial wall is shown to depend on the area involved and consequently upon the number of cells.

Reduction in size of an ascidian colony is shown to produce marked modifications in the structure of the ascidiozooids, one of which is the transformation of the oviduct into a brood pouch and the production of very few but very yolky eggs.

The development of each ascidian genus investigated is described in turn, commencing with the more primitive oviparous types and ending with those with the most modified development.

From a comparison of embryonic times, together with experimental results concerning the influence of various oxygen and carbon-dioxide tensions upon development, it is concluded that the rate of development up to the tadpole stage is determined by the ratio of the free surface-area to the volume of the egg, so long as the proportion of yolk to cytoplasm remains unchanged. It is also concluded that the ratio (volume)/(free surface-area) of the egg and embryo controls the developmental rate through its control of the rate of respiratory exchange, and that the carbon-dioxide tension at the surface and through the protoplasm is of much greater importance than the oxygen tension. Increase in proportion of yolk to cytoplasm has the same effect as increase in proportion of volume to surface-area.

The detailed analysis of development is divided into two main sections, the conclusions from which are given separately. The first division includes the forms in which variation concerns only egg-volume, the second in which the yolk/cytoplasm ratio also varies.

A. (Volume)/(free surface-area) ratio variable, yolk/cytoplasm ratio constant (p. 292).

a. The rate of cleavage, of gastrulation, and of differentiation of larval tissues varies directly with the (volume)/(free surface-area) of the egg and embryo (pp. 293–301).

b. Cell-division ceases at an early stage in the larval structures and organs, the cell-numbers remaining constant and cell-sizes varying with the egg-volume. The rate of differentiation of the notochord, tail muscle, neural tube, and sense-organs is also controlled by the (volume)/(free surface-area) (p. 305).

c. In the permanent organs and tissues cell-division continues until a minimum size is reached, so that the cell-number of each kind varies directly with the

volume of the egg. The developmental rate at first is proportional to the (volume)/(free surface-area), but after gastrulation the rate of cell-division becomes progressively accelerated with increasing egg-volume. In any case the full number and minimum sizes of the cells are not reached until after the tadpole stage has been passed (pp. 306–308).

d. The organization of the tadpole, both with regard to larval and permanent organs, is identical whatever the volume of the egg. Organization must therefore be relatively independent of cell-size and cell-number (p. 308).

e. Post-larval development is primarily a continuation of cell-division in the permanent tissues until a maximum number and minimum size is attained. As in the larval tissues, the cessation or slowing down of division is followed by a relatively long period of differentiation (pp. 308–310).

f. The number of protostigmata or number of rows of definitive stigmata first appearing is determined by the number of cells (or since the same minimum cell-size is attained in all forms, by the length) of the anteroposterior axis of the branchial wall. The subdivision of a protostigma by transverse tongue bars is determined by its growth beyond a certain length. If the number of cells along the dorso-ventral axis of the branchial wall exceeds a certain number before perforation occurs, rows of definitive stigmata appear from the first, in place of a corresponding number of protostigmata (pp. 311, 312).

B. Yolk/cytoplasm ratio variable.

a. An increase in proportion of yolk to cytoplasm retards the rate of development independently of egg-size (pp. 312, 313).

b. Increase in egg-volume and in yolk proportion when evenly distributed does not affect the cell-stage at which gastrulation occurs, nor the type of gastrulation. When yolk is accumulated more in the vegetative than in the animal half of the egg, cleavage becomes unequal and gastrulation epibolic (p. 301).

c. Increase in proportion of yolk to cytoplasm, whether equal or unequal, decreases the rate of cleavage, of gastrulation, and of differentiation of the larval structures. After gastrulation the rate of cleavage and development of the permanent tissues becomes progressively accelerated (pp. 314–317).

d. At the time of liberation of the tadpole the permanent trunk region may be very highly differentiated. In the extreme case of *Diplosoma* the first blastozooid may be almost functional before the embryonic period comes to an end (pp. 317–321).

e. The relatively small egg of *Botrylloides* is a case of secondary reduction in size, a reduction that has not affected the total amount of yolk per egg. Placentation is only apparent and there is no need to postulate extra-embryonic nutrition (pp. 321–323).

The general conclusions based upon sections A and B are that increase in egg-volume or increase in proportion of yolk to cytoplasm induces a telescoping of development, and that the telescoping is due both to a retardation of the rate of

differentiation of the larval tissues and to an acceleration in the rate of cell-division in the permanent tissues. The development of the larval chordate structure and of the permanent ascidian structure are virtually independent of one another. This is confirmed by the facts recorded in a previous study (Berrill, 1929) that in the Molgulidae the development of the larval chordate organization may be completely suppressed without interfering with the normal development of the permanent structure.

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