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THE EYES

AND

SUBNEURAL GLAND OF SALPA

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BY

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AN ABSTRACT OF A

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PREFACE.

The original paper of which this is a partial abstract described the anatomy and development of the eyes and subneural gland in both the solitary and the chain forms of Cyclosalpa pinnata (Forsk.), and compared these organs in this species with the similar organs in the following species:—

Cyclosalpa Chamissonis (Brooks), solitary and chain forms.

Salpa cylindrica (Cuv.), solitary and chain forms.

S. runcinata-fusiformis (Cham.-Cuv.), solitary and chain forms.

S. africana-maxima (Forsk.), solitary and chain forms.

Thalia democratica-mucronata (Forsk.), solitary and chain forms.

Pegea scutigera-confederata (Cuv.–Forsk.), solitary and chain forms.

P. scutigera-confederata, var. bicaudata (Quoy and Gaimard), chain form.

Iasis cordiformis-zonaria (Quoy and Gaimard), solitary and chain forms.

I. costata-Tilesii (Quoy and Gaimard), solitary and chain forms.

I. hexagona (Quoy and Gaimard), solitary and chain forms.

The homology of the ganglion of Salpa with the nervous system of other groups of Tunicates was discussed, and the conclusion reached that Salpa's ganglion is homologous with the ganglion of an adult Ascidian rather than with the elongated nerve cord of the Ascidian tadpole. The nervous system of Salpa at an early stage of its development closely resembles that of Doliolum. Salpa africana-maxima retains certain features of this Doliolum stage in the adult.

The eyes were found to be new structures not homologous with the eye of the Ascidian tadpole, or with the pineal eye of Vertebrates. The eyes of Pegea scutigera-confederata and the variety bicaudata were shown to be simpler than those of the other Salpidæ. The Cyclosalpas have the most highly developed eye. Observations were described which seemed to indicate a rich innervation for the ciliated funnel of Salpidæ.

Any homology between the neural gland of Ascidians or Pyrosoma and that of Salpa was shown to be doubtful, although the condition of the gland in Phallusia mammillata suggests a possible genetic connection between the neural glands of Ascidians and Salpa.

In appendices, Bütschli's[1] and Göppert's[2] papers upon the eye of Salpa were discussed.

The present paper is an abstract of those portions of the original publication which dealt with the anatomy and development of the eyes and of the neural gland of Cyclosalpa pinnata.

[1] O. Bütschli, "Einige Bemerkungen über die Augen der Salpen"; in Zool. Anz., Sept., 1892, No. 401.

[2] E. Göppert, "Untersuchungen über das Sehorgan der Salpen"; in Morph. Jahrbuch, Bd. 19, Heft 3.

INTRODUCTION.

At Professor Brooks' suggestion I undertook a study of the eye of Salpa in connection with his more extensive study of the genus. In the course of the work I discovered that Salpa shows a more or less well-developed subneural gland, a point of so much interest that I include a description of this organ in the present paper. I am indebted to Professor Brooks for the material upon which I worked and for the use of his own prepared sections of the embryos and developing stolons of Salpa pinnata. During the progress of this work I have received much assistance from him as my instructor, and I take this opportunity to express to him my warmest thanks.

The extensive collections of Salpa made by the United States Fish Commission, which were placed at the disposal of Professor Brooks, were the material upon which the investigations here recorded were made.[1]

METHODS.—Tunicate tissue is so easily manipulated that the simplest methods have given the best results. For staining, Kleinenberg's hæmatoxylin was used almost exclusively. For surface views the specimens were studied unstained. Dissociation was effected in a mixture of glycerine and acetic acid strongly tinged with methyl green.

Up to the present time no comparative study of the eyes of the different species of Salpa has been made. Perhaps this is not strange, for the group Salpidæ is so highly specialized that a knowledge of the eye of this group is not likely to throw much light upon the relation between the visual organs of the Chordata in general. A very casual glance at the structure of the eye in

[1] I have followed for the most part the nomenclature given by M. P. A. Traustedt: Spolia atlantica. Bidrag til Kundskab om Salperne, in Vidensk. Selsk. Skr., 6 Raekke naturvidenskabelig og mathematisk, Afd. II, 8.

the different species of Salpa is enough, however, to show that such a comparative study is likely to prove of considerable value within the group.

So far as I can learn, the ciliated funnel is the only structure as yet described in Salpa that has been regarded as homologous with any part of the subneural gland of Ascidians.[1] In the course of the present paper I shall describe certain other structures, till now undescribed (see footnote), that seem to have some relation to the Ascidian gland itself and to the lateral ducts from the gland of Phallusia mammillata to the peribranchial chamber.

[1] In a preliminary notice of this paper I gave a brief description of the organs here described at greater length.

SECTION I.—EYES.

DESCRIPTIVE.

The most noticeable feature of the anatomy of the eye of Salpa is its quite uniform shape throughout the solitary forms of the different species, and the strongly contrasted diversity of form that it shows in the chain individuals of these same species. These diverse forms are constant and characteristic for each species. In no case does the shape of the eye in the chain form agree with the shape in the solitary form of the same or any other species. The variations in the histological structure must be described in detail. The eye of the chain individual is closely related to that of the solitary Salpa, for it passes through an ontogenetic stage corresponding in shape to the adult condition of the latter. The eye of the solitary Salpa must then be regarded as the type from which the eye of the chain Salpa has diverged to a greater or less extent in the different species. The eye of the chain Salpa is not, however, in all cases a simple structure with a single origin, as is the case in the solitary form, but in several species distinct eyes are developed, or new portions of distinct origin are added to that part of the eye which is homologous with the eye of the solitary form.

First I will describe the adult eye of the solitary Cyclosalpa pinnata and its development, as the type to which to refer. Then I will describe the adult eyes of the chain form of the same species and trace their development.

THE ANATOMY OF THE EYE OF CYCLOSALPA PINNATA, SOLITARY FORM.

On the dorsal surface of the ganglion of the solitary form of Cyclosalpa pinnata there is a ridge shaped like a horse-shoe, with the open end of the horse-shoe anterior. This ridge, like the ganglion, is composed of a cellular peripheral portion and a non-cellular core; the cellular portion of the one being continuous with that of the other, while the core of the ridge is continuous with the central, non-cellular portion of the ganglion (Fig. 1). The height of the ridge above the ganglion is a little greater than its width. On the anterior face of the posterior part of the ridge and on the inner faces of its two anterior limbs, the cells are modified to form the retina of the eye. The eye, then, in every part faces toward the mid-dorsal point of the brain. The retina is formed from the more dorsal cells of the inner half of the optic ridge (that half toward the mid-dorsal point of the brain). The cells of the outer half of the ridge and of the ventral part of the inner half exactly resemble the cells of the ganglion.

As seen in Fig. 1, the ectoderm lies on the outer and dorsal surfaces of the optic ridge, but does not descend to the ganglion between the anterior limbs of the ridge. Between the dorsal surface of the ganglion, the optic ridge and the ectoderm there is a space (*oc*) which is a blood lacuna. This space, the optic chamber, is nearly shut off from the space in which the ganglion lies by a membrane (*z'*) which stretches from the ganglion, at the base of the optic ridge, out on all sides to the ectoderm. On the mid-line in front of the ganglion there is a perforation of this membrane, through which the circumganglionic blood space freely communicates with the optic chamber. The delicate membrane that clothes the ganglion is continued over the optic ridge. The membrane that closes off the optic chamber from the space in which the brain lies is continuous, on the one hand, with the membrane that covers the ganglion and eye, and, on the other hand, with the basement membrane upon which the ectoderm cells abut. That portion of the ectoderm which covers the eye and bounds the optic chamber I propose to call the optic sheath (Fig. 1, *os*).

The histological structure of the retina is the same in all regions. Fig. 1 shows a cross-section of the ganglion and of the two anterior limbs of the optic ridge. Fig. 2 is a more enlarged drawing of the retinal portion of the left limb shown in Fig. 1. The retina consists of three kinds of cells: (1), rod cells; (2), intermediate cells; (3), pigment cells. The rod cells (Fig. 2, *r* and *s*) are elongated columnar; their long axes being parallel to the horizontal plane of the body of the Salpa. They each consist of two portions: *s*, a thin-walled, lightly staining, outer portion (away from the core of the ridge) containing very finely granular protoplasm and a large nucleus with nucleolus and very apparent chromatin network; and *r*, a thick-walled, deeply staining, inner portion abutting on the next inner layer (2). The deep stain of the inner third of the rod cells is due to the thickness of the cell walls, which take the stain much more readily than the protoplasm does.

In the second layer (2), (Fig. 2, *i*) no cell boundaries can be made out, but the presence of a number of nuclei exactly resembling the nuclei of the peripheral portion of the ganglion indicates the cells of the second layer, for which I propose the name *intermediate cells of the retina.* The cell boundaries in the peripheral portion of the ganglion are no more visible than in the intermediate layer of the retina. These intermediate cells are ganglion cells which have remained unaltered during the development of the eye from the cells of the brain. In each section the number of intermediate cells is about equal to the number of rod cells. I have been unable to study live Salpas, so could not make successful macerations. I am convinced, however, that the intermediate layer of the retina is not a multinucleated mass of protoplasm, but is composed of true cells. In a partially successful maceration of the peripheral layer of the hardened ganglion, whose cells exactly resemble in appearance the intermediate cells of the retina, I could make out that the ganglion cells were of irregular shape and had, in some cases, as many as three processes. In Salpa runcinata-fusiformis, chain form, the cells of the intermediate layer are distinct and each shows usually a single process toward the rod cells.

The third layer of the retina (3) is the pigment layer (Fig. 2, *p*), composed of cells so full of pigment granules and so closely massed together that no structure can be made out. A study of their development and a comparison with other species show them to resemble, save for the pigment they contain, the cells of the intermediate layer of the retina or the ordinary cells of the ganglion. These pigment cells are arranged in a semicircle enclosing the intermediate cells and the inner ends of the rod cells.

I did not find the innervation of the eye in the solitary Cyclosalpa pinnata, but in the solitary Salpa democratica-mucronata (Fig. 3), the outer, thin-walled ends of the rod cells receive fine fibers that come from the dorsal part of the ganglion, apparently pushing out from the non-cellular core, through the peripheral cellular portion. It is probable that the eye receives fibers also directly from the dorsal cells of the ganglion. The distinctive histological character of the retina is seen from this description to consist of but two kinds of modification of the ordinary ganglion cells: first, the pigmentation of certain cells otherwise unmodified; second, the more complicated differentiation of the rod cells. No lens is ever present. The eye must be a very efficient light-perceiving organ, but the arrangement of the rod cells, the total absence of any lens and the simple character of the whole organ seem, beyond doubt, to show that it can give no perceptual image.

THE DEVELOPMENT OF THE EYE OF CYCLOSALPA PINNATA, SOLITARY FORM.

At a time in the development of the embryo, when the central cavity of the nervous system has just been obliterated, and the central cells of the ganglion are degenerating to form the central, non-cellular core, the cells destined to form the optic ridge push up from the dorsal surface of the ganglion. These cells are arranged, from the first, in a ridge having the characteristic horse-shoe shape, the open end of the horse-shoe being anterior. Salensky[1] describes the embryonic solitary Cyclosalpa eye as double; the two halves, right and left, being connected by a posterior transverse band. This description is a little misleading, for the size of the posterior curved part of the retina and optic ridge is fully as great as, and in most species a little greater than that of either of the two anterior limbs, and it is an equally important part of the eye. The eye forms a continuous curve a little greater than a semicircle, and is a single structure in its adult form and in its origin. In the chain form of different species the type is departed from and the eye is in two cases strongly bilobed, as will be shown further on.

[1] "Im Nervenganglion kann man nun zwei Theile unterscheiden: einen unteren—das eigentliche Ganglion—und einen oberen—die Anlage der Augen. Letztere setzt sich vom ersteren durch eine Einschnürung ab und verlängert sich nach vorn und hinten in zwei solide Fortsätze—die Anlage der Augen. Die Augen sind durch eine mittlere Brücke mit einander verbunden."

The ridge increases in size as the ganglion grows. As the central cells of the ganglion degenerate, the cells of the core of the ridge also degenerate; the two areas not being separate, but being, from the first, continuous with each other. Fig. 4 represents a section of the ganglion and eye at a time when the degeneration described has made some progress. It is a vertical transverse section through the central part of the ganglion. On the dorsal surface are seen sections of the two anterior limbs of the developing optic ridge.

At a considerably later period the retina cells begin to assume their characteristic appearance. The first noticeable change is the enlargement of the most peripheral layer of cells over the dorsal portion of the ridge. They elongate and become columnar, with their long axes dorso-ventral (Fig. 5). Ventral to the rod cells there are about three times as many cells having the character of the ordinary ganglion cells. About one-third of these will remain unmodified to form the intermediate cells of the retina. At about this time the other two-thirds begin to become pigmented, and, a little later, the walls of the inner third of each rod cell become thickened and so stain more deeply. The adult condition is reached by an increase in the size of the rod cells, by a greater thickening of the walls of their inner ends, by a greater deposit of pigment in the inner cells of the retina, and by a shifting of the retinal area from the dorsal surface to the dorsal part of the inner surface of the ridge. (Compare Fig. 5 with Fig. 1.) This change of position seems to be caused by the greater growth of the outer face of the optic ridge, pushing dorsalward that edge of the retina which was most distant from the mid-dorsal point of the brain (*p* in Fig. 5). Of course, by this shifting the long axes of the rod cells, which were originally vertical (Fig. 5), become horizontal (Fig. 1).

THE ANATOMY OF THE EYES OF CYCLOSALPA PINNATA, CHAIN FORM.

The histological structure of the retina of the dorsal eye of the chain form of Cyclosalpa pinnata agrees closely with that of the solitary form, save that no intermediate layer of cells is present between the rods and the pigment cells, *i. e.* all the cells of the retina except the rod cells have become pigmented. The shape of the eye, on the other hand, differs greatly from that found in the solitary form. There are present in the chain Cyclosalpa pinnata two pairs of small eyes, till now undescribed,[1] in addition to the well-known unpaired dorsal eye.

The Unpaired Dorsal Eye.

This is situated on the dorsal surface of the ganglion (Figs. 6 and 7) in a position similar to that occupied by the eye of the solitary form, except that it extends beyond the anterior face of the ganglion, only the posterior third of the eye lying upon the brain (Figs. 6, 7, 8 and 9). It consists of two almost distinct portions, the larger of which ($e' + e''$) may be described as a horse-shoe with the open side of the horse-shoe posterior, differing in this respect from the eye of the solitary form, which has its open side anterior. The posterior ends of the two posterior limbs (e') are enlarged, as shown in Fig. 6, so that the description "horse-shoe shaped" is not strictly applicable to this portion of the eye. The second, smaller portion (e''') lies in the curve of the anterior part of this horse-shoe. It is elongated transversely, reaching from one limb of the horse-shoe to the other (Figs. 6, 7, 8 and 9). It is connected to the rest of the eye only by a number of spindle-shaped cells, binding the anterior face of the second mass to the posterior face of the anterior portion of the main body of the eye (Figs. 8 and 9). In any one longitudinal section of the eye four or five of these spindle cells appear. This eye, like the eye of the solitary form, lies immediately beneath the ectoderm, in a chamber wholly shut off from the space in which the ganglion lies, except for a narrow opening on the mid-line in front. The optic chamber is, then, a blood space connected with the blood sinus in which the brain lies. In Figs. 8 and 9, which are longitudinal sections, and Figs. 10 and 11, which are cross-sections, the shape of this cavity is shown. The membrane, which intervenes between the optic chamber and the space in which the ganglion lies, is shown in all four figures at z'. The rupture of

[1] In my preliminary note of this paper I described briefly the structure, position and development of these smaller eyes.

this membrane in Fig. 8 is the point of connection between these two blood spaces. The membrane is seen to be continuous, on the one hand, with the delicate membrane around the ganglion and, on the other hand, with the membrane upon which the cells of the ectoderm rest. Figs. 8 and 9 show the characteristic infolding of the ectoderm between the anterior part of the eye and the dorsal surface of the ganglion. The only parts of the eye that abut on the ganglion are the posterior ends of its two posterior limbs. All the rest is separated from it by a double fold of ectoderm. In Fig. 11, which is a vertical cross-section of the ganglion and eye, through the region designated by *e′* in Fig. 9, there is shown the double fold of ectoderm, *k*, separating the eye, *e′*, from the ganglion. The delicate membrane that clothes the ganglion is continued over the whole eye (Fig. 9).

The arrangement of the histological elements is different in different regions (see Figs. 8 and 9). In the posterior limbs, *e′*, the rod cells are dorsal and the pigment, *p′*, ventral. In the anterior curved part of the eye, *e″*, this arrangement is nearly reversed; the pigment, *p″*, being on the dorsal and posterior faces, while the rods are on the ventral and anterior faces. Where these two regions, *e′* and *e″*, meet at the antero-lateral angles of the eye, the pigment layer of the one bends toward, but is not quite continuous with that of the other; the two almost meeting on the inner face of the eye, *i. e.* on that face which looks toward the mid-dorsal point of the ganglion. There is, then, a twisting of the main body of the eye, causing the pigment layer, as we pass from behind forwards, to face first ventrally, then toward the middle line, and finally dorsally and posteriorly. On the other hand, the same twisting causes the rod cells to face first dorsally, then laterally, and at last anteriorly and ventrally.[1] In the second, smaller portion of the eye (*e‴*), which lies in the anterior curve of the main portion, the rod cells face posteriorly toward the open end of the horse-shoe, while the pigment layer faces anteriorly, lying close to the pigment layer of the anterior part of the main body of the dorsal eye.

[1] I do not mean that there has been an actual twisting, but that there is seen in the eye a spiral arrangement of its elements such as would result from twisting. The development (see below) shows there has been no actual twisting.

The eye is innervated by two optic nerves that arise in the non-cellular core of the ganglion (Figs. 9, 10 and 12, *on*). The fibers run in two bundles, one on each side, over the posterior and dorsal faces of the two posterior limbs of the eye, some of them here entering the clear ends of the rod cells. Further forward, at the point where the relative position of rod cells and pigment cells is reversed, the fibers on each side divide into two bundles; one bundle going directly to the clear ends of the rod cells of the second, smaller portion of the eye (Fig. 8); the other passing around the inner side of the eye, below the secondary portion, to innervate in the same way the rod cells of the anterior portion of the eye (Fig. 21, *on''*). The origin of the two optic nerves is shown in Fig. 10, which represents a cross-section of the ganglion, through the region designated by *on* in Fig. 9. Fig. 12 is an enlarged drawing of the section of the left limb of the eye shown in Fig. 11. In this figure the optic nerve, *on*, appears on the dorsal surface; ventral to this are the rod cells, with their clear ends, *s*, containing large nuclei, and their thick-walled, deeply staining ends, *r*; ventral to these and abutting directly upon them is the layer of crowded pigment cells, *p*, so full of pigment granules that no structure can be made out. Over the whole surface of the retina the delicate membrane, *z*, is seen.

In one case the dorsal eye was removed from a hardened specimen of the chain Cyclosalpa pinnata and, after maceration in Haller's fluid, was gently torn with teasing needles. A bit of the smaller portion of the eye, *e'''*, was in this way isolated with some of its nerve fibers still attached. Fig. 13, which represents an optical section of this piece, shows very clearly the separate fibers connecting with single rod cells. This was even more clearly shown in more finely teased portions.

In Fig. 14 is seen a section of a portion of this eye cutting the thick-walled ends of the rod cells at right angles to their long axis. The walls of adjacent cells are so closely pressed together that they seem to form a single continuous network. In my partially successful macerations of the hardened eyes I could see in several cases a slight separation of the rods of adjacent cells, but the preparations were not sufficiently satisfactory to draw.

(See Fig. 13, which shows imperfectly the same thing.) The appearance indicated that this seeming network is not a continuous structure, but rather that each cell with its thickened walls is a separate structure, and that the deceptive appearance of a network is caused by the close apposition of the thick walls of adjacent cells. This is, of course, what we would expect, reasoning from analogy with the rod cells of other eyes.

In the same figures is noticed the protoplasmic core of each rod cell, which penetrates clear to the extremity of the thick-walled end of the cell. Within these protoplasmic cores are seen here and there (Fig. 14) round, or slightly oval, homogeneous, deeply staining bodies somewhat resembling nuclei. These are found only in the thick-walled ends of the cells, and each cell contains one of them. They seem not to be true nuclei, but to correspond to the very similar bodies found in the rod cells of the eyes of other animals, *e. g.* the pycnogonids (Morgan), insects ("secondary nuclei," Patten), scorpions (Lankester and Bourne), and probably also to the refractive globules in the cones of the retina of birds.

The Two Pairs of Smaller Eyes.

The four smaller eyes of the chain form of Cyclosalpa pinnata are arranged in two pairs, one pair lying on the posterior face of the ganglion on each side of the middle line, the other pair lying just below the posterior ends of the two posterior limbs of the unpaired dorsal eye. Fig. 15, which represents part of a longitudinal, vertical section of the ganglion, shows one of each pair of smaller eyes, *ex* and *ey*. Fig. 16, a vertical cross-section of the ganglion, shows the two eyes of the posterior pair. They lie imbedded in the midst of the smaller cells of the ganglion, just dorsal to the zone of origin of the nerves that arise from the brain. These eyes consist simply of rod cells which exactly resemble, except in size, the rod cells of the large dorsal eye. These cells are arranged in a hemisphere with their thin-walled, lightly staining ends posterior and their thick-walled, deeply staining ends toward the center of the ganglion. The membrane of the posterior face of the ganglion touches the posterior ends of the rod cells (Fig. 15). In the specimens studied, all of which were hardened in acid reagents, no pigment was found in the region of these eyes. In the live Cyclosalpa it may be that certain of the ganglion cells near the base of the rods are lightly pigmented; but this pigmentation cannot be very decided, for if ever present in specimens studied, it had been dissolved by the same reagents that had left apparently unimpaired the pigment granules in the pigment cells of the large dorsal eye. In another species, Iasis costata-Tilesii, the smaller eye is pigmented.

The structure of the dorsal pair of small eyes is the same as that of the posterior pair. In this case, however, the rod cells, which are arranged in a hemisphere, have their nucleated ends pointing toward the center of the ganglion and their thick-walled ends abutting on the pigment layer of the large dorsal eye (Fig. 15). The right one of the dorsal pair of small eyes lies at the base of the right optic nerve, on the right side of and a little in front of it. The left one is situated in a corresponding position on the left side. The pigment layer of the unpaired dorsal eye is separated from the rod cells of each small dorsal eye by the delicate neural membrane that intervenes (Fig. 15). This may or may not prevent the small dorsal eyes functioning as light-perceiving organs in connection with the pigment of the large dorsal eye. It indicates that the small dorsal eyes cannot be regarded as parts of the larger unpaired eye.

THE DEVELOPMENT OF THE EYES OF CYCLOSALPA PINNATA, CHAIN FORM.

Dorsal Unpaired Eye.

The early stages of development of this eye in the chain form are almost identical with those in the solitary form. See Fig. 17, which represents a vertical transverse section through the middle of the ganglion at the time of the earliest appearance of the rudiment of the eye. The rudimentary optic ridge is designated by *e*. It first appears at a time when the central cells of the ganglion are commencing to degenerate. It has, from the first, the characteristic horse-shoe shape seen in the eye of the solitary Cyclosalpa. (Compare with Fig. 4, which represents an anterior section of the developing eye of the solitary form.) The horse-shoe shape of the rudiment of the eye is very soon lost, but it is always present for a short time. I have examined serial sections of the ganglia of more than one hundred individuals of about the age represented in Fig. 17, and have always found the same appearance of a definite, though slightly developed, horse-shoe shaped ridge. This appearance is so constant and uniform that we can safely say *the eye of the chain Cyclosalpa passes through a stage when it corresponds in shape to the eye of both the adult and embryonic solitary Cyclosalpa pinnata.* The central cells of the ganglion and the cells of the core of the ridge degenerate at the same time, as is also the case in the solitary Cyclosalpa.

Very soon the horse-shoe shaped arrangement of the eye cells is lost. They are, from the first, close pressed to the ectoderm. Soon after the appearance of the optic ridge the ectoderm arches up over the ganglion, carrying with it the cells of the ridge. The commencement of this process is seen in Fig. 18. The eye cells lose their connection with the ganglion, except that the non-cellular core of the ridge is pulled out into long fibers that bind the eye cells to the ganglion. The rudiment of the eye is now a thickened disk of cells, close pressed to the ectoderm, with fibers connecting the center of its ventral face with the non-cellular core of the ganglion. As development proceeds the anterior edge of the disk approaches the brain till it comes in contact with it (Fig. 19). While the anterior edge shifts its position, the posterior edge retains its former place, the disk becoming in this way nearly perpendicular to the dorsal face of the ganglion. The nerve fibers which, from the first, connected the center of the disk with the non-cellular core of the brain, now, of course, lie along the posterior face of the perpendicular disk (Fig. 19, *on*). During its change of position the disk remains in connection with the ectoderm, causing the latter to approach anteriorly almost to the surface of the ganglion. The posterior edge of the disk, during this shifting of position, curls over backwards, forming the first rudiment of the second mass of retinal tissue which lies in the anterior curve of the main body of the adult eye (e''' in Fig. 19). The whole eye continues to bend forward till it comes to lie horizontal with the originally anterior edge of the disk posterior and the originally posterior edge anterior. (Compare Figs. 19, 20 and 21.)

The histological differentiation of the retinal elements, the change of form of the eye and its shifting from a perpendicular to a horizontal position, proceed simultaneously, all three processes becoming complete at the time when the circle of chain Cyclosalpas is set free from the mother-stolon. As in the solitary Cyclosalpa pinnata, the rod cells are the first to distinguish themselves, appearing at the same time in all regions of the eye. Their cell boundaries become distinct. Soon they elongate, becoming columnar (Fig. 19). After a very short interval the deeper staining of their inner ends shows the cell wall of this portion to be somewhat thickened (Fig. 20, *r*). The adult histological condition is reached by the greater elongation of the rod cells, an increase in the size of their nuclei, a greater thickening of the cell walls of their inner ends, and by a very dense deposit of pigment granules in the cells of the pigment layer of the retina. This pigmentation does not show in any of the young Cyclosalpas still attached to the stolon up to the time of the formation of the terminal wheel about to be set free. It must then be deposited rapidly between the time when this wheel is formed and the time when it is set free from the stolon of the solitary Cyclosalpa.

During the change in the histological character and in the position of the eye there is a concomitant change of form. The change from the very early horse-shoe shaped ridge on the dorsal surface of the brain to the thickened disk close pressed to the ectoderm and connected with the brain only by nerve fibers, has already been noted. We have seen also that the originally posterior edge of this disk curls over backwards to form the rudiment of the second portion of the eye seen in the anterior end of the main body of the adult eye (e''' in Figs. 19, 20 and 21). For a long time this rudiment is connected to the main body of the eye by a considerable mass of cells resembling the ordinary cells of the ganglion, *i. e.* by cells that still retain their primitive character (Fig. 21). When the wheel of chain Cyclosalpæ is about to separate from the stolon this mass of cells differentiates into three portions, one forming the pigment layer of the anterior portion of the main body of the eye (Fig. 21, p''), another forming the pigment layer of the secondary part (e''') of the eye; the cells of the third intervening portion becoming elongated to form the spindle cells that bind the secondary part of the eye to the main body (Fig. 9, *q*). While the differentiation of this mass of cells in the three directions mentioned is taking place, the posterior end of the now horizontal eye is dividing, longitudinally, in a vertical plane to form the two posterior limbs seen in the adult eye. At the stage shown in Fig. 20 no indication of a division into two limbs is visible. Later, when the young wheel is formed, but is still attached to the stolon, the division is complete, though the two limbs of the eye are not separated by so great a space as in the adult. The eye of such an embryo is shown in longitudinal section in Fig. 21.

While the developing eye is shifting from a perpendicular to a horizontal position it remains attached to the ectoderm. The ectoderm is thus folded back upon itself, forming the double fold seen in the adult between the eye and the ganglion. (Compare Figs. 20, 21 and 9.)

THE SMALL PAIRED EYES.

The two pairs of small eyes are formed at a very late period. No trace of them is found in the chain Cyclosalpa still attached to the stolon. Although their development has not been observed, they undoubtedly develop from the small ganglion cells, in the position they occupy when fully formed. Since in them no pigment layer or intermediate layer is distinguishable, the only change in the ganglion cells necessary to produce them would be a modification of certain of these cells into rod cells, after the manner of the development of the rod cells in the large eye of the chain or solitary form.

Section II.—THE ANATOMY AND DEVELOPMENT OF THE SUBNEURAL GLAND IN SALPIDÆ.

So far as I can learn, the ciliated funnel is the only organ in Salpa that has been regarded, up to the present time, as homologous with any portion of the subneural gland of Ascidians. This has been universally regarded as homologous with the ciliated funnel of Ascidians which serves in most species as the orifice of the duct from the gland proper, and perhaps in some species as a sense organ also. There are present in Salpa certain other structures that I regard as homologous with other portions of the subneural gland of Ascidians, Pyrosoma and Doliolum. Before entering on the description of the condition in the adult I wish to take up the development of these organs in Cyclosalpa pinnata.

The Development of the Subneural Gland and the Organs connected with it in the Chain Form of Cyclosalpa pinnata.

In an early stage of the development of the nervous system, long before any trace of the eye appears, the cavity of the brain and the lumen of the funnel open freely into each other by a wide duct, so short and wide as to hardly deserve the name duct. There is no distinction in histological character between the cells of the funnel, duct and brain; nor is there any indication, either in the thickness of the walls, or in any other feature, of the boundaries between funnel, duct and brain. The ventral wall of the posterior part of the neural canal becomes thickened. This corresponds to the thick ventral wall of the "visceral portion" of the larval Ascidian nervous system, *i. e.* that portion which gives rise to the subneural gland. Later, the cells of the dorsal wall of the posterior part of the neural canal begin to multiply rapidly; some of them keeping their original arrangement as an epithelium bounding the canal; others, much more numerous, push up toward the ectoderm to form the dorsal portion of the adult ganglion. This proliferation of cells is greatest, for a time, at the most posterior part of the dorsal wall of the neural canal. The brain cavity has now a thick ventral wall, and a dorsal wall about twice as thick as the ventral. Fig. 22 shows one stage in the development of the ganglion of the solitary Cyclosalpa pinnata. It will answer equally well for the chain individual.

At the time of the first appearance of the rudiment of the dorsal eye the three regions, funnel, duct and brain, are clearly distinguished, the duct being a small round tube connecting the lumen of the funnel with the cavity of the brain (Fig. 18). The description so far would answer almost equally well for either Salpa, Doliolum or Pyrosoma, except that the latter two do not have the ventral wall of the neural canal thickened. The brain cavity at this time is much flattened dorso-ventrally, forming a narrow slit separating the ventral third of the brain from the dorsal two-thirds. As the cells of the dorsal wall of the neural canal push up to form the dorsal part of the ganglion they do not at once make a solid mass, but they leave between themselves fine, anastomosing, lucanar canals (Fig. 17), some of which open inward to the neural canal *f*, while others open outward to the cavity around the brain. These lacunæ are not mere chance spaces, but have a quite definite appearance and persist for some time. Soon the brain becomes solid, the lacunæ and also the central canal disappearing. When this change is nearly completed (Fig. 18), we see a condition almost exactly resembling that found in a nearly mature Doliolum. There is a slight antero-ventral process containing a remnant of the disappearing brain cavity, *f*. This is still connected by a very fine canal with the wide lumen of the funnel. The lumen of the canal does not show in the section drawn. In the later development the brain cavity and the duct wholly disappear, and the lumen of the funnel is the only remnant of the neural canal. The duct disintegrates as if pulled apart by the elongation of that region of the body, reminding one of the elongation of the duct in Doliolum, by which the cells are pulled out into an interrupted pavement epithelium. Neither in the half-developed chain individual nor in the adult can any trace of a duct opening into the funnel be found.

Certain other structures develop later that seem to bear some relation to the lateral communications between the subneural gland and the peribranchial chamber, described for Phallusia mammillata. At a time when the embryonic eye-disk has taken its perpendicular position (see the section on the development of the eyes of Cyclosalpa pinnata, chain form, and Fig. 20) the wall of the branchial chamber, which up to this time has been close to the ventral surface of the brain (Fig. 17), begins to separate from the brain. Two small areas remain contiguous, however, with the brain, one situated at the right, the other at the left of the mid-ventral point of the ganglion (Fig. 23). As the wall of the branchial chamber separates more and more from the surface of the ganglion, those portions of this wall which are adjacent to the two areas of adhesion are gradually drawn out into two tubes reaching from the ventral surface of the ganglion to the branchial chamber (Fig. 24). The adult condition is reached by the considerable growth of these tubes, which become greatly coiled, and by the flattening out into a hollow disk of the portion of each tube contiguous with the brain. Figs. 25 to 27, serial sections in a longitudinal, vertical plane, and Fig. 28, a cross-section, show the adult condition. In the former series Fig. 25 is nearest the middle line of the body. The structure of the cells lining the tubes and hollow disks does not surely indicate their function. They are smaller than the cells of the brain, irregularly cubical in form, have well defined nuclei surrounded by finely granular protoplasm which, under very high magnification, shows minute vacuoles. These may or may not indicate secretory activity. The homologous cells in Salpa africana-maxima are almost surely glandular, so it is probable these cells in Cyclosalpa pinnata are more or less functional in secretion.

In close connection with the enlarged ends of the tubes just described there are four masses of cells, two on each side. One of these two masses, the more anterior (Figs. 25 and 28, *b′*), is composed of large cells with large nuclei, resembling closely the large ganglion cells that lie in the brain, in the zone of origin of the nerves (cf. Fig. 23, *n*). The more posterior mass, *b*, is composed of small cells with small nuclei, having the appearance of the ordinary cells of the periphery of the brain. These masses of cells develop simultaneously with the lateral tubes (Figs. 23 and 24) as outgrowths of the ventro-lateral areas of the brain. What present function they may have I am unable to say.

EXPLANATION OF PLATES.

Reference Letters.

b = small celled, ventro-lateral outgrowth from the ganglion toward *h*.

b' = large celled, ventro-lateral outgrowth from the ganglion toward *h*.

bc = blood corpuscle.

bl = blood lacuna.

cf = ciliated funnel.

cr = non-cellular core of the optic ridge.

d = lateral duct of the subneural gland.

dl = dorsal lamina (gill or its anterior curtain-like projection).

do = opening of the lateral duct of the subneural gland into the branchial chamber.

e = eye or rudiment of the eye.

e' = basal portion of the unpaired dorsal eye of the chain form.

e'' = apical portion of the unpaired dorsal eye of the chain form:

e''' = secondary portion of the unpaired dorsal eye of the chain form.

ex = one of the dorsal pair of small eyes in the chain Cyclosalpa pinnata, or its homologue in other species.

ey = one of the posterior pair of small eyes in the chain Cyclosalpa pinnata, or its homologue in other species.

f = canal of the central nervous system.

g = ganglion.

gc = non-cellular core of the ganglion.

gs = space in which the ganglion lies.

h = disk-like enlargement of the neural end of *d*.

i = intermediate cell, or cells, of the retina.

k = epidermis.

k' = wall of branchial or peribranchial chamber.

l = lacunar canal in the immature ganglion.

n = nerve.

nc = large ganglion cells in the zone of origin of the nerves.

oc = optic chamber.

on = optic nerve.

on' = fibers of the optic nerve which innervate *e'*.

on'' = fibers of the optic nerve which innervate *e''*.

on''' = fibers of the optic nerve which innervate *e'''*.

os = optic sheath.

p = pigment cell, or pigment layer.
p' = pigment cell, or pigment layer of e'.
p'' = pigment cell, or pigment layer of e''.
p''' = pigment cell, or pigment layer of e'''.
q = spindle-shaped cells binding e''' to e''.
r = thick-walled portion of rod cell.
r' = thick-walled portion of rod cell of e'.
r'' = thick-walled portion of rod cell of e''.
r''' = thick-walled portion of rod cell of e'''.
s = thin-walled portion of rod cell.
s' = thin-walled portion of rod cell of e'.
s'' = thin-walled portion of rod cell of e''.
s''' = thin-walled portion of rod cell of e'''.
up = unpigmented spot.
z = neural membrane.
z' = membrane separating the optic chamber from the space in which the ganglion lies.

The *orientation* of the figures is indicated by the following signs:
——>: the arrow points anteriorly.
——o: the club-shaped rod points dorsally.

The two are often combined in this way—o—>

All figures are drawn with the camera and reduced to one-third, linear measurement.

EXPLANATION OF PLATES.

All figures except Fig. 3 portray portions of Cyclosalpa pinnata.

Figure 1, a transverse section through the ganglion and the anterior limbs of the eye. Solitary form, adult. × 105 diameters.

Fig. 2, a more enlarged drawing of one of the limbs of the eye shown in Fig. 1. × 330 diameters.

Fig. 3, a parasagittal section of the ganglion and eye of Thalia democratica-mucronata, solitary form, showing also one of the disk-like portions of the subneural gland, *h*. × 160 diameters.

Fig. 4, a transverse section through the anterior limbs of the horse-shoe shaped rudiment of the eye and through the ganglion and adjacent parts. Embryo of solitary form. × 105 diameters.

Fig. 5, a transverse section through the ganglion, eye and adjacent parts in an advanced embryo of the solitary form, showing also the disk-like enlargements of the lateral tubes of the subneural gland. The section cuts the two anterior limbs of the eye. × 105 diameters.

Fig. 6, a dorsal view of the eye, ganglion and nerves of the adult chain individual. × 78 diameters.

Fig. 7, a lateral view of the same. × 78 diameters.

Figs. 8 and 9, longitudinal, vertical sections of the ganglion and unpaired eye of the adult chain individual. Fig. 8 represents the section nearer the mid-line. The three regions of the unpaired eye are shown, e', e'', e'''. × 105 diameters.

Fig. 10, a transverse section of the ganglion through the region marked *on* in Fig. 9. Chain form, adult. × 105 diameters.

Fig. 11, a more anterior section from the same series, cutting the eye through the region marked e' in Fig. 8. × 105 diameters.

Fig. 12, a more enlarged drawing of the right limb of the eye shown in Fig. 11. × 152 diameters.

Fig. 13, an optical section of a piece of the second, smaller portion of the unpaired eye, e''', separated by teasing, and having some of the fibers of the optic nerve still attached. Chain form, adult. × 105 diameters.

Fig. 14, a part of a horizontal section through the region marked e' in Fig. 8. It shows the thick-walled ends of the rod cells in cross section. Chain form, adult.

Fig. 15, a parasagittal section of the antero-dorsal part of the ganglion, showing one of each pair of smaller eyes, *ex* and *ey*. The position of the large unpaired eye, e', is indicated. Chain form, adult. × 240 diameters.

Fig. 16, a transverse section of the posterior part of the ganglion of the adult chain form, showing the posterior pair of small eyes, *ey*. × 160 diameters.

Fig. 17, a transverse section of the ganglion and first rudiment of the optic ridge in a young chain individual. The anterior limbs of the horseshoe shaped optic ridge are indicated by *e*. × 200 diameters.

Fig. 18, a sagittal section of the ganglion, unpaired eye, *e*, and ciliated funnel, *cf*, of a slightly older individual. × 200 diameters.

Fig. 19 (older stage), a parasagittal section of the ganglion and eye. The eye, *e*, has assumed an almost vertical position; along its posterior face the fibers of the optic nerve, *on*, are seen. The dorsal edge of the optic disk is curling over backward to form the second, smaller portion of the unpaired eye, *e'''*. × 200 diameters.

Fig. 20 (still older stage), a parasagittal section of the ganglion and unpaired eye. The rod cells have acquired definite cell walls and are commencing to elongate. × 200 diameters.

Fig. 21, a slightly parasagittal section of a yet older eye. The eye is now nearly horizontal; its three regions are clearly defined. The manner of innervation of these three regions is clearly shown. The individual from which this section was taken was one member of a small wheel-shaped colony that had just formed on the end of the stolon, but was not ready to be loosed.

Fig. 22, a sagittal section of the ganglion of a young embryo of the solitary form, showing how the dorsal part of the adult ganglion is formed from the dorsal wall of the originally thin-walled neural canal. × 176 diameters.

Fig. 23, a parasagittal section of the ganglion of a chain individual at a stage of development corresponding to that represented in Fig. 17, showing the commencement of the formation of a lateral tube of the subneural gland, *h*. × 160 diameters.

Fig. 24, a parasagittal section of the ganglion of a chain individual at a stage of development corresponding to Fig. 18, showing a later stage in the formation of a lateral tube of the subneural gland. × 160 diameters.

Figs. 25-27, parasagittal sections (not quite vertical) of the ganglion and adjacent parts in an adult chain individual, showing the subneural gland, *d* and *h*, and the ventro-lateral outgrowths from the ganglion, *b* and *b'*. Fig. 25 is nearer the mid-line. × 105 diameters.

Fig. 28, a transverse section of the ventral half of the ganglion and adjacent parts in an adult chain individual, showing the relation of the subneural gland to the ventro-lateral outgrowths from the ganglion. × 105 diameters.

Fig. 1

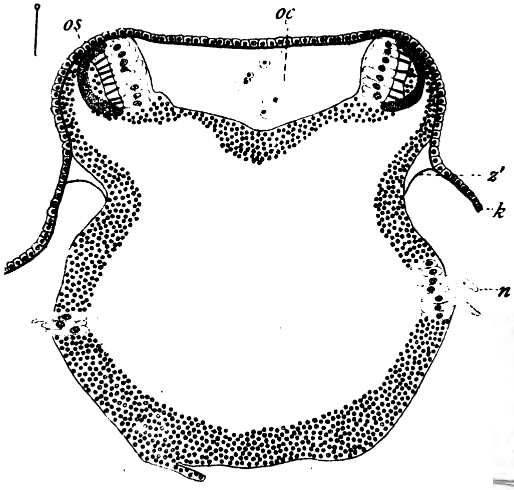


Fig. 2

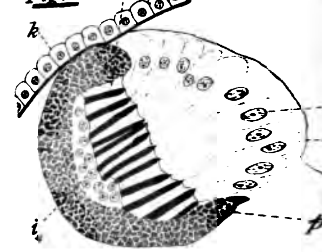


Fig. 3

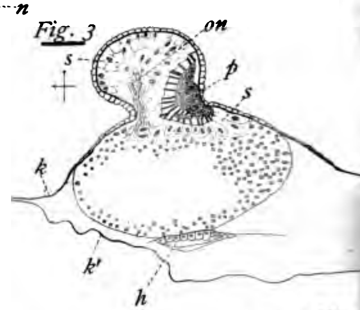


Fig. 6

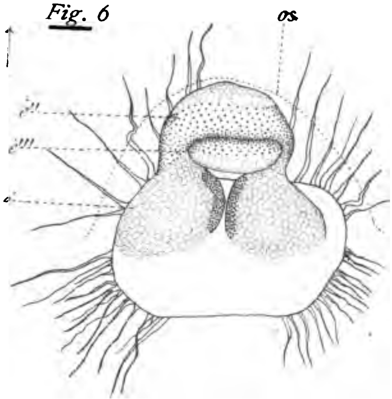


Fig. 14

Fig.

Fig. 11

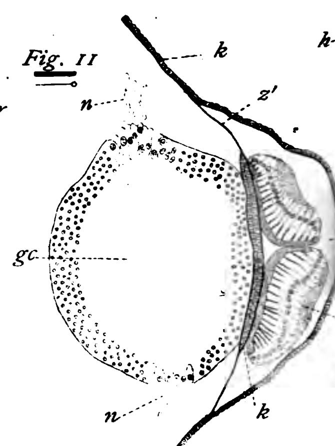
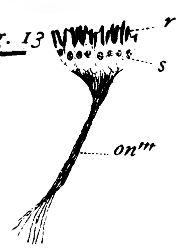


Fig. 13



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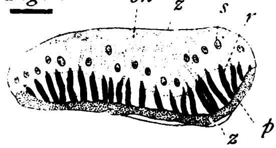

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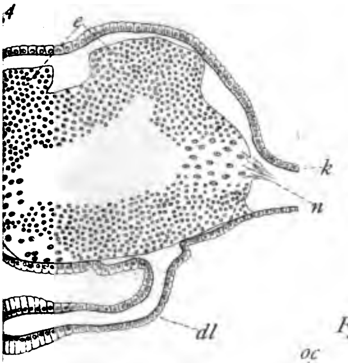
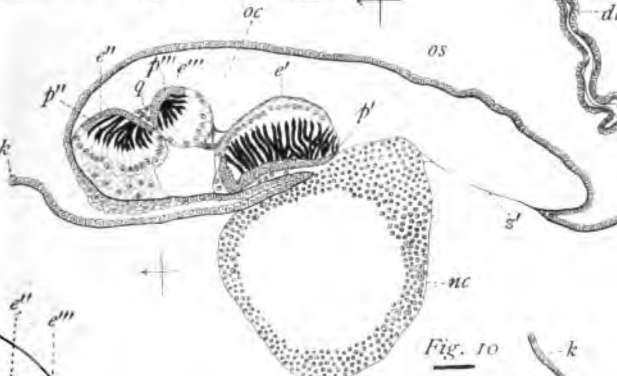


Fig. 5

Fig. 8



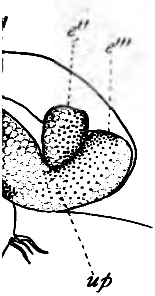
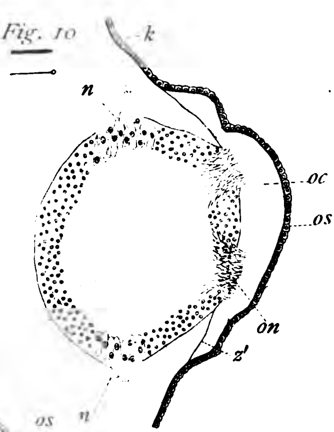


Fig. 10



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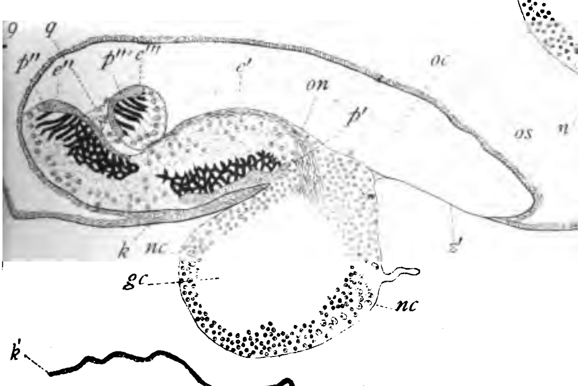

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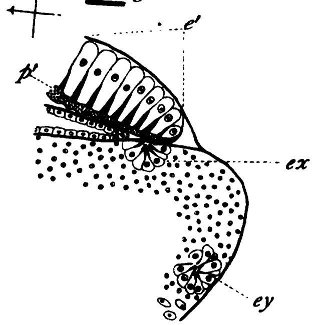


Fig. 16

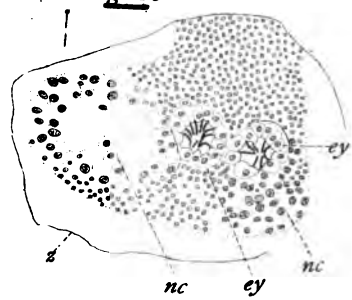


Fig.

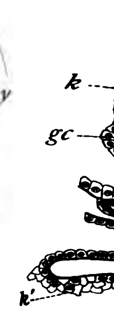


Fig. 19

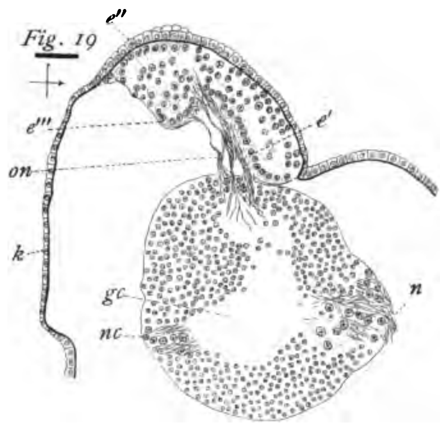


Fig. 20

Fig. 25

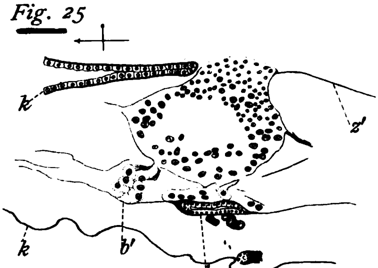


Fig. 26

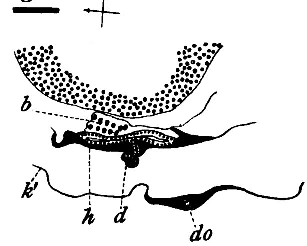
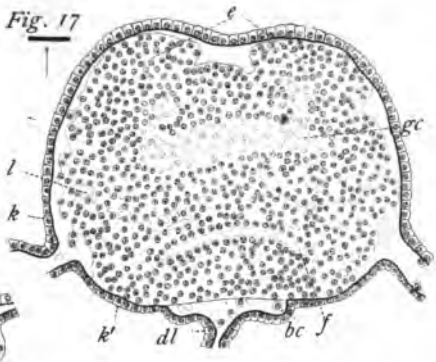


Fig. 17



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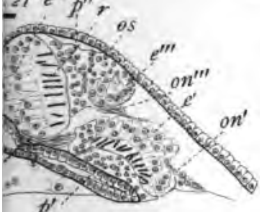

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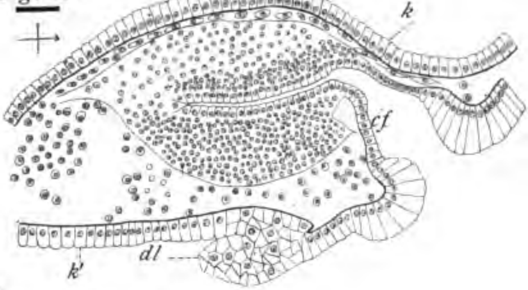


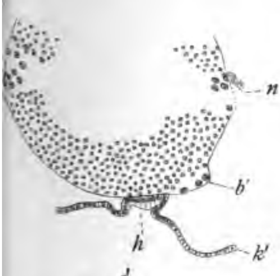


Fig. 24

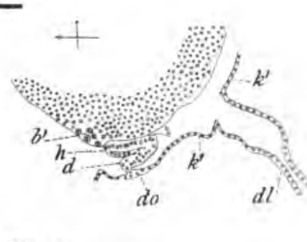
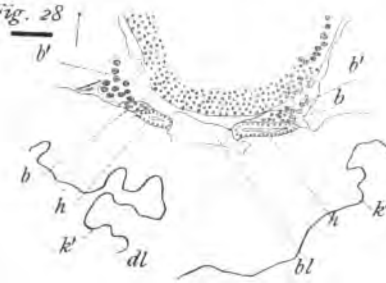


Fig. 27

Fig. 28



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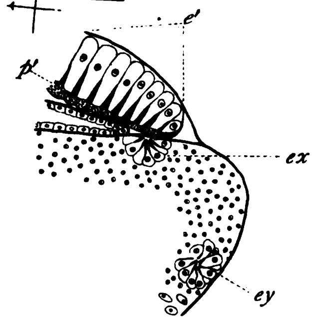

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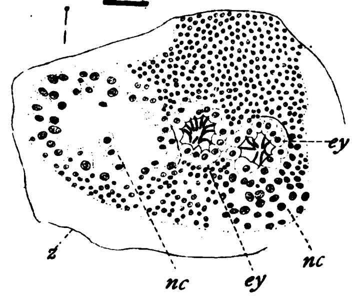
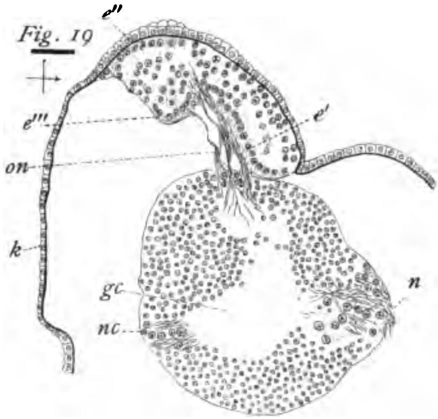


Fig. 1

Fig. 19



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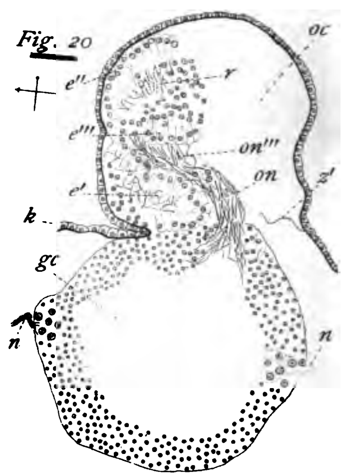

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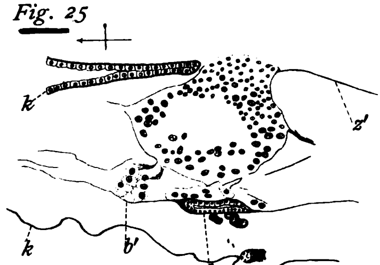

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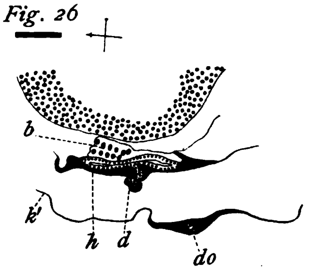

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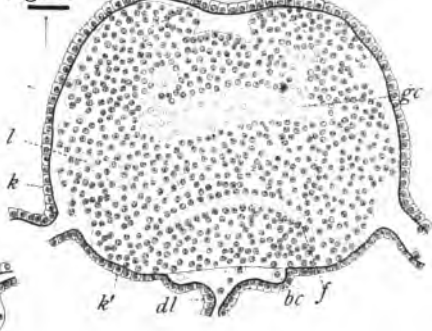

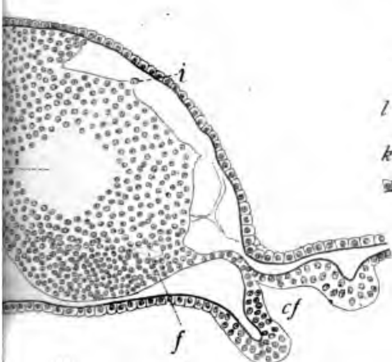

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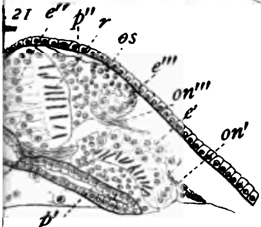
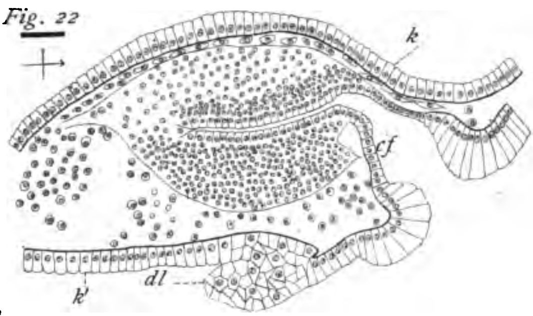
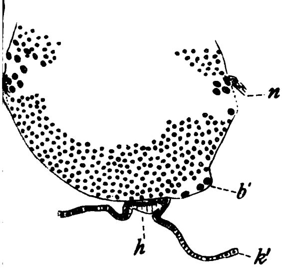


Fig. 22





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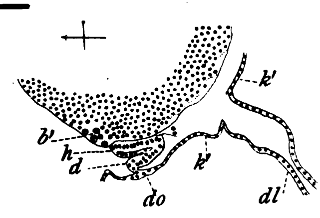

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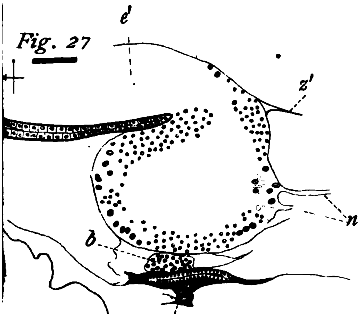

Fig. 28