On the Dendritic Appendage of the Urogenital Papilla of a Siluroid.

By

S. Hirota, *Rigakushi.**

Zool. Inst., Science College, Imp. Univ., Tōkyō.

With Plate XXXVI.

In spite of the fact that all the siluroid fishes included in the genus *Plotosus* have long been known to possess a peculiar dendriform appendage behind their urogenital papilla and that its external features have repeatedly been described, only imperfect knowledge exists of its internal structure and physiological significance. It

* It is my painful duty to record here the death of the author of the present article. Some time before his last illness, Mr. Hirota placed in my hands the manuscript of the article, which had already been so far worked over that after his death, I found myself able to put them into the final shape for publication. The alterations that I have taken the liberty of making are only such as in my opinion bring out his ideas more clearly and would, I believe, have been accepted by him if he had been alive. In no case have I wittingly run counter to his ideas. At the same time I must ask the reader's kind consideration of the circumstances if some parts are found to be not as full as may seem desirable. I can not let this opportunity pass without saying that we have lost in Mr. Hirota a co-worker of great promise. His published articles besides the present one are as follows:—

1. On the Sero-Anmiotic Connection and the Foetal Membranes in the Chick. *Jour. Coll. Sci., Imp. Univ. Japan Vol. VI. 1894. English.*
2. On the Fauna of the Ogasawara (Bonin) Islands. *Zool. Mag., Tōkyō, Vol. VI., Nos. 68 and 69, for June* and *July, 1894. Japanese.*
3. On the Loss of Weight in the Fowl's Egg during Incubation. *Zool. Mag. Tōkyō, Vol. VI., No. 74 for Dec. 1894. Japanese.*
4. Anatomical Notes on the 'Comet' of *Linckia multifora*, Lam. *Zool. Mag. Tōkyō, Vol. VII., No. 78 for April 1895. Japanese and English.*
5. Notes on a Scink with an Accessory Tail. *Zool. Mag. Tōkyō, Vol. VII., No. 81 for July 1895. Japanese and English.*

Besides these, he published several short notes in the Zoological Magazine of Tōkyō.

Zool. Inst., Sci. College, Imp. Univ. Tōkyō.

K. Mitsukuri.

is true that J. BROCK [1] made a microscopical study of the appendage in question in *Plotosus anguillaris* (BLOCH) LACEP., but as his materials were confined to four immature specimens, the results of his investigation were not entirely satisfactory.

Last year, at the suggestion of my friend Mr. T. KITAHARA, I undertook a new investigation of the appendage in *Plotosus anguillaris* already studied by BROCK. This fish is easily obtainable at the Marine Biological Station at Misaki throughout all seasons, so that the supply of my materials was practically unlimited, and I was able to examine the organ in a large number of fresh as well as alcoholic specimens of both males and females in various stages. The following notes containing the results of my investigation are, I regret to say, purely morphological, but it is hoped that they may throw some side light on the physiological function of the organ, for the determination of which future research is however still needed.

Before going further, I wish to express my deepest indebtedness to Prof. Dr. MITSUKURI for kind supervision of my work and to Mr. T. KITAHARA for valuable advice.

External Parts.

As a full description of the external parts of the appendage seems at the present day hardly needed, I shall introduce here only such remarks on the general features of the organ as will make what follows intelligible along with others about it in its fresh state and in its early stages.

Fig. 1, Pl. XXXVI. represents in their natural size the appendage and the adjacents parts in a fully grown individual.

1) Brock, J., Ueber Anhangsgebilde des Urogenitalapparates von Knochenfischen. *Zeit. f. wiss. Zool.* Bd. 45, 1887.

The appendage occupies a comparatively wide space behind the urogenital papilla and is so situated as to be more or less protected by the paired pelvic fins. It is very soft and pliable, and is in its natural state blood-red in colour. It is, as a whole, divided into two symmetrical halves, each of which is again subdivided into numerous thin lobes of various sizes. Figs. 3–5 represent three different aspects of one and the same specimen: Fig. 3 is the dorso-anterior, Fig. 4 the ventral, and Fig. 5 the dorsal, view. The whole organ is lodged in a depression (Fig. 2) of the body proper, corresponding in shape to the dorsal surface of the organ, in a manner represented in Fig. 10. On a ventral view, the urogenital papilla anteriorly, and the front end of the caudal fin posteriorly, are received between the two divisions of the appendage. At the bottom of the depression just mentioned, there is a collar-like structure (*collar* Fig. 2) surrounding a central pit (marked *fissure*, Fig. 2). Into this pit is inserted the stalk of the appendage by which it is connected with the body-proper and which is situated rather nearer the anterior end of the organ. There is between the stalk and the collar a distinct canal all around except at the anterior median line where the stalk is adherent by a narrow strip to the urogenital papilla. The size of the appendage may vary in different individuals of the same stage. The largest found among about four dozen specimens of adult individuals is 8 mm. in length and 12 mm. in breadth, while the individual to which it belongs measures 180 mm. in total length.

If we examine a lobe or leaf of the appendage under a magnifying lens, we observe numerous more or less round deep pits of different sizes on its surface (Fig. 6). Examined in a fresh state, these pits are found to be in meshes of a fine net-work of capillaries as represented in Fig. 7. Also in the same state numerous branching blood-vessels are seen in the interior, (Fig. 11) although they are rather obscure in

the thicker parts. In fact, the characteristic colour of the organ is caused by these blood-vessels and capillaries.

The dendritic appendage is found equally developed in the two sexes as was, according to Brock, ascertained by Kner in 1855 ; in fact the sex can be determined only by an examination of the generative organ. The appendage is also found from the very earliest stages of growth being simpler in structure, the earlier the stage. For instance, Fig. 9 represents somewhat enlarged the organ belonging to the very young individual shown in Fig. 8 in its natural size. It is already divided into two more or less symmetrical halves but each half consists only of a few lobes marked with shallow pits. It is at this stage coloured not only by blood but like the body proper by fine dots of black pigment which becomes dispersed and almost invisible in the adult.

In passing, I may remark that Brock's Figs. 1 and 2 appear to me somewhat unfortunate as representations of nature. His Fig. 1 almost makes me think that the species he observed is different from mine,[1] but the difference probably arises from the unsatisfactory state of his preserved specimens.

Internal Structure.

When we cut the organ in question into serial sections, we soon observe that it is built up of two concentric zones, of which the outer or the cortex consists of a parenchyma-like epidermis containing also slime cells and well developed glandular cells, while the inner zone or the corium consists of smooth muscular

1) For specific identification, I have mainly relied on Günther's *Cat. of the Fishes in the Brit. Museum*, but have also referred to Desmarests' *Hist. Nat. de Lacepède* and to Schlegel's *Fauna Japonica, Poiss.*

fibres containing blood-vessels, lymph-cavities and nerve-bundles. Particulars concerning each of these zones are briefly stated in the following paragraphs.

The Cortex.

In describing the cortex it is convenient to consider separately the parenchyma-like epidermis and the glandular cells, although the two stand in close relations with each other. The parenchyma-like epidermis is, as shown in Figs 20 and 21, formed of densely packed simple epidermal cells with ordinary round or oval nuclei and of scattered bottle-shaped slime cells (*sl*) with short ducts. In or near the stalk of the organ, the epidermis forms a thick continuous stratum and is the sole component of the cortex in that part (Fig. 18). In the more distal portion, *i.e.*, in the thin lobes of the appendage, it is interrupted by numerous pits in which it suddenly thins itself out and at their bottom exposes the heads of underlying glandular cells to be spoken of immediately (Fig. 20). The slime cells are found only in those regions where the epidermis attains a certain thickness and are entirely absent from the pits.

Longitudinal sections of the basal portion of the appendage show that the epidermis is directly continuons with that of the body proper (Fig. 18). In both parts, the epidermis is exactly alike in structure, except that in the appendage it is interrupted by numerous pits, the bottom of which is always occupied by glandular cells. In the body proper, these glandular cells are entirely absent, although quite a different sort of gland-cells known as goblet cells are present (Figs. 18 and 19). The slime cells are few in number in the stalk but they can be traced in longitudinal sections right to the body proper without any great gap in the series. They are, in the appendage and the body

proper, exactly alike not only in shape and occurrence but also in the fact that they are distinctively stained by Kleinenberg's hæmatoxylin solution.

Let us next examine the glandular cells in the pits which form such a characteristic feature of the appendage. As Figs. 20 and 21 show, they are uncommonly large cells, always filled up with finely granular substance and containing a single oval nucleus in about the middle of their length. In a section, they are seen standing side by side in a single row along the bottom and sides of every pit and exposing their heads directly into the cavity of the pit, although the nuclei of the epidermal cells are seen here and there scattered over them. A group of the glandular cells belonging to one pit forms a sort of cup, more or less distinctly separated from neighbouring groups or cups by the intervening epidermal cells, capillaries, etc. (Figs. 19 and 20); so that the glandular cells do not form a single continuous sheet as supposed by BROCK. It is evident from the above description that the secretory product of these glandular cells is discharged directly into the cavity of the pit through the interruptions of the epidermis. In specimens killed with chromic acid or chromo-acetic acid, the granular contents are well stained with alcoholic or watery solutions of such aniline dyes as eosin, methyl-blue, and saffranin, but do not absorb Kleinenberg's haematoxylin solution which differentiates the slime cells.

In the body proper as well as in the basal portion of the stalk, there are found, as I have mentioned above, instead of such true glandular cells, a number of large ductless goblet-cells at the inner part of the epidermal layer (Figs. 18 and 19). In the fully developed stage, these two kinds of cells are distinguished from each other by the following points :—

a) The glandular cells of the appendage form definite groups,

while the goblet cells are scattered or at least separated from one another by interstitial cells.

b) The goblet cells are usually multi-nucleated — containing sometimes as many as four nuclei — while the glandular cells have never more than one (Figs. 18 and 21).

c) The goblet cells which are deeply imbedded in the epidermis have no opening, while the glandular cells are directly exposed externally.

d) The goblet cells are always much larger than the glandular cells, being often as much as ten times in volume (Fig. 19).

e) The glandular cells are without exception filled with finely granular thick contents, while the contents of the goblet cells are partly or entirely very coarse-grained.

f) As Fig. 19 shows, there is no gradual transition between the goblet, and the glandular cells, an abrupt replacement taking place.

In a young animal, such as is represented in Fig. 8, the glandular cells of the dendritic appendage are as yet only slightly larger than the surrounding epidermal cells, but they are already directly exposed at one end in the bottom of diminutive pits (Fig. 22), although it is doubtful whether they are actually functional or not. In such a stage differences between these cells and the goblet cells are not as clear as in the adult, but it is noteworthy that the former are found in groups, while such is never the case with the goblet cells in any stage. It would, however, require further embryological research to decide whether the glandular cells are metamorphosed goblet cells or are independently developed in this particular organ.

The first writer who pointed out the presence of the pits or "Krypten" and of the glandular cells in the appendage is BROCK. In his sections, he, however, found ordinary epidermal cells in the

bottom of the pits overlying the glandular cells, and he was at a loss to account for the way by which the secretory product of the glandular cells was discharged into the cavity of the pits. He supposed that the condition found in his sections was due to the fact of his specimens being immature, and that in the adult, the epidermal cells were removed from the bottom of the pits, thus exposing the glandular cells. This statement is somewhat remarkable, as even in specimens much younger than those studied by him, the glandular cells are freely exposed at the bottom of the pits, as I have stated above (see Figs. 8 and 22). It is possible that his sections were cut somewhat obliquely and thus failed to show the true state of things. The glandular cells are also stated by BROCK to form a continuous layer, whereas in reality every group is discontinuous from the adjacent ones.

The Corium.

The inner zone of the dendritic appendage consists of smooth muscular fibres containing nerves, large blood vessels, and irregularly branched lymph-cavities. From sections we learn that this inner zone attenuates in thickness from the stalk towards the tips of the lobes. In the latter position, it is thicker in the middle than towards the edges. In longitudinal sections of the basal portion of the stalk, showing the connection between the appendage and the body proper (Fig. 18), we observe that the peripheral part of the muscular zone is directly continuous with the corium of the body proper, while the middle part runs deeply inwards to attach itself to the ventral apophyses of the vertebræ, mainly to those of the second and third caudal vertebræ and partly to those of the first [1] and fourth.

Blood is supplied to this appendage by a pair of arteries which

1) I designate the first vertebra which forms the hæmal arch by a bony bar the first caudal vertebra. It is perhaps the third according to Brock's nomenclature.

arise at the same level from the dorsal aorta behind the first hæmal arch (Fig. 10). Their courses may vary more or less in different individuals: both of them may run in behind (as is represented in Fig. 10), or before, the coalesced apophyses of the second caudal vertebra or one of them may pass before, and the other behind, the same apophyses. They may send off small branches before entering the stalk of the appendage. In all the cases I have examined, I have found them symmetrically paired in the basal portion of the stalk (Fig. 13). As they proceed centrifugally, they divide and subdivide in the usual manner, until they end in capillaries forming the network around the pits of the lobes.

The venous vessels which arise from the capillaries become gradually larger as they proceed centripetally by the union of smaller vessels. In the distal portion of the stalk (Figs. 14 and 15), they are already united into two main trunks, each coming from the corresponding half of the appendage. Sooner or later before entering the body proper, these two are also fused into one (Fig. 13). In the body proper it passes behind or before the ventral apophyses of the second caudal vertebra and opens into the cardinal vein at about the level of the origin of the arteries (Fig. 10), and in company with a pair of veins coming from the abdominal wall on both sides. Figs. 13–16 represent selected transverse sections of the stalk between its base where it is attached to the urogenital papilla and the point where it divides into two, and Fig. 17 is a horizontal section cut through the plane indicated by the letter (*a*) in Fig. 3. In such transverse sections, it is natural that some of the blood vessels should be cut obliquely or one and the same vessel should appear two or three times in one section on account of its being bent or curved in its course. But in spite of these circumstances it is clear from these figures that all the larger vessels belong to the venous system and are monstrously large

in proportion to the arterial system as well as to the appendage itself.

The nerves are supplied to the appendage from a pair of spinal nerves sent out from the first caudal vertebra (Fig. 10). These spinal nerves give near the root of the appendage one branch on each side of the body which enters directly into the appendage and innervates it. They run similar courses to the arteries but begin to branch sooner than the latter. I have been unable to trace these bundles into their final twigs, but I have recognised them in sections near the tips of the lobes (Fig. 17).

Besides these blood-vessels and nerves there is observed in the inner zone of the appendage still another system of cavities, *i.e.*, that of the ill-defined lymph-cavities (Figs. 13–16). In serial sections it can be traced in the body proper to the dorso-median lymph-cavity surrounding the aorta and the cardinal vein. Near the base of the stalk (Figs. 13 and 14), it may be branched into a pair or be simple, but on its further course, (Figs. 15 and 16) it sends out here and there several irregularly shaped branches. It is bounded by an endothelial membrane while it passes through the connective tissue of the body proper, but in the appendage from the base of the stalk upwards, there exists no longer a definitive boundary. Owing to this circumstance, the cavities have often collapsed by bad treatment and the lymph-cavity appears then as if it ended blindly near the root of the stalk. It can be traced for a certain distance towards the lobes of the appendage but in the latter it vanishes in the loose fibrous tissue.

All these structures of the corium are equally well observed in various younger stages. Even in such a stage as is represented in Fig. 8, there are already recognisable in the cross-sections of the stalk a pair of arteries, a vein of considerable size, and a lymph-cavity of suitable proportion in the same relations with one another as in the adult (Fig. 12). The only noteworthy peculiarities of such a stage are the

apparent absence of nerve-bundles and the presence of pigment cells. The first circumstance is perhaps owing to the difficulty of distinguishing nervous from muscular fibres in such a young stage, while the second circumstance is due to the small size of the organ in which the pigment cells have not yet begun to disperse. At all events, the general plan of the structure of the inner zone can be made out in such an early stage.

The inner zone has already been examined by BROCK. The lymph-cavities as well as nerve-bundles seem to have escaped his notice while he considered the veins as the most important part of the organ. He says:—"Das ganze Organ wird vom Stiel bis in seine feinsten Verzweigungen von ungeheuer entwickelten Bluträumen entgenommen, die zum Theil noch mit geronnenem Blute dicht gefüllt sind (Fig. 3 s). Diese Bluträume sind begrenzt von mächtigen Balken (Fig. 3 m), welche überwiegend in der Längsrichtung der einzelnen Zotten angeordnet sind, so dass die Bluträume polygonale langegezogene Maschen bilden, welche unter einander in offener Verbindung stehen * * *." These large blood-spaces he considers a part of the venous system. Then he proceeds to say "dass wir hier ein typische cavernöses Gewebe vor uns haben" and "dass ein Organ in dem wir cavernöses Gewebe in überwiegender Menge vertreten finden auch erektil sein muss." Thus, according to BROCK, the whole inside of the organ is, so to speak, nothing but large anastomosing blood spaces and under the circumstance the organ itself must be erectile. From the description which I have given above, it will not be surprising that I find myself unable to accept these statements of BROCK'S. It is true that in some longitudinal sections through such parts as are given in Fig. 16 where numerous branches of veins and of lymph-cavities are present we often meet with a picture which reminds us of Fig. 3 of BROCK'S paper, but if we trace the so-called "Bluträume"

and "Balken" in serial sections, we soon discover that the former are nothing but the swollen dendritic and not anastomosing branches of blood-vessels and that the latter are not bars but tissues separating the bloodvessels. The erectile nature of the organ seems therefore highly doubtful. From the description which I have given in the preceding pages it seems to me evident that the appendage in question is *a highly developed gland.* The enormous surface-extension of the organ and the unusual development of the venous system may be taken as the indications of its importance and activity. Its presence in both sexes and its early development must be taken into account in any explanation of its physiological significance. We are for the present obliged to leave it to future research to determine in what period of life or on what occasions it is actually functional and what service it then renders.

Explanation of Plate XXXVI.

Abbreviations: *A*, arteries; *cap.*, capillaries; *Depr.*, pits; *Gl.*, glandular cells; *Gob.*, Goblet cells; *Sl.*, slime cells; *u. g. p.*, urogenital papilla; *V*, veins.

Fig. 1. General view of the dendritic appendage and its adjacent parts. Nat. size.

Fig. 2. Represents the anus, the urogenital papilla, and the depression of the body into which the dendritic appendage is received, together with the stalk of the appendage cut at its base, thus exposing the collar and the central pit (fissure) at the bottom of the depression. × 2.7.

Fig. 3–5. Represent respectively the dorso-ventral, the ventral, and the dorsal, views of the dendritic appendage. The stalk is cut off at about its middle. × 2.7.

Fig. 6. Surface view of a lobe or leaf of the appendage under a magnifying lens Black spots represent pits. × 9.

Fig. 7. Surface view of a part of a lobe. Red lines represent capillaries and black areas pits. Highly magnified.

Fig. 8. Young *Plotosus*. Its dendritic appendage is represented in Fig. 9. Nat. size.

Fig. 9. Dendritic appendage of a yound *Plotosus* represented in Fig. 8. Fine black spots on the surface represent pigment. × 18.

Fig. 10. Represents diagrammatically the origin of blood-vessels (red lines) and of nerves (black line) which enter the dendritic appendage. An artery and a nerve on the opposite side are not represented.

Fig. 11. Surface view of a lobe, seen under a magnifying lens by transmitted light, showing blood-vessels.

Fig. 12. Transverse section of the stalk of the very young dendritic appendage shown in Fig. 9. The peripheral light area represents the cortex, the red rings arteries and the vein, and the light black ring the lymph-cavity.

Fig. 13–16. Selected transverse sections of the stalk. Fig. 13 is taken from that portion of the stalk where it is adherent to the urogenital papilla and Fig. 16 from the level where the appendage is bifurcated. There are 114 sections between Fig. 13 and 14, 39 between Figs. 14 and 15, and 55 between Figs. 15 and 16. Red and light black rings represent the same structures as in Fig. 12. Black dots represent nerve-bundles. Zeiss 2.aa.

Fig. 17. Transverse section of lobes through the line pointed to by the letter *a* in Fig. 3. Zeiss 2.aa.

Fig. 18. Longitudinal section of the basal portion of the stalk showing the connection between the dendritic appendage and the body proper. This section is somewhat to one side of the axis of the stalk, and in this particular case the collar-shaped skin-fold is rather rudimentary, though it is more marked in the median sections than in this. The upper end of the figure is directed towards the tail. Zeiss 2.aa.

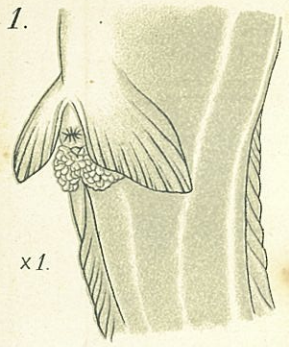
Fig. 19. Longitudinal section of the stalk showing the sudden replacement of the goblet cells by the glandular cells. Zeiss 2.BB.

Fig. 20. Transverse section of a lobe of the dendritic appendage in a fully grown individual. Zeiss 2.DD.

Fig. 21. Highly magnified view of a group of glandular cells. Zeiss 2.F.

Fig. 22. Highly magnified view of a part of transverse section of a lobe belonging to the dendritic appendage given in Fig. 9. Zeiss 2.F.

1.



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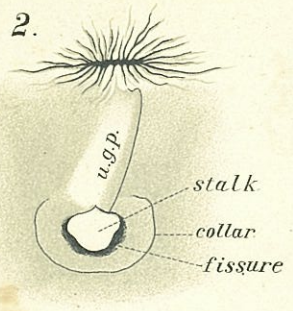

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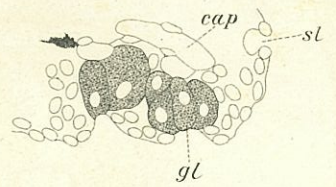
22. 2.F.



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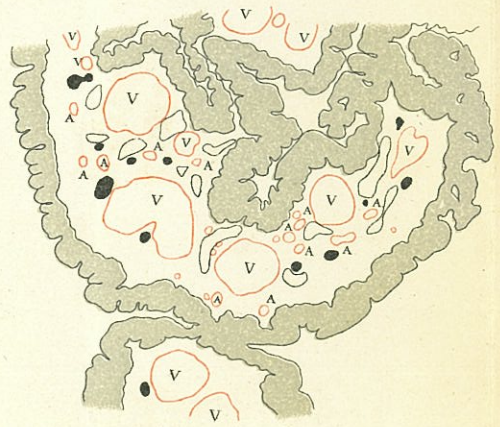

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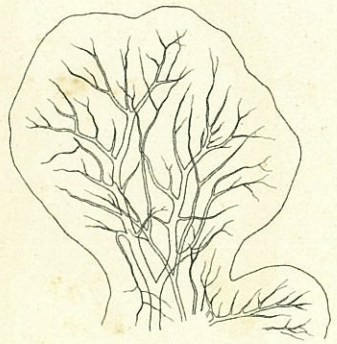

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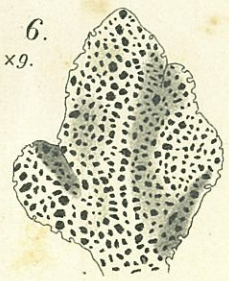

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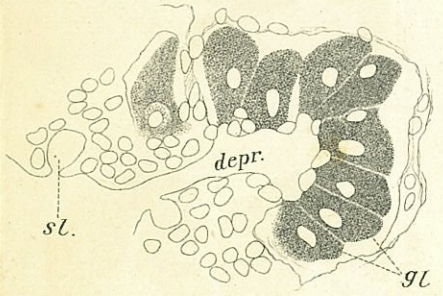
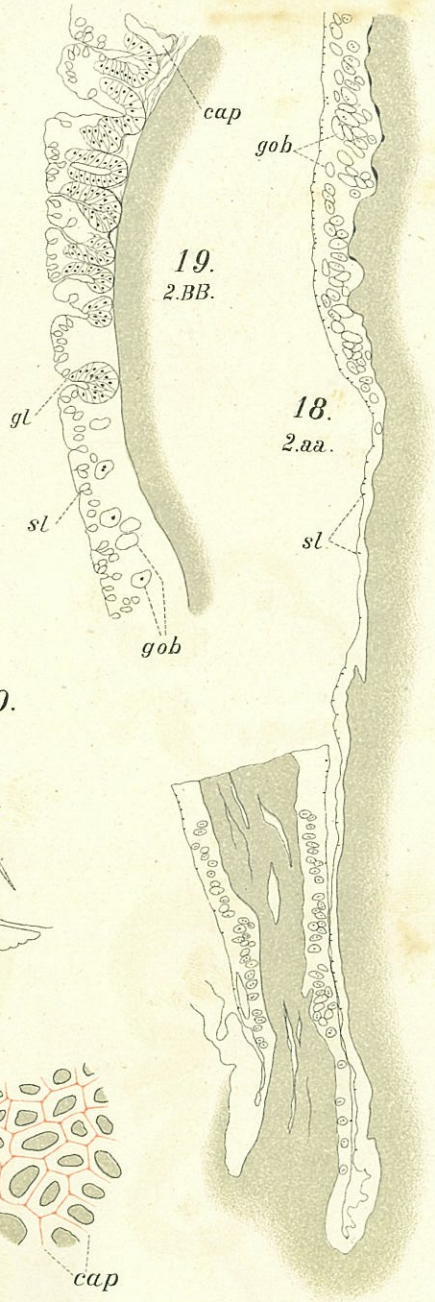


19. 2.BB.

18. 2.aa.



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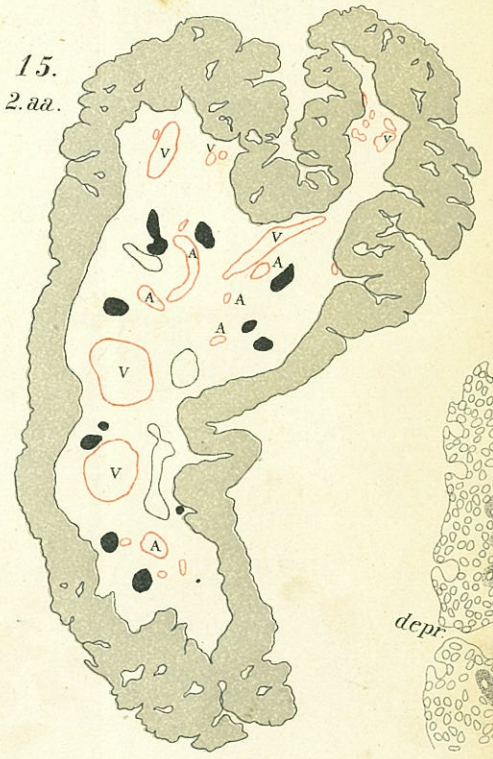

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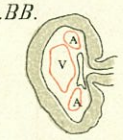

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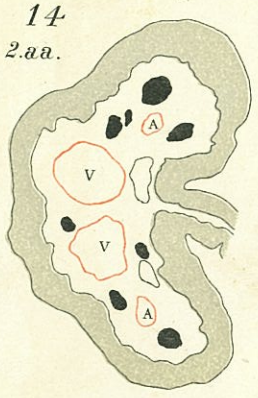
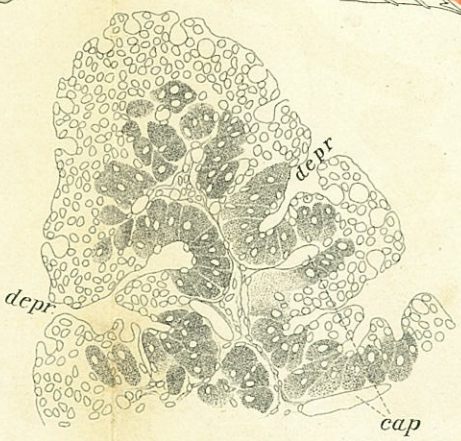


9. x18.

20. 2.DD.



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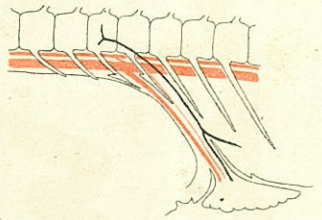

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