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EMBRYOLOGY AND EMBRYONIC FISSION
IN THE GENUS CRISIA.*

BY
ALICE ROBERTSON.

INTRODUCTION.

The processes of embryonic fission in the Cyclostomata were first made known a few years ago by Dr. Sidney F. Harmer. That investigator found that this unique process of reproduction of the embryo occurs in several somewhat distantly related genera of the subclass, viz., in *Crisia*, in *Lichenopora*, and in *Tubulipora*. The facts disclosed were so interesting and remarkable, that further study of the phenomena was deemed desirable, both for the corroboration of the results, and for the purpose of completing more of the details. The investigation reported in the following pages has been made upon *Crisia* only, several species of which occur abundantly in the vicinity of San Francisco Bay. The chief results of Dr. Harmer's investigations, that is, the discovery of the occurrence in this genus of a budding of the embryo, the separation of the buds from the mother embryo, and their ultimate transformation into free swimming larvæ, have been fully confirmed. Besides as thorough a study as possible has been made of the origin of the genital products, both male and female. Some unique features have been found in the origin and development of these elements, all of which may be interpreted as secondary modifications due to the high degree of colonial specialization to which these bryozoa have attained.

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R. BLANCHARD

PROF. FAC. MED. PARIS

Technique.—For this investigation, material has been collected each month, and twice in the month during the spring, when the tides were favorable. Although specimens have been secured from various localities, they have been regularly obtained from a locality known as Lands End, near the entrance of the Golden Gate, California. The results most relied upon have been obtained then, from material killed and fixed under the most favorable circumstances, *i.e.*, very soon after collection. The relatively thick calcareous ectocyst of *Crisia* makes it difficult to fix the tissues rapidly enough to prevent their shrinkage and consequent distortion. The most successful results were obtained by the use of a solution of hot corrosive sublimate. In most cases a solution of this with glacial acetic was used, in other cases, the hot corrosive sublimate alone. The specimens were allowed to remain in the fixing fluid only long enough to become penetrated, when they were washed in 50% alcohol containing iodine. After this, they were carried through the various grades of alcohol and finally preserved in 85% alcohol. The process of killing and fixing did not include decalcification. Such portions only as were required for mounting, were afterward completely decalcified. In the process of decalcification, much trouble is frequently experienced by the formation of bubbles of gas. It was found easy to avoid these, however, and the consequent tearing of the tissues, by decalcifying small pieces in a high grade of alcohol made weakly acid. The stains used were Delafield's and Ehrlich's hæmatoxylin with eosin; Benda's iron hæmatoxylin alone, and with eosin and fuchsin; and Auerbach's mixture of methyl green and fuchsin. Many other stains were experimented with, but these gave the most satisfactory results.

Four species of *Crisia* are more or less abundant in this region, *viz.*, *Crisia eburnea*, *Crisia geniculata*, *Crisia cornuta*, and a new species, *Crisia occidentalis*. A full description of this last species will follow in a later paper. Special reference is made in this paper to *Crisia eburnea*, although all the species have been studied more or less in regard to their method of reproduction. *Crisia eburnea* is certainly diœcious, the two kinds of genital products never being found in the same colony. This is thought to be true also of *Crisia occidentalis*, although the evi-

dence is less conclusive for this species. The other two, *Crisia geniculata* and *Crisia cornuta* are probably monœcious.

REPRODUCTIVE PROCESSES.

SEXUAL ELEMENTS.

1. *Origin of the Male Genital Products.*—*Crisia*, and perhaps other genera of the Cyclostomata, differ from the rest of the bryozoa in the production of the sexual elements. In young and growing colonies of this genus these products originate and are differentiated as such, *at the tips of the branches*. This can best be seen in the spring when the colonies are growing actively, and when the germinal tissue is in the healthiest condition. During the fall and winter months the tissue is thin even at the growing points, stains badly, and is in a degenerated state. In the latter part of February and throughout March, April, and May, however, both sorts of germinal cells are abundant, and form very conspicuous objects in all the young tips. The tissue at the growing points at this time forms a thick layer of "embryonic" cells closely packed together and staining deeply in hæmatoxylin. It is here differentiated into two layers which form the body wall, or lining of the zoœcia. Pl. XII, Fig. 1, represents the tip of a branch of *Crisia eburnea*, which has been decalcified, stained and mounted in toto. It consists of two series of zoœcia (z^1 and z^2) lying side by side. At the growing point (*gr. tis.*) the zoœcia are cut off alternately from the outer edges, the bases (*b.*) or proximal extremities of each pair being in contact, while their distal portions are separated by the bases of the next succeeding pair. The branch is thus somewhat flattened, having a dorsal (*d.*) and a ventral side (*v.*), and a right (*r.*) and a left (*l.*) edge. The growing point includes that portion which is anterior to the youngest pair of zoœcia and consists of two parts, (*a*) the layers of deeply staining cells (*gr. tis.*), and (*b*), the budding region. This latter is represented in Fig. 1 by young polypides (*pd. bd.*) in various stages of advancement. These portions are again shown in Pl. XIII, Fig. 18, which represents the tip of an actively growing branch containing, besides a developing ovicell (*ovl.*), a number of young polypides (*pd.*).

The cell layers which make up the body wall of a colony may be distinctly seen in section. Pl. XII, Fig. 2, represents a section from a growing tip of a male colony, in which the outer, or ectodermal layer consists of small rounded cells (*ec. cls.*), while the inner or mesodermal layer consists of much larger cells possessing a distinct large nucleus (*mes. cls.*). It is part of this inner layer which becomes modified into a germinal epithelium, (*ger. cls.*), and from which both ova and spermatozoa originate. Pl. XII, Fig. 3, is a section from the same series representing much the same characters. If these two sections be compared, the mesodermal cells in each (*mes. cls.*) are seen to be of various sizes. Many are of normal size (*mes. cls.*), while others are much larger, and constitute the cells of the male germinal epithelium (*ger. cls.*). In the germ cells, the nucleus and nucleolus have increased in size, and are surrounded by a layer of finely granular cytoplasm. The mesodermal cells which go to form the parietal layer are of various sizes and shapes, but of similar appearance. The ectodermal cells are either rounded or elongated, depending upon the portion of the tip in which they are. Near the edges, right and left, they are round, while near the middle they become much elongated and less numerous, (Pl. XII, Figs. 10 and 11, *ec. cls.*).

The relation between the polypide buds and the germinal tissue is shown in Fig. 4, a section from a male colony which represents several stages in the development of the polypides. At the anterior edge, in the angle toward the left (*l.*) the germinal cells may be seen (*ger. cls.*). Proximal to this point, a mass of cells represents the youngest polypide bud (*pd. bd. 2*), and below this there is an older bud (*pd. bd. 1*) in which the cavity of the stomach is formed (*st.*). As the distal portion of the branch continues to grow, the fully formed germinal cells are left behind at or near a point where a polypide bud forms, and in a male colony a few of these cells become attached to each bud constituting the testis of the developing polypides. In Pl. XII, Fig. 4, a number of large cells closely resembling the cells of the germinal tissue in size and appearance of the nucleus, are attached to the stomach of the older polypide bud (*pd. bd*

1., *tes.*). Below the stomach of the polypide (*pd.*) is a similar but larger mass of cells constituting the testis of that animal (*tes.*). If more of the branch of which Fig. 4 is a section could be shown, each succeeding polypide would be found to possess a corresponding structure. Examination of a series of polypides shows that the development of the testis proceeds with that of the polypide, the lower and hence the older polypides possessing the larger testis.

The spermatozoa, two of which are shown in Fig. 5, may be found clustered about large cells which are more or less abundant throughout the testis, or may be seen passing in a stream through the testis toward its distal portion, to a point at the base of the tentacles. Their actual egress was not detected, so that it is not known whether it occurs through a definite opening or only after the degeneration of the polypide, as is the case in most bryozoa. Harmer ('93) mentions the escape of the spermatozoa of *Crisia cornuta* through the aperture of the zoecium, but fails to state whether or not the polypide had degenerated. Hincks ('80) observed them passing in a stream through the intertentacular organ. The ectoprocts are not thought to have a sperm duct, the sperm escaping presumably through the orifice of a zoecium after the polypide has degenerated. Since in most cases ova and spermatozoa are produced in the same zoecium, either simultaneously or in succession, the necessity for a means of egress so that the one may reach the other is not so important. It is possible that in *Crisia* they may escape before the death of the polypide, and what evidence I have would indicate that those that mature do so while the polypide is still intact. In examining a quantity of material, however, the scarcity of ripe spermatozoa is very noticeable. In the spring, at least, the male genital products can be obtained in abundance and in various stages of development, but one searches almost in vain for spermatozoa. In a collection of preparations representing a hundred or more polypides, and made from material obtained during the season when the sexual elements are most abundant, in only *one instance* was ripe sperm found. Fig. 7 represents a section of living testis in a somewhat advanced stage of development, showing a typical arrangement and appearance of the

cells. These are in groups of darkly staining nuclei, sometimes arranged in large numbers around a central mass of cytoplasm, very frequently in groups of four nuclei imbedded in a mass of cytoplasm. (Pl. XII, Fig. 7, *tet*, and Fig. 7A.) The individual members of these tetrads consist sometimes of solid masses of chromatin, sometimes of an outer layer of chromatin surrounding a vacuole. Whether vacuolated or not, these probably represent stages in the development of spermatozoa—a development which apparently proceeds no further. Without making an exhaustive study of the spermatogenesis, it is, of course, impossible to state positively that degeneration of the testis occurs at this stage in the development of the sperm cells, and such a study has not been made; but in view of the evidence adduced, the suggestion that the testis does thus degenerate is worth consideration.

In examining branches of male colonies in which regeneration is taking place, the quantity of degenerated material in each zoecium is unusually large as compared with that found in other bryozoa. Such a mass of material is shown in Fig. 6, which represents a section of a zoecium containing a small regenerating polypide (*re. pd.*) and the remains of a degenerated polypide, the former occupant of the zoecium (*b. b.*). In this "brown body" two portions can be distinguished, a round, somewhat homogeneous mass representing the tentacles and alimentary canal of the degenerated polypide (*de. pd.*), and a long, tapering mass extending almost to the base of the zoecium representing, perhaps, the degenerated testis (*de. tes.*). This latter occupies the position of the testis and closely resembles it in appearance, both of the whole mass and of the individual groups of cells, among which the tetrads, both vacuolated and non-vacuolated, can be detected.

Comparing the early regenerating stages of male and female colonies, the quantity of material in the "brown bodies" in the latter is smaller than that in the male, and represents the degenerated polypides only. Each is at first a homogeneous mass which later disintegrates more or less, and falls into the base of the zoecium. In the later stages, when the regenerated polypide has attained its full growth, the difference in appearance of the

"brown bodies" of the sexes is not so apparent. In both the residue becomes pushed into the extreme base of the zoëcium and is packed into smaller space.

The evidence for degeneration which is afforded by the scarcity of spermatozoa, and by the resemblance between the "brown body" of the male colonies and the testis, is strengthened by its probable correlation with what occurs in the female colonies. Here, as will be shown, large numbers of ova are produced, but on account of the reproduction peculiar to *Crisia*, relatively few give rise to larvæ, hence a relatively small number of sperm are functionally necessary. Degeneration of the male genital product, if it occur, is to be regarded, then, as a secondary modification correlated with the fact that every egg that contributes to the perpetuation of the species produces, through embryonic fission, not one, but a great many colonies.

2. *Origin of the Female Genital Products.*—In the female colonies of *Crisia eburnea* the ova arise as do the male germ cells, from the mesoderm of the growing tip of the branches. They are *differentiated at the tip of the branches, and in no other part of the colony.* Pl. XII, Fig. 8, represents a section from the ventral side of a female colony, in which the two layers of the body wall are distinctly shown. Close to the anterior edge is a row of small round ectodermal cells (*ec. cls.*), forming the outer layer, while inside of this is a layer of larger cells possessing very large distinct nuclei, and constituting the mesodermal layer (*mes. cls.*). The cells of this layer perform various rôles in the economy of the colony, some giving rise to part of the parietal lining of the zoëcia, some being transformed into the mesenchymatous tissue of the branches (*mes. tis.*), and the remainder producing the germinal epithelium. If a comparison be made between Fig. 8 of a female colony, and Fig. 2 and 3 of a male colony, no difference will be recognized in the cells of this tissue. In both, the germinal cells are of the same size, and bear identical relations to the growing points. It was shown for the male colonies that the germinal cells are more numerous in the angles, right and left, of the tip. This is true also of the female colonies, as may be seen in Pl. XII,

Figs. 9 and 10, which represent serial sections from the growing tip of a female colony, each of which shows the accumulations of modified mesodermal cells in the angles of the branch (*ger. cls.*). They are found, too, at a time earlier than that at which the polypide bud appears. This is especially clear in Pl. XII, Fig. 11, representing a section of the bud-forming region of a female colony. At the anterior edge of the tip are the germ cells (*ger. cls.*), while proximal to these is a series of polypide buds in various stages of development. In the oldest bud (*pd. bd. 1*) the cavity of the stomach is visible (*st.*). No ova have united with any of these buds, and an examination of older portions of the branch does not reveal their existence in the older zoëcia. On the other hand, numberless sections prove that not only are single, detached ova produced at the anterior extremities of the branch, but it is in these places that the ovaries are located. Evidence of this is given in Pl. XII, Fig. 12, and Pl. XIII, Figs. 13 and 14, consecutive sections taken somewhat obliquely through the germinal region of the tip of a colony. The line of cells (*sep.*) in the three sections, represents different parts of the same septum. Fig. 12, the first section of the series, is composed mainly of cells forming the ventral wall, the heap of cells lying near the septum (*pd. bd. 2*) representing the outer layer of a polypide bud. Fig. 13 represents the same polypide bud (*pd. bd. 2*), while proximal to it is another (*pd. bd. 1*). Distal to the anterior bud (*pd. bd. 2*) in this section, five cells of an ovary are shown, one of which (*ov.*) has advanced considerably in development. Fig. 14 shows an ovum (*ov.*) from the same ovary, which lies in close proximity to a polypide bud (*pd. bd. 1*). From this point forward there extends to the tip of the branch, an almost unbroken line of ova, constituting an ovary. A similar condition is represented in Pl. XIII, Fig. 15, a section from another colony, where several ova lie close to the septum (*sep.*), within the cavity of the branch. These are in close proximity to a mass of small cells (*pd. bd. 2*), and constitute the older portion of an ovary (*ovy.*), which as succeeding sections show, extends forward to the anterior edge of the branch. Examination of a great number of series reveals the same condition, *i. e.*, the formation of groups of ova, or ovaries at the tips

of the branches. Such a precocious appearance of ova is reported in a few instances among the Cheilostomata (Calvet '00), but as far as I am aware no other case is known in which the *ovary itself* is thus precociously formed. The early appearance of the germ cells in *Crisia* is somewhat comparable to what takes place in the *Hydromedusae* ('90). In both classes of animals it is a secondary condition correlated with the subordination of the sexual individuals, and the assumption by the colony of the reproductive function.

Throughout the bryozoa the sexual elements are produced, as a rule, in the zoecia and in connection with the polypides. Thus, Nitsche ('69) found that in *Bugula* the ova arise from the inner surface of the endocyst of the younger zoecia. In the older zoecia he found the spermatozoa and in still older ones, the fertilized ova. Vigelius ('84) reports that in *Flustra membranacea-truncata* the genital products, both male and female, also arise from the endocyst of the zoecium, and Prouho ('92) in a series of observations upon the Ctenostomes, found essentially the same condition as far as the time and place of origin of the sexual elements are concerned.

More recently, Calvet ('00) has reported a series of observations upon no less than forty-four species of marine bryozoa. These studies have reference mainly to the Cheilostomata and the Ctenostomata, his study of the Cyclostomata having been very restricted. In one species of the Cyclostomata, viz., *Crisia denticulata*, he made some observations on the reproductive processes, corroborating the researches of Harmer on the fission of the embryo. In the list of species whose spermatogenesis he studied, he mentions two Cyclostomes: *C. denticulata* and *Tubulipora flabellaris*. In his discussion he makes no particular mention of them, however, merely including them in the list with others, in which he says the primitive sperm cells originate as in *Bugula sabatieri*, i. e., in the vicinity of the funicular cord in the lower portion of the zoecium. One can only infer that he made no investigation of the growing tips of these two species, and the study of the adult animals alone would certainly mislead one as to the time and place of origin of the spermatoblasts.

Calvet's study of ovogenesis in *Bugula sabatieri* reveals an

interesting similarity between the origin of the ova in that species and in *Crisia*. Thus, in the young tips, he finds large cells which he considers to be "éléments ovulaires." Furthermore, he finds these cells in a cavity of a branch, distal to the region where the polypide buds are found. He says: "Dans les blastozoïdes jeunes, soit par l'observation directe sur le vivant, soit par l'examen comparatif des coupes histologiques, on peut suivre pas à pas la genèse des différentes parties constitutives de l'ovaire adulte. Il n'est pas rare de rencontrer, parmi les éléments libres de la cavité d'un blastozoïde terminal renfermant un polypide à l'état de rudiment massif, un certain nombre de cellules qui, par leurs grandes dimensions et leurs caractères histologiques, se désignent déjà comme éléments ovulaires (Pl. V, fig. 7et9, *ovu*)."

His description of these cells leaves no doubt that they are eggs, and his figures show the close resemblance between them and the ova found at the growing points of a colony of *Crisia* (Pl. XII, Figs. 13, 14, and 15). This writer regards the ova which he finds at the growing points of *Bugula* as exceptional, and not as showing the ordinary method of their development. When so found they constitute merely the "anlage" of the future ovary, and in no case does he find the mature ovary outside of a zoecium containing a polypide. In this respect then, *Bugula* differs materially from *Crisia*, since in the latter genus the ova which appear among the free elements of the tips of the branches, constitute the ovaries, and it is here that the ovum matures, is fertilized, and unites with a young bud to form an ovicell.

There is much in confirmation of these observations on the early development of the genital products, and of their independence in their earliest stages, to be obtained from Harmer's investigations. That writer reports the finding of egg-like cells in the growing tips of *Crisia*, and says, "The fact that these eggs are commonly found in the growing points, leads me to suppose that several are produced in each fertile internode; apparently by a modification of the funicular tissue, and that their further development depends upon their entering into definite relation with a polypide bud." In *Tubulipora* ('98), he finds that eggs are abundant in the young lobes. He found them in many of the zoecia in connection with polypides and polypide

buds of every stage of growth. In *Lichenopora* ('97), he found but one egg, as a rule, in each colony, and always in the second or third zoëcium, and when the polypide was very young. In all these cases he regards the egg as "probably differentiated *in situ* from the outer layer of a young polypide bud," or, "The eggs appear as part of the polypide bud." Or again, "The eggs (of *Tubulipora*) are developed at a very early stage by the polypide buds, as in *Lichenopora* and *Crisia*." Furthermore, he found an egg-like cell $9.6\ \mu$ in diameter at the growing margin of a colony of *Lichenopora*. He did not feel sure that this was normal, although as he says, it recalls the condition in *Crisia*. In his study of embryonic fission this observer made no special study of the origin of the sexual elements. He explains the occurrence of ova at the growing margin as due either to the productiveness of the young buds, or as an unusual, perhaps abnormal phenomenon.

In *Crisia* one fails even in the height of the breeding season to find even a rudimentary ovary within the individual zoëcia, or elsewhere. What becomes of the relatively large number of ova? Do they all reach maturity? If not, what is their fate? In answer to these questions it is to be said that all ova do not produce embryos. According to their fate they fall into three classes. The first (*a*), comprises the relatively small number that produce embryos within an ovicell. The second (*b*), includes the small number which reach a *partial development* within the zoëcia, and the third (*c*), includes the remainder which *fail* of development entirely.

It has already been shown in the case of the male colony that proximal to the region where the germ cells are formed is the budding region, and further that in order that the male germinal cells may complete their development, they must become united with a polypide bud. (Pl. XII, Fig. 4, *pd. bd. 1.*) In a similar manner, in order that an *ovum* may reach maturity, it is necessary that a union should be effected with a polypide bud. In his account of the reproductive processes in the Cyclostomata, Harmer ('93) has shown that a peculiar relation must exist between a bud and an ovum, in order that an ovicell should be formed. He says: "One of these (egg-cells) acquires a close

relation to the potential alimentary canal of the ovicell polypide," that is, to a bud which without the intervention of an ovum would have developed in the ordinary fashion. And further, "This potential alimentary canal grows round the ovum, losing its previous form and becoming a compact multinucleated follicle surrounding the egg . . ." The study of a series of sections from an ovicell-bearing colony, shows that the relations entered into by the bud and ovum are of two sorts, each producing opposite results. In the first the *ovum* develops, while the bud is aborted. This includes all the cases of the first class (*a*) as given above, and represents the only relation recognized by previous observers. In the other, the *polypide* grows to maturity while the ovum is aborted, and includes the second class (*b*) above. To distinguish between the earliest stages of these two possible relations is extremely difficult, if not impossible, since before the cells of the bud become somewhat differentiated, there is no criterion by which it can be certainly known whether or not an ovicell will result. Thus, in Pl. XIII, Fig. 14, an ovum (*ov.*) is shown in close proximity to a bud (*pd. bd. 1*), but the outcome of this relation cannot be predicted. Again in Fig. 15 several ova are seen in close connection with a group of small cells (*pd. bd. 2*), but whether or not there is here an incipient ovicell, cannot be asserted. Can the union indicated by the proximal polypide bud of this figure (Fig. 15, *pd. bd. 1*) be interpreted as the beginning of an ovicell? This bud consists of a long column of cells having a somewhat definite arrangement, and caught at its proximal extremity is a large ovum. This, for a time, was thought to represent an incipient ovicell, but the conditions shown in Fig. 16 reveal its true meaning. But one bud (*pd. bd.*) is represented in this figure, and this has reached a stage of development similar to that shown for the proximal bud of the preceding figure (Fig. 15, *pd. bd. 1*). If we compare the arrangement of the cells of the bud in these two cases, with buds which represent early stages of undoubted polypide formation, the resemblance is strong, and there can be no doubt that these are stages in polypide development. Thus in Pl. XII, Fig. 11, are shown several instances of the earlier stages in the development of a polypide. The cells in the upper portion of the bud

arrange themselves in parallel lines forming the incipient tentacles (*pd. bd. 1* and *3 in. tent.*), while those in the lower portion form into a hollow sphere to produce the cavity of the stomach (*st.*). The proximal bud of Pl. XIII, fig. 15 (*pd. bd. 1*), and the anterior bud of Fig. 16 (*pd. bd.*), represent a stage in the development of polypides identical with those in Fig. 11. The significance of the union of ovum and polypide in these two cases is further revealed by the polypide just proximal to the young bud (Pl. XIII, Fig. 16, *pd. 2*). Here attached to the caecal end of the stomach of an adult polypide, is a veritable embryo (*emb.*) consisting of at least three cells. That these are blastomeres of an embryo, and not merely a bunch of ova, is shown by the condition of the nuclei. The two upper cells have apparently just completed their mitosis, and the nuclei are relatively small. The nucleus of the lower cell has lost its nuclear wall, and the cell is preparing for division. This case affords an explanation of those instances where an ovum is held by a delicate membrane at the proximal end of a column of cells, and represents a kind of union that may occur between a bud and an egg, but one in which *no ovicell results*. The next older polypide (*pd. 1*) possesses neither ovum nor embryo. Young embryos of two or three cells are not uncommon upon buds or young polypides near the growing points, although single ova attached to young buds and to adult polypides are of more frequent occurrence. This figure (Fig. 16) represents a typical section through the growing tip of a young colony. In the growing tissue, right and left, ova are more or less numerous. Proximal to this, the youngest bud frequently possesses an ovum, and below this, one or two polypides may carry a single ovum each, or a young embryo. The coexistence of polypide and embryo or ovum has not been previously noted in this subclass of bryozoa, and while it is probably an abnormal condition for *Crisia*, it is, perhaps, indicative of a more primitive method of reproduction. I have never observed this except at the height of the breeding season, when ova are being rapidly produced. In the older portions of the colony neither eggs nor embryos have been found, nor have larvæ been obtained, in any of the older zoecia. These embryos, apparently, never attain complete development, but are absorbed.

This kind of union was not recognized by Harmer, and as a consequence the instances which he offers as probable early stages of an ovicell are somewhat doubtful ('93, Pl. 22, Figs. 1 and 2). This is especially true of Fig. 1, which is probably an instance of this second relation.

The partial development of an embryo in connection with a polypide is interesting for two reasons. In the first place, it probably points to a more primitive method of reproduction, and in the second place, it is important for the light it throws on the time and place of fertilization.

In regard to the indications of more primitive conditions, it is clear, aside from the question of the origin of the ova, that in *Tubulipora* (Harmer, '98) ova occur in many of the zoëcia. Moreover, in this genus any zoëcium may become an ovicell, and usually several zoëcia of a colony become thus transformed. In the constant occurrence of eggs in the individual zoëcia, and in the direct transformation of the latter into ovicells, *Tubulipora* shows the least specialized condition of any Cyclostome whose history is known. In *Lichenopora* an ovum is found only in that young zoëcium which becomes the ovicell of the colony, and which Harmer designates as the fertile zoëcium. In this case specialization may be considered to have gone a step further in setting off a certain zoëcium to perform the function of an ovicell, and perhaps to produce the single egg which comes to maturity. In *Crisia* specialization has proceeded so far that the ovicell is at no time a zoëcium, although from its position in the internode it must be considered homologous with one. While the ova in this genus are a colonial production and always originate at the anterior edge of the branch, they are occasionally found in the individual zoëcia. Such instances may be regarded as representing an early tubuliporidan stage, or possibly a more primitive stage in which each zoëcium brought at least one ovum to maturity.

In regard to the time and place of fertilization, it may be said that since *Crisia* is dioecious the question arises as to the time when, and the manner in which the spermatozoa reach the ova. According to Harmer, fertilization probably takes place after the egg has been inclosed by its follicle and after the ovicell

has been started. He considers that the very thin wall of the anterior end of the ovicell is not impenetrable to the spermatozoa. If, indeed, the spermatozoa reach the ova at all, they must penetrate the tissues of the colony at some point. Whitman ('90) has shown that a method of impregnation somewhat similar to this is not uncommon in several groups of animals. In most of the cases he mentions the spermatozoa are forcibly injected through the cuticle, and wandering through the tissues, some succeed in reaching the ova. *Crisia* is covered with a calcareous layer which is pierced at intervals by pores that extend through the chitinous ectocyst beneath it. The epithelial cells of the body wall pass through these pores and spread out over the surface, forming a very thin layer upon it. These pores afford innumerable points where spermatozoa could effect an entrance. Moreover, near the growing tip the outer covering becomes thinner and the deposition of calcareous material does not keep pace with the growth of the branches, so that the growing points are covered with an extremely delicate chitinous layer only. Since, as has been shown, the ovaries are situated at the growing tips, it is practicable for fertilization to take place before, or at the time that the ovum becomes associated with the bud. The occasional occurrence of embryos in a zoëcium, as for example in the case shown in Pl. XIII, Fig. 16 (*emb.*), where cleavage has occurred, indicates fertilization thus early, as does also the early cleavage in an undoubted ovicell shown in Pl. XIII, Figs. 19 and 20. One of the blastomeres resulting from the first cleavage is shown in each of these figures (*bl.*). They are not surrounded by the cells of the polypide bud (*pd. bd.*), and yet the first division has taken place, so that cleavage occurs, apparently, at or before the time that the ovum is surrounded by the cells of the bud, and before the ovicell is formed.

The view that Harmer advances in regard to the time of fertilization is based upon his belief that the ovum is the product of the polypide bud ('97). He considers that only certain buds in each internode produce eggs, that these are equivalent to fertile polypides, and that they give rise to ovicells. The evidence from my own observations, however, proves that eggs are produced in every terminal internode, independently of either buds or poly-

pides, and that they become only secondarily united with buds. Moreover, it seems probable that *any* bud may form a union with an ovum, but that all such unions are not fertile, *i.e.*, do not produce embryos that give rise to larvæ. The view that fertilization may take place at a time earlier than that at which the ovicell is formed, and before the egg is surrounded by its follicle, is supported by the facts given above.

This brings us to the consideration of another possibility which correlates the probable degeneration of the male cells with a possible parthenogenetic development of the ovum. A most careful and thorough search has been made through both young and old portions of ovicell-bearing colonies for spermatozoa. None whatever were found, although their size is not so minute that they should be imperceptible with the high power of magnification used. The possibility of parthenogenesis has already been suggested by Smitt ('63), who, according to Claparède ('70) had observed the asexual development of the egg in the ovicells of *Crisia eburnea* and *C. aculeata*. Smitt's reason for supposing that the ova of several species of bryozoa develop parthenogenetically is mainly the failure to find spermatozoa. On this point Claparède remarks that from Smitt's account it seems probable either that the forms he reported upon are dioecious, or that parthenogenesis may occur in the bryozoa under certain circumstances. Of course, mere failure to find spermatozoa is insufficient ground upon which to base a belief in parthenogenetic development, and as a matter of fact, one of the species Smitt mentions, *viz.*, *C. eburnea*, is dioecious. At the same time the evidence here given of degeneration of the testis adds weight to this suggestion, and the small number of spermatozoa compared with the vigorous growth of testis is not only remarkable, but may be correlated with the small number of ova that reach maturity either partial or complete. It is possible that this degeneration may be carried so far as to produce no mature spermatozoa whatever, or so few that their rôle in the economy of reproduction is reduced to the lowest degree.

Instances of the third class of ova (*c*), *i.e.*, those that fail of development, may be found in sections of the extremity of a branch where ova are frequently found in various positions,

sometimes upon the tentacle sheath of a developing polypide, sometimes lower down upon a septum, and sometimes free in the mesenchyme which fills the interior of the tip. In this last situation they frequently possess long processes which suggest that they have an amœboid motion. Their position, however, is to be attributed not so much to their own movement as to the fact that the tip has grown away from them, and has left them suspended in the network of interior cells. Pl. XIII, Fig. 17, represents a section in which two such ova have been thus left behind (*ov.*) and which, like those embryos which reach only a partial development, are absorbed. Measurement shows that the ova decrease in size as their distance from the growing point increases, and in the lower zoœcia no eggs are found, they having gradually disappeared.

A number of measurements of ova in various positions, *e. g.*, those in the ovaries, those on young buds or polypides, and those free in the different portions of the internode, shows that much variation in size occurs, but that these variations follow a regular law. Thus a gradual growth can be traced from the very small ova at the anterior edge of the tip, 5.4 μ in diameter, to older ones measuring 10.8 μ , 14.4 μ , and 18 μ . A parallel growth of the nucleus also occurs, those ova whose diameter is 10.8 μ possessing a nucleus of 7.2 μ , while those whose diameter is 14.4 μ and 18 μ have a nucleus measuring 10.8 μ .

The eggs attached to buds or polypides are, as a rule, larger upon the younger buds, and gradually diminish with the development of the bud. Instances are found where the ovum attached to the bud measures 21.6 μ with a nucleus 10.8 μ in diameter. A frequent size upon young buds is 18 μ , while upon older buds and polypides it diminishes to 11.7 μ and 10.8 μ , with nuclei varying in size from 9 μ to 7.2 μ . If the ovum develops even partially (Fig. 16, *emb.*), the blastomeres of the embryo, while large apparently, are smaller than the larger ova. In the instance shown in Fig. 16, *pd. 2*, the boundaries of the blastomeres are somewhat indistinct. One of them, however, measures 14.4 μ , while its nucleus is only 3.6 μ . Here, although the size of the blastomere as a whole equals that of some of the ova, the nucleus is much smaller. The outlines of

the others are too indistinct for measurement. As a whole they are smaller than the upper blastomere, their nuclei measuring about 5.4μ . The ova which fail of development and are free in the various portions of the internode, vary in size from 10.8μ to 7.2μ . Of these the smallest are invariably found at the greatest distance from the tip.

It is thus seen that ova increase in size from their origin at the anterior edge of the tip to the proximal border of the ovary. If, at this point, they unite with a bud, they may continue to increase somewhat in size. If the bud develops into a polypide, the ovum either becomes an aborted embryo or is absorbed without further development. Those ova which form no union with a bud are frequently found in the lower portion of an internode, much diminished in size. Those which develop in ovicells will be discussed later.

The data afforded by the preceding observations show that the time at which the genital products appear, both male and female, is much earlier than that at which the buds arise. The place of origin of each has also been shown to be different, and that the close relation existing between bud and ovum at a later period is secondary. Furthermore, it is shown that any bud may form a union with an ovum, *i.e.*, the possibility of a union between genital product and bud is the same for both males and females. As a matter of fact, however, every bud in a female colony does not unite with an egg, nor conversely does every egg succeed in uniting with a bud, a large number of ova undergoing degeneration. Of those ova which effect a union with a bud only a relatively small number give rise to larvæ, *i.e.*, become inclosed in ovicells. It seems probable, then, that certain buds only possess the possibility of developing into ovicells, *viz.*, those which arise at that point in the internode where the ovicell is found. Any or every internode then has the possibility of being a fertile one. The questions are, Why does not every internode possess an ovicell? And why do some unions result in only a partial development of an embryo and no ovicell? What the determining factor is, is not known. A struggle seems to ensue between the two elements, bud and ovum, the one obtaining ascendancy over the other. The result

may be due in part to the *time* at which the union is effected, *i.e.*, if the bud has already got started toward the formation of a polypide, the momentum of growth may be so great that the development of the egg has no power to change or hinder it. Whereas if the union takes place early enough, before bud differentiation has begun, the embryo gains the ascendancy, and an ovicell results.

DEVELOPMENT OF THE PRIMARY EMBRYO.

The Ovicell.—Development of the embryo in *Crisia* takes place within a special structure, the ovicell. Smitt ('65) first called attention to the fact that the ovicell of *Crisia* develops according to the same laws as zoëcia, and Harmer has shown that in several genera of the Cyclostomata it is homologous with a zoëcium. The reasons for these conclusions are first, in *Crisia* the ovicell occupies a position in the internode similar to that of a zoëcium. In *C. eburnea* there are ordinarily seven zoëcia in an internode, so that ovicell-bearing internodes consist of six zoëcia and an ovicell, the latter taking the place of the second or third zoëcium.* Second, within the ovicell is found a bud which is equivalent to that found in a zoëcium. In the latter this develops into a tentacle sheath and the alimentary canal of a polypide, in the ovicell, into a tentacle sheath and the follicle inclosing the embryo. Third, in *Lichenopora* and *Tubulipora* the ovicell originates in an actual zoëcium. In the former it is the second or third zoëcium of the colony and functions as a brood pouch only after the degeneration of the first polypide; in the latter, any zoëcium may become an ovicell, and after it has already had one or two occupants.

Pl. XIII, Fig. 18, represents in optical section a decalcified tip containing a young ovicell (*ovl.*) in the so-called "funnel stage", in which is a very young embryo (*emb.*), and the beginning of the tentacle sheath (*tent.*). Starting with the chitinous articulation (*art.*) at the base of the internode, the ovicell is found in this instance to occupy the place of the third zoëcium.

*This statement may seem inconsistent with that made on p. 134 relative to the difficulty in locating early ovicell stages, but determinateness in the position of the ovicell is not accompanied by constancy in its occurrence, relatively few internodes possessing ovicells.

In the rear of the ovicell the continuation of the internode appears in the form of young buds. These would have eventually grown beyond the ovicell and have constituted the remaining zoëcia of that internode. Just what stage of development this embryo has attained, it is difficult to say, but judging from others of similar size and appearance it probably consists of three or four blastomeres.

Early Cleavage Stages.—It was remarked above that the earliest stages of ovicells are difficult to distinguish. In the sectioned material, no instance has occurred in which a single ovum is contained within an undoubted ovicell. There are many cases of juxtaposition of ovum and group of cells, but as has been shown the interpretation of this relation is not always possible. It is true that at an early stage an ovicell can be detected by its size, but on sectioning material that could be thus distinguished, cell division has always been found to have occurred. Since in *Crisia eburnea* the ovicell occurs in the proximal portion of the internode, usually in the place of the second or third zoëcium, it would seem relatively easy to secure the early stages by preparing in large numbers the young tips of colonies in active reproduction. This method was adopted, but without success in obtaining an undoubted ovicell containing an ovum previous to cleavage. In all of the earliest stages secured, the first cleavage at least had occurred, and there are reasons for supposing that cleavage usually occurs before the ovicell is definitely set off. Pl. XIII, Figs. 19 and 20, are consecutive sections of one of the three youngest ovicells obtained. The embryo consists of two blastomeres, one being represented in each figure (*bl.*). These latter are large ovum-like bodies imbedded in cells and lying distal to a mass of elongated cells which represent the polypide bud of an ordinary zoëcium (*pd. bd.*), and which seem to be arranging themselves around the embryo to inclose it. The cells of the embryo possess a large vesicular nucleus, and in size and appearance bear so close a resemblance to ova, that the question arises whether they may not be such. The strongest evidence that they are the result of cleavage is found in the relative size of nucleus and cell. Measurements of a large number of ova show that the ratio of the size of the

whole cell to that of the nucleus is 2:1.5 or less, whereas in the blastomeres it is 2:1 or more. This latter rule holds in the present case. Thus in Fig. 19, although the blastomere is as large as many ova, *i.e.*, 14.4 μ in diameter, its nucleus is only 7.2 μ , while in Fig. 20 the blastomere measures 9 μ with a nucleus of 3.6 μ . In the first the ratio is just 2:1, in the second it is slightly greater. Additional evidence that these bodies are not ova is afforded by the difference in their rate of growth since cleavage. In a second instance an ovicell in the same stage contained an embryo of two blastomeres still adhering to each other as if division had but recently occurred. The cells of this embryo are relatively very small, the two measuring 14.2 μ , about as much as a single ovum. The cells of the bud have much the same appearance and bear the same relation to the embryo as those shown in the bud of Figs. 19 and 20. That the latter represent an early stage in the development of the embryo is further shown by the fact that the blastomeres are not yet surrounded by the cells of the bud (*pd. bd.*). Nevertheless that some time has elapsed since cleavage occurred is shown again by the presence of the small cells between the blastomeres. Furthermore, the separation of the blastomeres shows that cell division takes place some time previous to or following very close upon the formation of the ovicell. In this ovicell there is yet no appearance of the tentacle sheath, the two lines of cells extending downward from the anterior border being those that form the vestibule (*vest.*).

A somewhat later stage of embryonic development is represented in Pl. XIV, Fig. 21. Here the embryo (*emb.*) contains at least three blastomeres which are not only surrounded by the follicle but are pushed apart and separated by the interior cells. The beginning of the tentacle sheath is shown in the layer of cells separating from the distal surface of the bud, the cavity formed between the outer surface of the bud and this layer (*tent.*) being the cavity of the tentacle sheath (*tent. cav.*). Here again the blastomeres have the same ovum-like appearance as in the two-cell stage, but they are smaller, the larger of them being 10.8 μ in diameter, and the other two about 7.2 μ . In this stage the cells between the blastomeres are smaller than those in

a similar position in the two-cell stage. The separation of the blastomeres and the interpolation of small cells is a characteristic of the early stages of *Crisia*, and in most older stages than the two-cell stage the blastomeres divide quite independently of one another. Pl. XIV, Fig. 22, represents a four-cell stage in which again are shown the separation of the blastomeres and the interpolation of the follicle cells (*sm. fl. cls.*). This ovicell is further interesting as showing the characteristics of the follicle cells. These now surround the embryo so that it lies in the center of a sphere consisting of a number of concentric layers composed of cells which form a net-work by the union of their protoplasmic processes (*fl. cls.*). In the interior of the spherical follicle the four blastomeres of the embryo may be distinguished by their larger size (*bl.*). The other cells of the interior (*sm. fl. cls.*) are of various sizes, those nearest the embryo being the smaller, those nearest the inner layer of the follicle, the larger. An examination of a large number of specimens shows that the multiplication of the small cells is accompanied by a diminution in number of the cells of the concentric layers. The former seem without doubt to be derived from the latter and to represent a stage in their absorption. Pl. XIV, Fig. 23, represents an embryo in the eight-cell stage, only four blastomeres being visible in this section. The separation of the cells of the embryo is clearly brought out, the blastomeres being perfectly distinguishable by their larger size and their different staining capacity. The increase in the number of small interior cells is noticeable as is also the decrease in the follicle inclosing the embryo.

This separation of the blastomeres continues to be a striking feature of the embryonic development of *Crisia* until about the twenty or twenty-four cell stage when the blastomeres unite to form a more or less compact ball. Harmer ('93, '97 and '98) has shown that it is characteristic of this and also of other genera of the Cyclostomata viz., *Lichenopora* and *Tubulipora*. In a recent paper, Braem reports a somewhat similar method of cleavage for *Plumatella*. According to this writer the egg of *Plumatella* consists of two quite distinct parts, an outer granular zone, and an inner zone containing the nucleus.

It is the latter only which takes part in cleavage and from which the blastomeres are formed. At the first cleavage the plane of division does not pass entirely through the egg, even of that part out of which the embryo is formed, and as a consequence the first two blastomeres, while being connected at one pole, fall asunder at the other. The undivided portion, called the middle piece (*mittelstück*), remains intact through the two, four, and eight-cell stages, while the blastomeres are widely separated at the animal pole. In the meantime the granular zone disintegrates more or less, its granules become larger, and nuclei appear between the free ends of the blastomeres. It is in the sixteen-cell stage that the resemblance between the embryos of *Plumatella* and *Crisia* is closest. At this time the middle piece disappears and the blastomeres being set free completely separate from each other. They continue to increase in number, although not regularly, while in the spaces between them are numbers of small cells. With further increase in the number of blastomeres, the small cells gradually decrease in number until, in the twenty-four cell stage the blastomeres having united into a ball, the small interpolated cells disappear almost entirely. From this point development proceeds in the regular manner. A comparison of the series of figures I to V in Fig. 104, Pl. IV, of Braem's paper, with Figs. 22, 23, and 24 of this paper will show the similarity of the cleavage in the two cases. The resemblance consists not only in the separation of the blastomeres but in the appearance between them, as if shoving them apart, of numerous small cells resembling those similarly situated in the embryo of *Crisia*. The function of these cells in both cases is probably identical, *i. e.*, they serve as nourishment for the embryo. As in *Crisia* the interpolated cells gradually disappear and the blastomeres unite at about the twenty or twenty-four cell stage into a solid ball.

The Ball Stage.—From the twenty-cell stage onward the embryo of *Crisia* forms, as has been said, a more or less compact ball. Pl. XIV, Fig. 24, represents an embryo measuring $43\ \mu$ in diameter and containing from sixty to seventy blastomeres which have united into a ball, although still surrounded

by the original follicle (*f.*). In this case the small follicle cells have not disappeared but may be seen packed together in the space around the embryo in the cavity of the follicle (*sm. fl. cls.*). Numbers of mesenchymatous cells forming a net work are present in the cavity of the tentacle sheath.

Pl. XIV, Fig. 25, represents a much older stage. This embryo is a compact ball with a well differentiated outer layer. Its greatest length is $150\ \mu$, while the size of the separate cells varies from $5.4\ \mu$ to about $8\ \mu$ or $9\ \mu$. At higher magnification these larger cells are shown to be in division, but mitosis does not seem to occur more actively in one part of the embryo than another. The absence of the follicle is very noticeable at this stage, but that its loss is probably more gradual than has so far been indicated, is shown by Pl. XIV, Fig. 26. This is a section of an ovicell of *Crisia occidentalis*, in which the embryo has attained about the development of that in Fig. 25. Here a portion of the original follicle remains in the chain of cells lying below the embryo (*fl. cls.*). These cells occupy the position and have the appearance of the follicle cells of other embryos, possessing the enlarged nuclei with scattered chromatin granules. In this ovicell a number of other cells are present below the embryo which represent a possible source of a second follicle (*sec. fl. cls.*). These latter are most numerous in connection with a chitinous tube (*chi. t.*) which extends from a septum (*sep.*) below the embryo to the base of the ovicell. In development this tube begins as a layer of chitin below the young embryo then consisting of only a few cells. Later the chitinous layer becomes more extensive and assumes a cone shape, the apex of which, with the continued growth of the ovicell, extends to the proximal extremity of the ovicell. Meantime a chitinous ring forms immediately below the embryo (*chi. r.*), dividing the ovicell into two parts. The tissue lining the ovicell is continued over the septum into the tube, and throughout its extent and in close connection with it there appears numerous large cells often possessing two or three nuclei, resembling the giant cells (*gi. cls.*) found in the ovicell of *C. ramosa*. The interior of the tube is filled with a net-work of deeply staining cells that extends above the septum and around the embryo. The chitinous ring or

septum,* (*chi. r.*) probably serves as a supporting structure to keep the embryo from passing downward into the narrow portion of the ovicell, but the whole tube seems to be related to the great development of the second follicle in this species. In *C. eburnea* the follicle of the adult ovicell consists of a relatively small number of cells scattered among its contents (Pl. XV, Fig. 28). In *C. occidentalis*, however, the second follicle is a mass of cells in which the embryos and larvæ are imbedded (Pl. XV, Fig. 29). With the disappearance of the spherical follicle and the appearance of a second follicle, the embryo attains a relatively enormous size before budding begins.

The Secondary Embryos:—An early budding stage is shown in Pl. XIV, Fig. 27. This is drawn to the same scale as Figs. 25 and 26, and a comparison with these two figures will give an idea of the great size which the embryo attains, this one being 200 μ . in its longest diameter. As the embryo increases in size it comes to occupy a higher position in the ovicell, moving upward apparently to the point where the walls are more widely expanded. This is especially noticeable in *Crisia cornuta* where the ovicell is widest at the distal end. The embryo is not anchored in any way in *C. eburnea*, and is often found at the top of the ovicell close against the valvular closure (Pl. XV, Fig. 28, *prim. emb.*) Buds are formed at various places on the body of the embryo. In the case represented in Fig. 27, two somewhat irregular processes project distally, from the extremities of which small portions are constricted (*sec. emb.*). These are not the only budding regions, however, for on other parts of the surface outgrowths occur which as other sections reveal, are incipient buds (*in. bd.*) At the proximal extremity there are a few cells which the examination of preceding sections shows belong to another bud (*sec. emb.*). There are instances also where the first buds are constricted from the extremities of long arms extending proximally through the whole length of the ovicell. The primary embryo frequently possesses a somewhat rounded triangular form, and the buds are

*The septum found in the base of the ovicell of *C. occidentalis* is probably homologous with the chitinous articulation occurring on each zoecium of this and the related species, *C. geniculata* and *C. cornuta*. Evidence for this homology will be given in a later paper.

given off at the apices. This is contrary to the observations of Harmer who finds that the primary embryo of *Crisia ramosa* buds only at the distal extremity. Calvet ('00) also represents the same condition for *Crisia denticulata*.

The buds of the primary embryo, from whatever portions of the body they arise, constitute the secondary embryos and from them the free swimming larvæ develop. When first set free the secondary embryos of *Crisia eburnea* consist of a small number of cells united into a solid ball, and varying in size from 25 μ to 35 μ in diameter, containing approximately from 55 to 65 cells. Redivision of the secondary embryos has not been observed in this species. In *Crisia occidentalis*, however, there occurs not only the formation of secondary embryos by budding, characteristic of *C. eburnea*, but also, in some cases, a redivision of these to form tertiary embryos. In these cases the primary embryo breaks up into large masses of cells, the secondary embryos, which in turn, become budding centres, from which tertiary embryos arise, these ultimately becoming the ciliated larvæ. This is illustrated in Pl. XV, Fig. 29, which represents a section of an almost adult ovicell of *C. occidentalis*. On examination of the series of sections to which this figure belongs, it is seen that the ovicell contains a few fully developed larvæ (*lar.*). The presence of these indicates that the primary embryo had budded off a few secondary embryos at an early period, and that, later, it divided almost simultaneously into a number of embryos. Some of these may have undergone no further division, while others notably the masses *a* and *b*, divided into tertiary embryos. The method of division in these cases is different from that which takes place in *C. eburnea*, although the result is the same. At the point where the division is about to occur, the nuclei arrange themselves into two linear series parallel to each other, or almost so. In this way two or more masses are formed which round up, separate from each other, and become the tertiary embryos. Many instances of this method of division are shown in the series of which Fig. 29 is a section. In the mass of cells, *x*, such a process is taking place. Pl. XV, Fig. 30 represents an embryonic mass, taken from another ovicell, showing two tertiary embryos (*ter. emb.*) which are forming from a large secondary

embryo. In *C. eburnea*, neither in the primary embryo nor in the buds when first set free, is there any differentiation into cell layers. As the primary embryo increases in size, the cells upon the surface become more compactly arranged, the inner cells forming a loose, spongy mass. The secondary embryos of *Crisia denticulata*, according to Calvet, possess two distinct layers, an outer containing large nuclei, and an inner containing much smaller nuclei surrounding a central cavity. This is true even before the buds are detached from the parent. This central cavity persists and forms part, at least, of the general cavity of the first individual of the new colony. When the secondary embryos of *Crisia eburnea* are first set free they do not differ histologically from the primary embryo. No cavity is present, the cells being heaped together in a somewhat irregular way. When a cavity appears it is not at first lined by a distinct layer of cells as is the case in *C. denticulata*. By the time the ovicell has completed its growth it is filled with larvæ of various sizes and in various stages of advancement. Fig. 28 is a section through an ovicell which is almost mature, *i.e.*, one in which the larvæ outnumber the embryos and will soon be set free. In this instance many of the larvæ have attained their full development and are confined in their narrow quarters only until the valvular membrane can be perforated. The larger larvæ possess long cilia, which fact suggests that either they move bodily through the ovicell, or that the vibrations of their cilia set up currents which carry the smaller bodies about. It is not uncommon to find the secondary embryos remote the length of the ovicell from the primary embryo, showing that the contents of the ovicell must have been in motion during life. The size of the larvæ seems to be pretty constant, at least in a given species. Those of *C. eburnea* measure about 86μ in diameter, while those of *C. occidentalis* are somewhat larger, measuring 107μ . The opacity of the living ovicell prevents any study of the living contents while the ovicell is intact. But if a living ovicell be crushed in a drop of sea water, a very interesting scene is presented. The larvæ dart away and swim about with great activity. Smaller ciliated balls move about in clusters. The color of the whole mass, larvæ, embryos, and cellular

tissue, is yellow. Perhaps the most interesting sight is the primary embryo which floats out with the rest of the material and frequently becomes isolated. It may easily be obtained by the dissection of a living ovicell, or from a stained decalcified ovicell dissected in a drop of oil. In the latter case the embryo is a more compact and clearly defined mass than in the former, but the characteristic features of both are the same. Projecting from the surface in various directions protuberances appear which are the buds of the secondary embryos.

Near the top of the ovicell represented in Fig. 28, the primary embryo appears much reduced in size, but still budding actively. As budding continues the primary embryo decreases in size, both as a whole, and in the size of its individual cells. This may be seen by comparing Figs. 27 and 31, the latter representing the primary embryo of Fig. 28 drawn to the same scale as that in Fig. 27. This, as has been said, measures 200 μ in length, while the older embryo (Fig. 31), measures but 71 μ in length. In the older embryo cell boundaries are less distinct, and the cells are more closely massed together. In examining a number of ovicells, primary embryos are frequently found much smaller than this, and much smaller than the contained larvæ. Thus in one instance the primary embryo measures 50 μ and the adult larvæ 86 μ . This ovicell contained a number of secondary embryos 25 μ in diameter. The secondary embryos in the older ovicells average slightly smaller than those in the younger. It seems extremely probable for several reasons that the primary embryo is completely used up in the process of budding. Evidence for this is found in the gradual decrease in size of the embryo resulting from its continued activity in budding. Again, the instance of *Crisia occidentalis* (Fig. 29) in which the primary embryo divides into a large number of secondary and tertiary embryos, so that no one of the masses present can be called the primary embryo, and in which each mass of cells is apparently either redividing or is transforming into a larva, is strong evidence that no portion of the original embryo is left over. Further, complete series of sections of ovicells are obtained in which no primary embryo can be found, although larvæ and half grown, secondary embryos are abundant, and the aperture of the ovicell

is still unperforated. Finally, although empty ovicells are remarkably scarce, yet in one instance at least, a complete series was obtained which possessed neither larvæ, nor embryos, the interior containing nothing but a fine network and some degenerated cells. The evidence seems to be conclusive, then, that the whole of the primary embryo is converted into larvæ.

The number of larvæ to which a colony of *Crisia* gives rise is probably not less than is produced by other bryozoa although *Crisia* produces few mature eggs. As far as the evidence from my observations is concerned all the larvæ found in the ovicell, arise from one egg. Both Harmer and Calvet, however, believe they have evidence that more than one ovum may develop simultaneously within a single ovicell. Harmer ('97, Pl. 9, Fig. 25), represents two young embryos whose blastomeres are still separated, which he considers are the result of the development of two eggs. While this may be true, there is a possibility that the conditions presented may have resulted from the blastomeres of the two-cell stage of a single ovum having become so widely separated that each has gone on to develop into a separate embryo. The numerous recent experimental demonstrations of the power of independent development possessed by the blastomeres, and this too, in ova whose blastomeres normally retain their connection with one another, renders this hypothesis the more probable. Calvet figures a similar condition (Pl. 10, Fig. 15) which he considers affords undoubted evidence of the presence of two ova and of their simultaneous development within a single ovicell. Here again the facts may be differently interpreted. The two embryos may represent the individual development of two blastomeres which had become separated in the two-cell stage and had not reunited, or it may be an instance of a condition similar to what occurs in *Crisia occidentalis*. The two large masses, the two so-called primary embryos, may be two secondary embryos, and the smaller masses arising from these, may be tertiary embryos. The production of tertiary embryos is reported for *Lichenpora* and *Tubulipora*, but has not been previously found in *Crisia*. In the species in which it undoubtedly occurs, *Crisia occidentalis*, there is more or less variation, and it will not be surprising to find it in all species of the genus.

The protection and nourishment afforded the embryo of *Crisia* are typical of the Cyclostomata, and are paralleled to a certain extent among the Ctenostomata and the Phylactolamata. According to Prouho, the Ctenostomes are, as a rule, viviparous, the different genera showing degrees of this condition varying from the primitive state exhibited by *Alcyonidium duplex*, where the young are sheltered during a portion of their development only, to that found in *Pherusa tubulosa*, for example, where several embryos develop in the tentacle sheath of a degenerated polypide. Joliet ('77), who studied the living animal, has given the most detailed account of the process. He shows that in *Valkeria cuscuta*, another Ctenostome, upon the degeneration of a polypide there appears in the zoëcium both an egg and a new bud. The latter grows into an immature polypide, but develops a tentacle sheath and the muscles belonging thereto. The small polypide soon degenerates while into the newly formed tentacle sheath the egg finds its way, and there develops into an embryo and ultimately into a larva. In both *Crisia* and *Valkeria* the development of the embryo is accompanied by the destruction of the polypide, and in both the embryo develops inside of the tentacle sheath newly produced to receive it, in the one case in a highly modified zoëcium, in the other, in an old unmodified one.

The developmental processes of the Phylactolamata as exhibited by *Plumatella* show a closer resemblance in some respects to those of *Crisia*. According to Braem an ovary and a bud develop simultaneously on the body wall, the bud differing from an ordinary polypide bud in the possession of a high columnar layer and a flattened mesodermal layer. One of the cells of the ovary grows larger than the others, and partly by increase in its size, partly by pressure from behind, it approaches the side of the bud, pushes through it and becomes enveloped by it. This bud which according to Braem, Kraepelin ('93) and others is homologous with an ordinary polypide bud, now performs the function of a broodsac or oëcium, and shelters the embryo until it develops into a larva. The origin of the ovary of *Plumatella* appears to be similar to that in *Crisia* in its independence of a polypide. The suggestion of Braem, however, in regard to the relation sustained by the ovary of *Plumatella* and the bud which

forms the oecium is probably true, viz., that ovary and bud together constitute the equivalent of a sexual animal, the nutritive portion of which, the polypide, has undergone a change of function. The complete envelopment of the egg by a polypide bud is similar in the two cases, but in *Plumatella* this bud is set off structurally at an early stage, whereas in *Crisia* any bud may be thus set apart, no structural difference between it and an ordinary bud being at first discernible.

In its main features, the processes of embryonic fission as described by Harmer for *Crisia ramosa* and other Cyclostomes have been confirmed by this investigation, while certain additional facts and individual variations have been noted. Observations have also been made on the origin of the sexual elements and their secondary union with the polypide buds. The results may be summarized as follows:

1.—In the genus *Crisia* the sexual elements are produced in both male and female colonies, at the edge of the growing tips of the colony. The germ cells arise from the mesodermal layer, and are differentiated at a point anterior to the budding zone, and at a time earlier than the origin of the buds.

2.—In the male colonies of *Crisia eburnea* a few of the primitive germ cells attach themselves to each bud as it arises, and these form the beginning of the testis. In a majority of cases degeneration of the testis probably occurs before the spermatozoa become mature.

3.—In the female colonies the ovaries are produced at the anterior edge of the young tips. As in the male colonies, in order that the germ cells may reach maturity, it is necessary that they unite with a polypide bud. In this case one of two results may follow:

a.—The ovum may develop into an embryo, while the polypide bud as such, becomes aborted.

b.—The polypide bud may develop, while the ovum either degenerates at once or soon after it has passed through the early cleavage stages.

Many ova are produced which never form a union with a polypide bud. These soon degenerate.

4.—From the time the ovum leaves the germinal epithelium there is a steady increase in its size until it reaches the boundary of the budding region. If here it forms a union with a polypide bud, the size increases somewhat until division occurs. If after this union is effected, the polypide bud develops, the ovum gradually grows smaller. Those ova which fail of development, decrease in size as they become more remote from the ovary.

5.—Fertilization, if it occurs, takes place before or near the time at which the union of bud and ovum is effected. In view of the probable degeneration of the testis the possibility of parthenogenetic development is suggested.

6.—During its development the embryo within an ovicell becomes gradually inclosed by the bud which forms into a spherical follicle consisting of several concentric layers of cells.

7.—A characteristic feature of the early cleavage of *Orisia* is the complete separation of the blastomeres. This continues up to the twenty or twenty-four cell stage when the blastomeres unite into a more or less compact ball.

8.—The separation of the blastomeres is accompanied by the penetration between them of numbers of small cells, and by the diminution of the concentric layers of the follicle. With the continued growth of the embryo, the follicle being absorbed by the embryo gradually disappears.

9.—The primary embryo attains a size many times that of the original ovum before it divides to form the secondary embryos. In *C. occidentalis*, the secondary embryos divide to form tertiary embryos which develop into ciliated larvæ. At the close of its proliferation the primary embryo itself becomes a larva.

University of California,

May, 1903.

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LIST OF ABBREVIATIONS USED IN THE PLATES.

<i>art.</i> —articulation.	<i>mes. tis.</i> —mesodermal tissue.
<i>b. b.</i> —brown body.	<i>ov.</i> —ovum.
<i>b.</i> —proximal extremity of a zoöcium.	<i>ovl.</i> —ovicell.
<i>ba. cls.</i> —ball of cells.	<i>ovy.</i> —ovary.
<i>bl.</i> —blastomere.	<i>pd.</i> —polypide.
<i>chi. r.</i> —chitinous ring.	<i>pd. bd.</i> —polypide bud.
<i>chi. t.</i> —chitinous tube.	<i>prim. emb.</i> —primary embryo.
<i>d.</i> —dorsal side of internode.	<i>r.</i> —right side of internode.
<i>de. cls.</i> —degenerated cells.	<i>re. pd.</i> —regenerating polypide.
<i>de. pd.</i> —degenerated polypide.	<i>sec. emb.</i> —secondary embryo.
<i>ec. cls.</i> —ectodermal cells.	<i>sep.</i> —septum.
<i>emb.</i> —embryo.	<i>sm. fl. cls.</i> —small follicle cells.
<i>fl.</i> —follicle.	<i>st.</i> —stomach.
<i>fl. cls.</i> —follicle cells.	<i>t. cls.</i> —cells of the tube.
<i>ger cls.</i> —germinal cells.	<i>tent.</i> —tentacle.
<i>gi. cls.</i> —giant cells.	<i>tent. cav.</i> —cavity of the tentacle sheath.
<i>gr. tis.</i> —growing tissue.	<i>ter. emb.</i> —tertiary embryo.
<i>in. bd.</i> —incipient bud.	<i>tes.</i> —testis.
<i>in. tent.</i> —incipient tentacles.	<i>tet.</i> —tetrads.
<i>l.</i> —left side of the internode.	<i>v.</i> —ventral side of the internode.
<i>lar.</i> —larva.	<i>vest.</i> —vestibule.
<i>m.</i> —membrane.	<i>z.</i> —zoöcium.
<i>mes. cls.</i> —mesodermal cells.	

All drawings made with the aid of a camera lucida, and all figures except 1 and 18, by the use of Zeiss oculars and objectives.

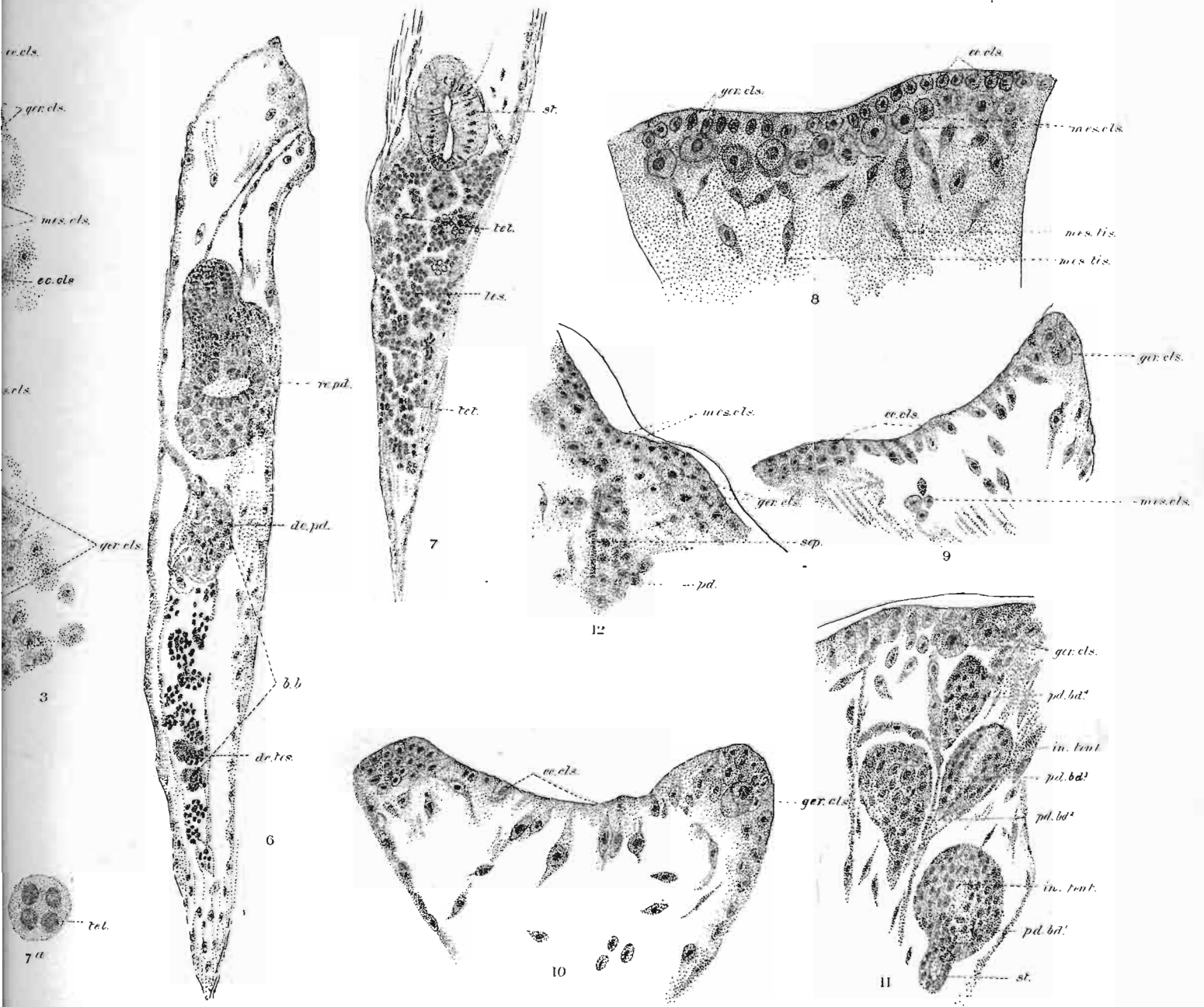
PLATE XII.

- Fig. 1.—Portion of a young decalcified internode of *Crisia eburnea* showing the growing tissue (*gr. tis.*), the budding region (*pd. bd.*), and the alternate arrangement of the zoëcia (*z.*).
- Fig. 2.—Section from the tip of a male colony, close to the edge, right or left, showing the character of the cell layers, the small round ectodermal cells (*ec. cls.*), the larger mesodermal cells (*mes. cls.*), and a few cells of the germinal epithelium (*ger. cls.*). × 600
- Fig. 3.—Section from the same series as the preceding, showing practically the same cell layers at a point nearer the middle of the tip where the ectodermal cells are thinning out and are becoming elongated (*ec. cls.*). × 600
- Fig. 4.—Section from a male colony through the budding region. In the angle toward the left edge of the branch, are a number of germinal cells (*ger. cls.*). Proximal to this is a young polypide bud (*pd. bd. 2*), still lower down is an immature polypide (*pd. bd. 1*) possessing a stomach (*st.*), and an incipient testis (*tes.*). × 600
- Fig. 5.—Two spermatozoa from a ripe testis of *C. eburnea*. × 2500
- Fig. 6.—Section of a zoëcium from a male colony showing a regenerating polypide (*re. pd.*), and below this a "brown body" (*b. b.*) extending to the base of the zoëcium. The brown body consists of a homogeneous mass of yellowish brown degenerated cells, the remains of the polypide (*de. pd.*) and the testis (*de. tes.*). × 600
- Fig. 7.—Section through a zoëcium containing a normal testis. Distally, the stomach (*st.*) of the polypide is shown, while extending into the base of the zoëcium is the testis (*tes.*) in which the cells are arranged in scattered groups of various sizes. Numerous groups of four nuclei (*tet.*) are visible. × 600
- Fig. 7A.—Group of four nuclei (*tet.*) in a mass of cytoplasm. × 2500
- Fig. 8.—Section from the growing tip of a female colony showing the two cell layers of the body wall, the outer or ectodermal layer (*ec. cls.*), consisting of small round cells, the inner or mesodermal layer (*mes. cls.*) consisting of larger cells, part of which gives rise to the germinal epithelium (*ger. cls.*), part to the spindle-shaped mesenchymatous tissue (*mes. tis.*). × 600
- Figs. 9 and 10.—Serial sections from the same tip as the preceding. The ova are accumulated in the corners (*ger. cls.*). × 600

PLATE XII.—(Continued.)

Fig. 11.—Section through the bud forming region of a female colony, showing the relation of the polypide buds and the germ cells. The latter (*ger. cls.*) are differentiated at a point anterior to that where the buds form. Four buds are shown, in the older of which (*pd. bd. 4*) the cavity of the stomach has formed (*st.*). The cells above the stomach are arranged in somewhat regular rows, and represent incipient tentacles (*in. ten.*). These are again shown in the third polypide bud (*pd. bd. 3, in. ten.*).
× 600

Fig. 12.—Section from the ventral side of a female colony, showing the cells of the zoëcial wall, the outer layer of a polypide bud (*pd. bd.*) lying close to the septum (*sep.*) which separates two zoëcia. The germ cells (*ger. cls.*) are prominent in the germinal tissue (*ger. tis.*). × 600



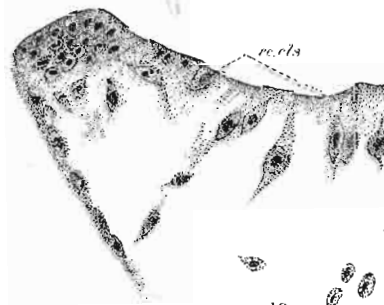
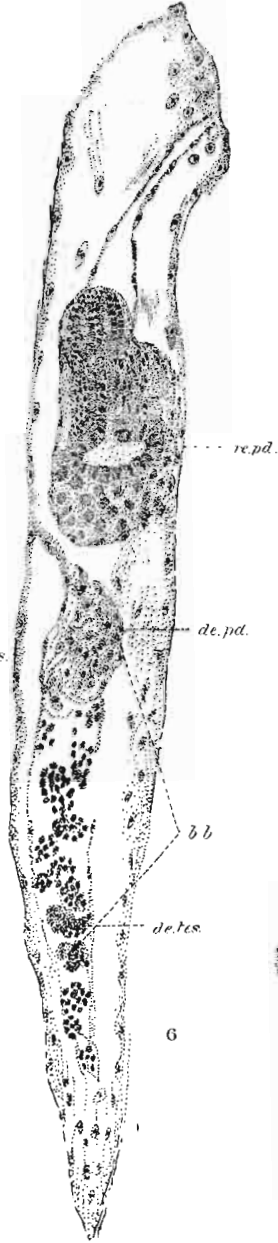
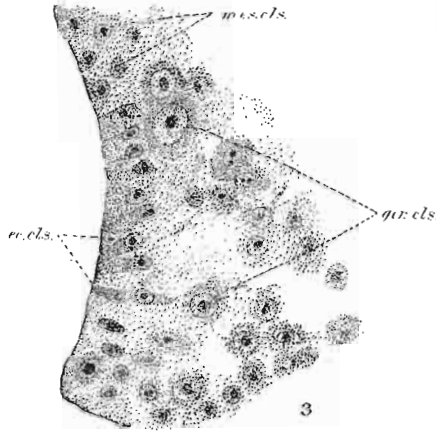
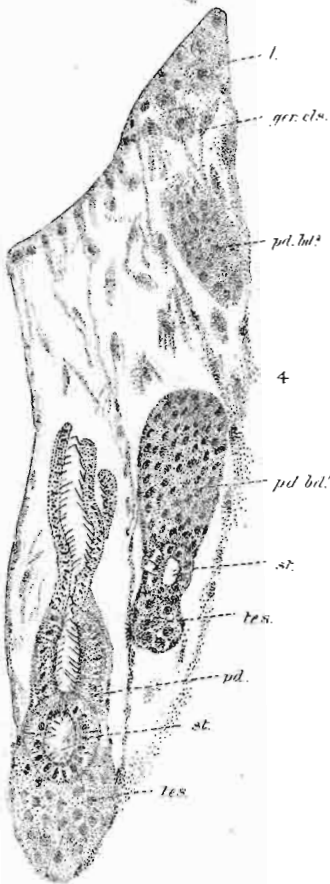
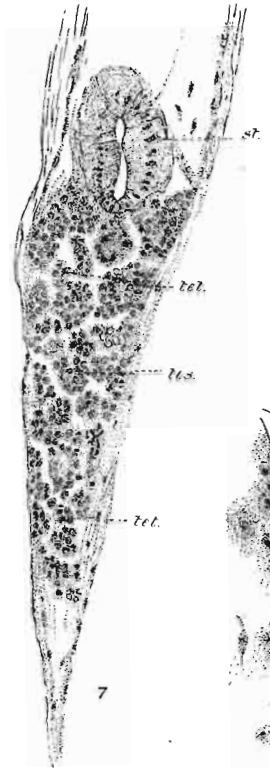
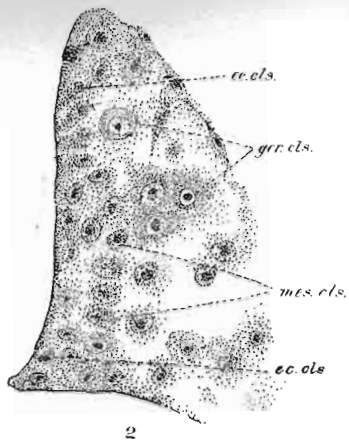
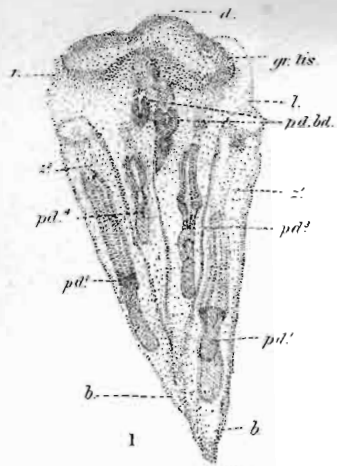
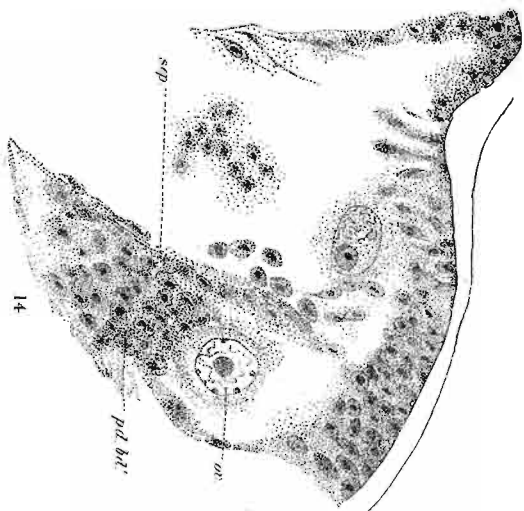


PLATE XIII.

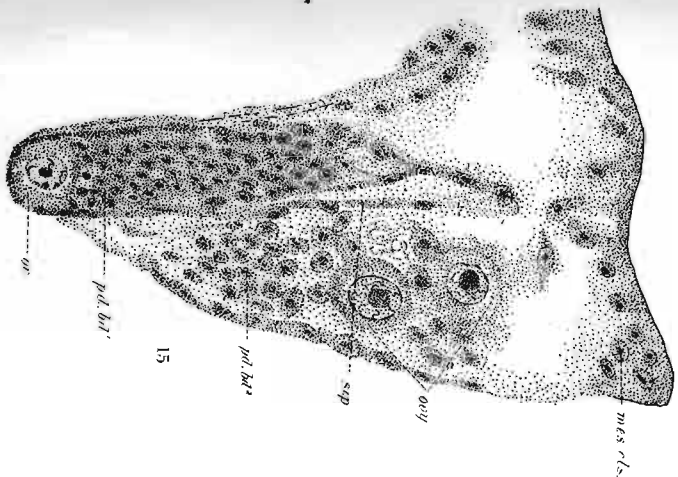
- Fig. 13.—A section which follows the preceding in consecutive order, representing a portion of the same septum (*sep.*) and of the same polypide bud (*pd. bd. 2*). There are shown besides a portion of another bud (*pd. bd. 1*) and numerous large ova constituting an ovary (*ovy.*). × 600
- Fig. 14.—A section following the preceding in consecutive order, showing a portion of the septum (*sep.*), and in the cavity of the branch a large ovum (*ov.*) in close proximity to a polypide bud (*pd. bd.*). × 600
- Fig. 15.—Section through the tip of a female colony, representing a part of an ovary (*ovy.*), a few cells of a young bud (*pd. bd. 2*) and a portion of an older bud (*pd. bd. 1*) to the proximal extremity of which a large ovum is attached (*ov.*). × 600
- Fig. 16.—Section through the tip of another colony, showing two ova in the germinal epithelium of the anterior edge (*ov.*), a polypide bud (*pd. bd.*) with an ovum attached to its proximal extremity. In the next older zoëcium is an adult polypide (*pd. 2*) with a small embryo (*emb.*) attached to the œcal end of the stomach, and in the succeeding zoëcium is a still older adult polypide (*pd. 1*). × 600
- Fig. 17.—Section through the tip of a female colony showing two ova (*ov.*), which are attached by long processes to the interior the branch. These have formed no union with a bud and would have degenerated. × 600
- Fig. 18.—Decalcified internode of *Crisia eburnea*, containing an ovicell (*ovl.*) in an early stage of development. At the proximal extremity is the articulation (*art.*), by which the internode is connected with the branch. Arising from the articulation are two zoëcia (*pd. 1* and *pd. 2*), while the ovicell takes the place of the third zoëcium. At the distal extremity of the branch, two or three buds are forming (*pd. bd.*). The ovicell contains a young embryo (*emb.*), and a tentacle sheath (*tent.*).
- Fig. 19.—Section of a young ovicell, containing an embryo in the two-cell stage. This figure contains but one blastomere (*bl.*), not yet surrounded by the cells of the polypide bud (*pd. bd.*). × 600
- Fig. 20.—Section immediately following Fig. 19, showing the second blastomere (*bl.*) of the embryo, a portion of the elongated cells of the polypide bud (*pd. bd.*), and the beginning of the vestibule (*vest.*) × 600



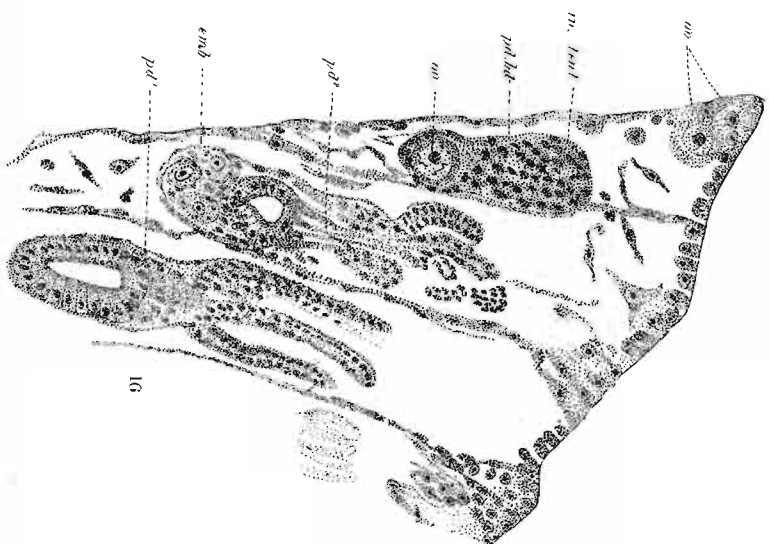
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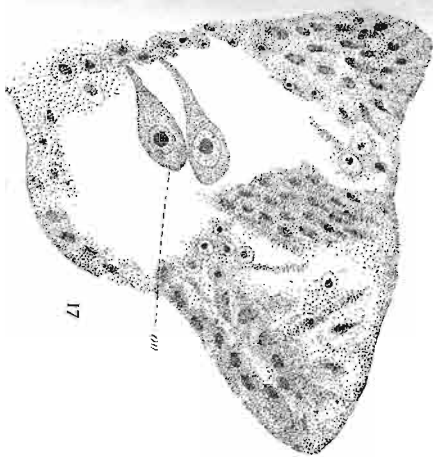
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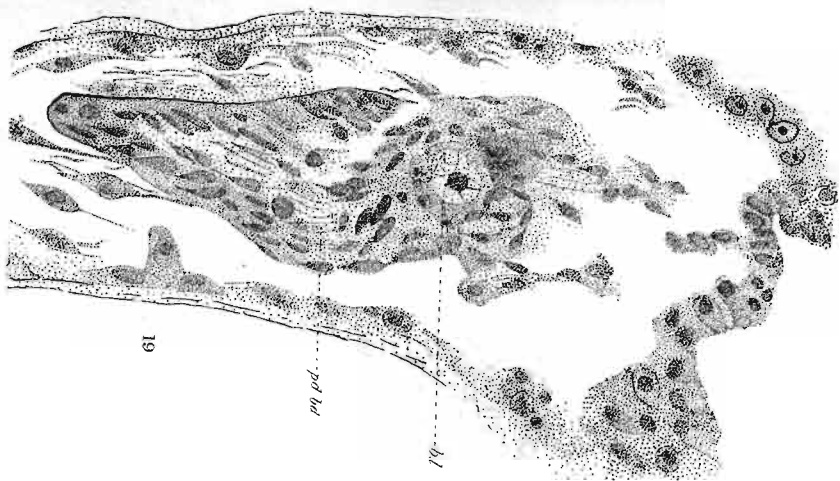
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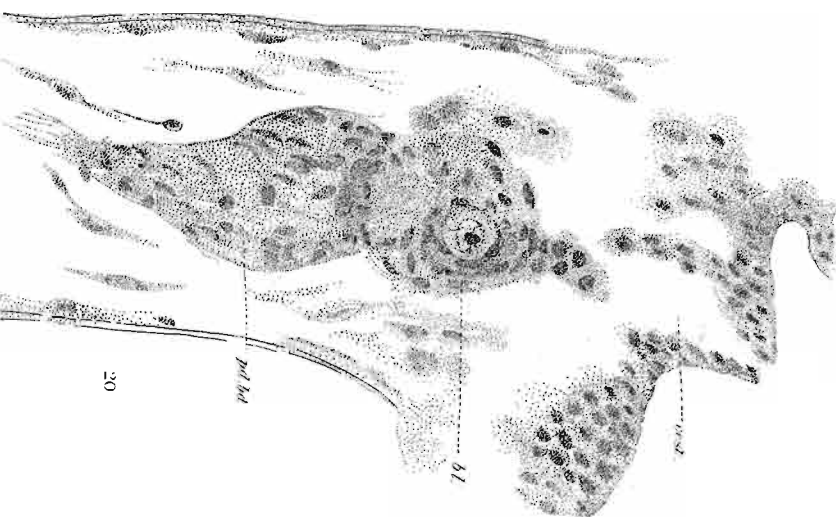
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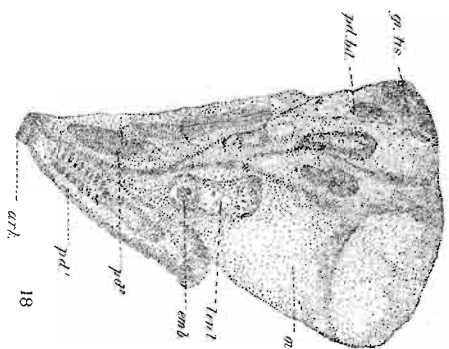
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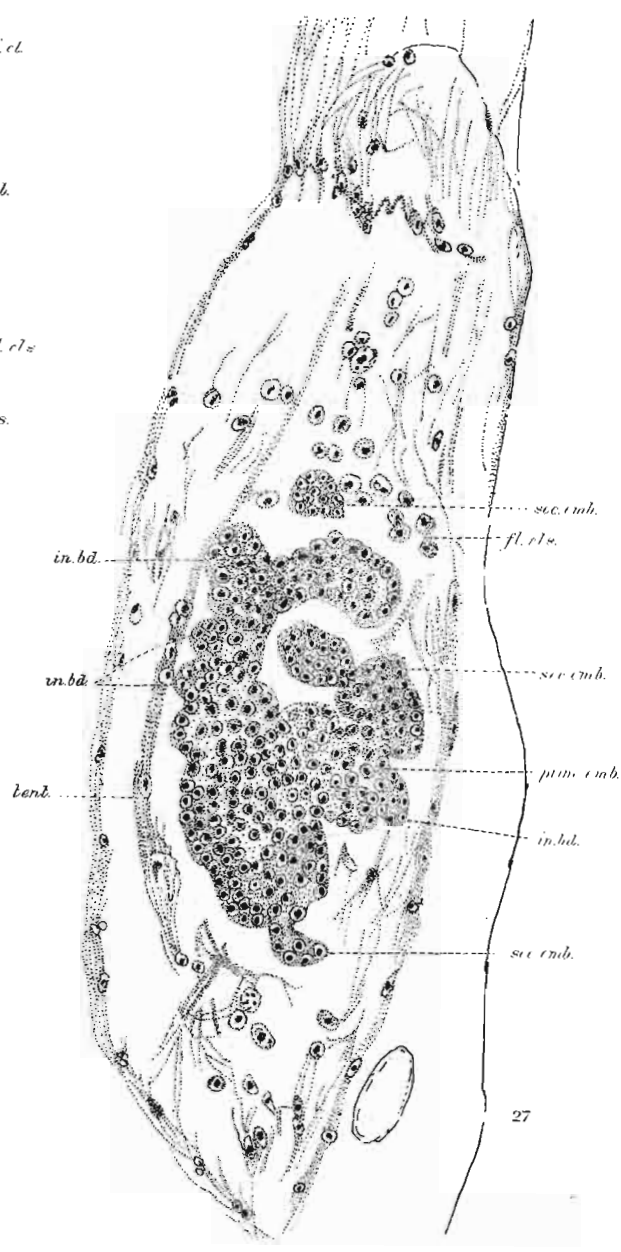
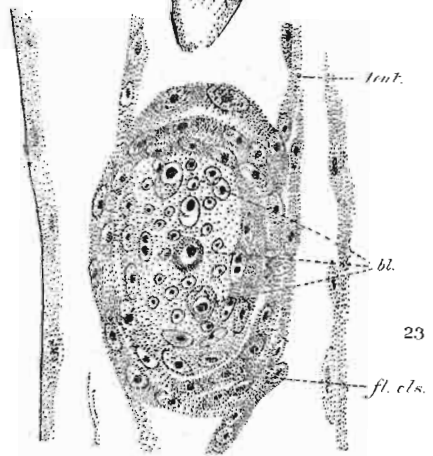
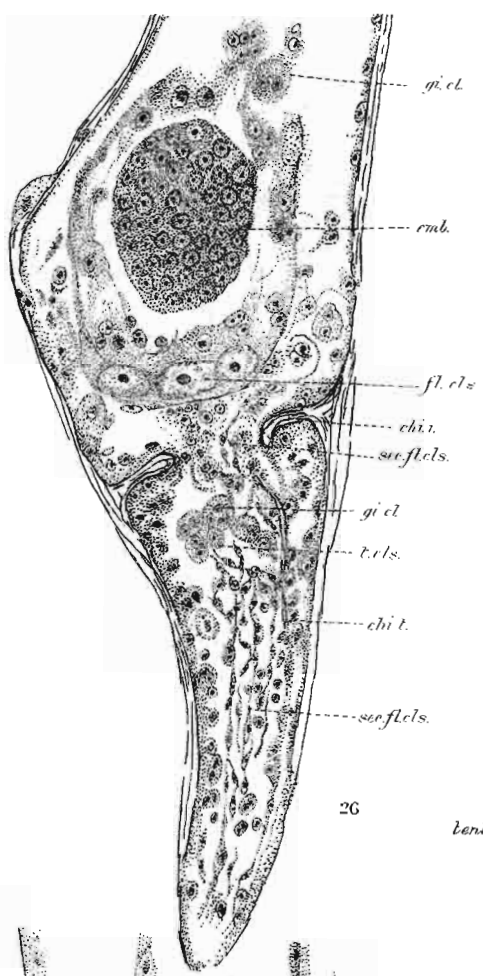
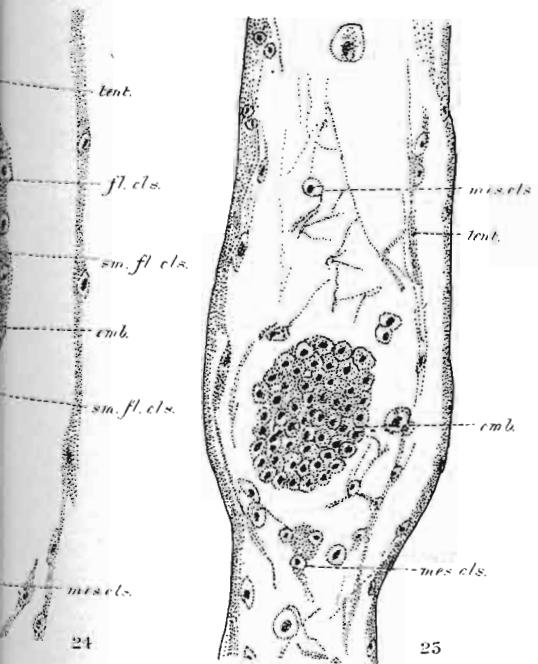
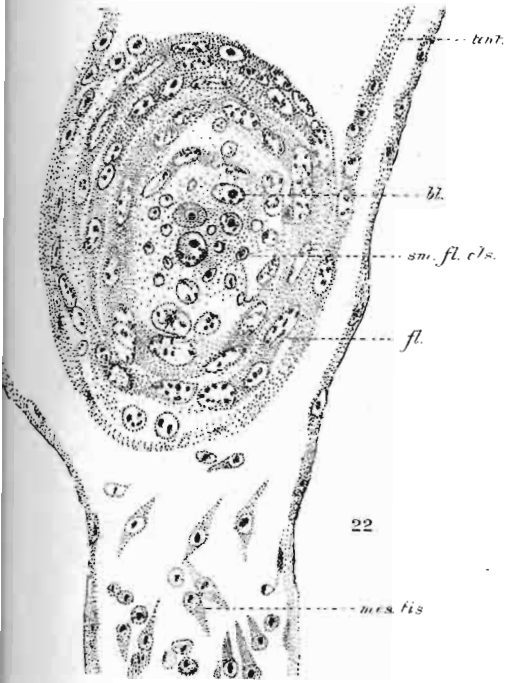
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PLATE XIV.

- Fig. 21.—Section of an ovicell of *C. eburnea* containing an embryo in the three-cell stage. The outer layer of cells represents the tentacle sheath (*tent.*), and the cavity between it and the follicle (*fl.*) is the cavity of the tentacle sheath (*tent. cav.*). × 600
- Fig. 22.—Another four-cell stage of *C. eburnea*, in which the blastomeres are separated (*bl.*) and between them are numerous small cells (*sm. fl. cls.*). The spherical follicle (*fl. cls.*) is diminished, the tentacle sheath is well developed (*tent.*), and below the embryo in the proximal portion of the ovicell are numbers of mesenchymatous cells (*mes. tis.*). × 600
- Fig. 23.—Section of an ovicell showing four blastomeres of an embryo in the eight-cell stage. The concentric layers of follicle have decreased (*fl. cls.*), while the small cells (*sm. fl. cls.*) interpolated between the blastomeres have greatly increased. × 600
- Fig. 24.—Section of an ovicell containing an embryo whose blastomeres have united to form a ball (*emb.*) which is still surrounded by the follicle (*fl. cls.*). Close to the embryo are a number of the small follicle cells (*sm. fl. cls.*). × 600
- Fig. 25.—An advanced stage in ovicell and embryo formation. The follicle cells have disappeared, and within the tentacle sheath above and below the embryo are a number of cells of the mesenchyme (*mes. tis.*). × 600
- Fig. 26.—Section of a ball stage of *Crisia occidentalis* representing an embryo at about the same stage of advancement as that in the preceding (Fig. 25). A portion of the original spherical follicle yet remains (*fl. cls.*). Below the embryo is the chitinous septum (*chi. r.*) separating the ovicell into two parts. The chitinous tube (*chi. t.*) contains large numbers of cells forming a network. Among them are numbers of multinucleated or giant cells (*gn. cls.*). × 600
- Fig. 27.—Section of an ovicell of *C. eburnea*, containing a budding embryo (*prim. emb.*), and a number of secondary embryos (*sec. emb.*). At various points on the surface of the primary embryo are a number of projections, indicating the formation of buds (*in. bd.*) or secondary embryos. The follicle is represented by a number of scattered cells (*fl. cls.*). The tentacle sheath is intact (*tent.*). × 300



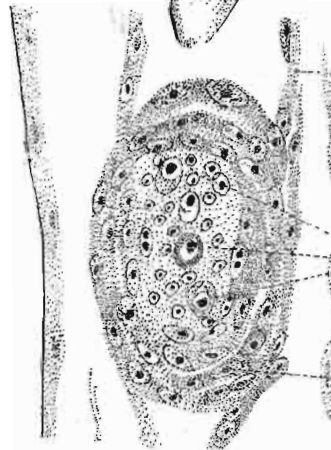
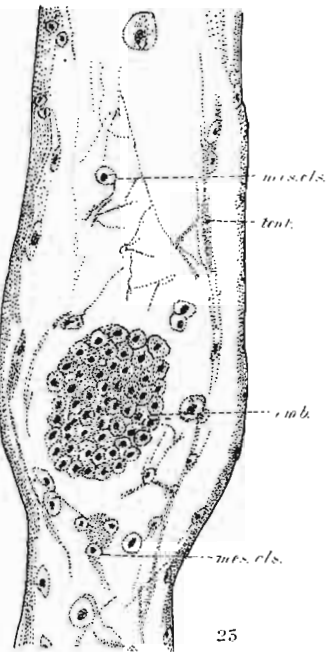
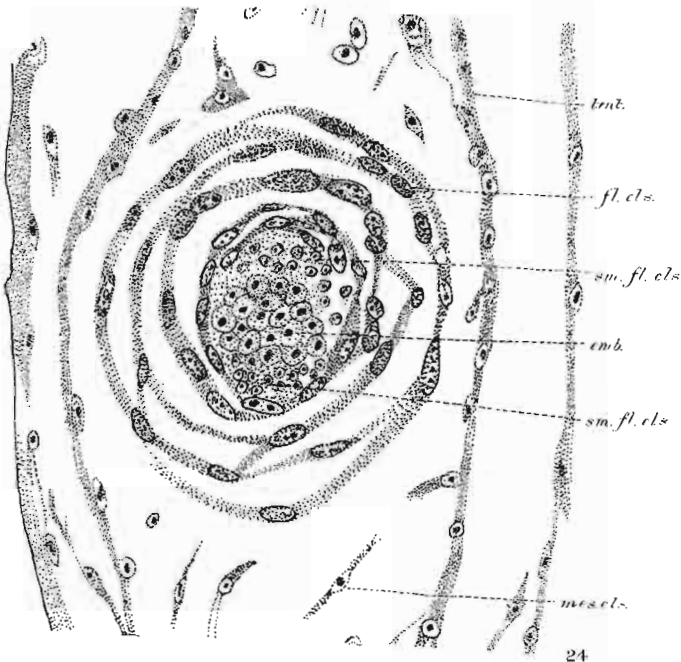
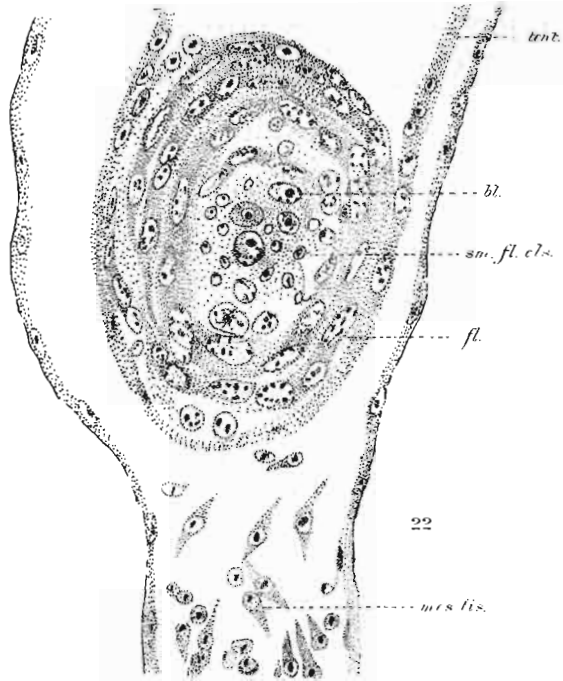
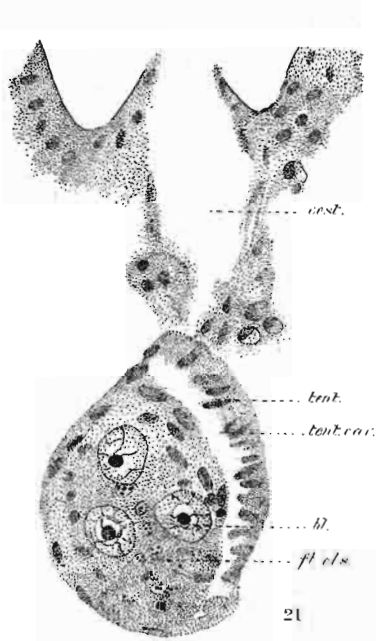


PLATE XV.

- Fig. 28.—Section of a mature ovicell of *C. eburnea*, in which the larvæ outnumber the embryos. The primary embryo (*prim. emb.*) lies at the distal end of the ovicell, still giving off buds (*sec. emb.*).
× 200
- Fig. 29.—Section of an ovicell of *C. occidentalis*, showing the formation of tertiary embryos (*ter. emb.*), and the large amount of follicle (*fl.*). Tertiary embryos are forming from a number of budding centers (*bd. c.*), which are large secondary embryos. × 250
- Fig. 30.—A single budding centre or secondary embryo from another ovicell of *C. occidentalis*, in which the two tertiary embryos (*ter. emb.*) are forming. × 2500
- Fig. 31.—The primary embryo of Fig. 28 drawn to the same magnification as that of Fig. 27, to show the reduction in size of the former.
× 300

