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# STRUCTURE AND FUNCTION OF THE FEMALE GENITAL SYSTEM OF *BOMBYX MORI* WITH SPECIAL REFERENCE TO THE MECHANISM OF FERTILIZATION.<sup>1)</sup>

By

Seinosuke Ômura

## I. Introduction

Since the announcement of DZIERZON's theory on the mechanism of sex-determination in bees the structure and function of the female genital system of insects has attracted the attention of entomologists. In Lepidoptera, in spite of being preceded by the wide anatomical studies of STITZ (1902) and others on the female genital system, the experimental studies made by KLATT (1920), MICHAEL (1923) etc. on mating, fertilization and oviposition are far from being errorless, suggesting a lack of fundamental studies. Unexpected difficulty is often encountered in the study of genetics and in practical silkworm culture, unless a fundamental knowledge of the structure and physiology of the female genital organs is first obtained. The present work is directed to a detailed analysis of the female genital system of *Bombyx mori* to trace the whole history of the spermatozoa ejaculated into the bursa copulatrix with special reference to the mechanism of fertilization.

The materials were obtained mostly from the Sericultural Laboratory of our University and the necessary experiments were carried out at the room temperature of 25°C unless otherwise stated. For the histological study CARNOY's, CHAMPY's and PETRUNKEWITSCH's fluids were used as the fixatives according to the various purposes and the staining was done with DELAFIELD's haematoxylin or HEIDENHAIN's ironalumhaematoxylin.

The present work was carried out under the direction of Professor

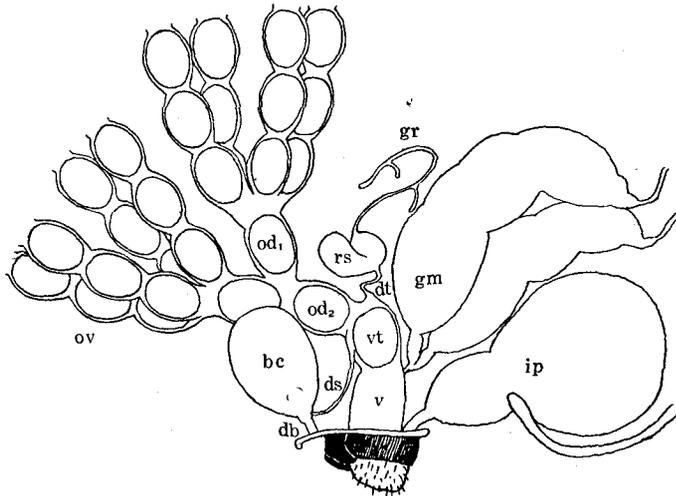
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1) The general matter of the present work was delivered at the sixth Nippon Nôgakukai congress (ÔMURA, 1935).

E. KAWAGUCHI to whom the writer wishes to express his sincere gratitude for invaluable suggestions and criticisms. He is also indebted to Professor T. INUKAI who kindly looked over the manuscript before publication and offered many criticisms. Thanks are also due to Professor K. NAGAI who directed him in formulating the new nomenclature.

## II. Structure of the organs

1. *General construction.* The nomenclatures hitherto employed by various authors for the female reproductive system are in good agreement contrary to the case for the male organs. Text-Fig. 1 shows



Text-Fig. 1. A schematic figure of the reproductive system of a mated female just before oviposition. bc...bursa copulatrix with a spermatophora, db...ductus bursae, ds...ductus seminalis, dt...ductus tortuosus, gr...glandula receptaculi, gm...glandula mucosa, ip...intestinus posterior, od<sub>1</sub>...oviductus geminus, od<sub>2</sub>...oviductus communis, ov...tubus ovarius, rs...receptaculum seminis filled with spermatozoa, v...vagina without egg, vt...vestibulum with an egg.

schematically the reproductive system of the female just after the mating and before the oviposition. In the figure may be seen the paired ovaries, each of which consists of four ovarian tubes, connecting with the oviductus gemini which are covered with a thin muscle layer and

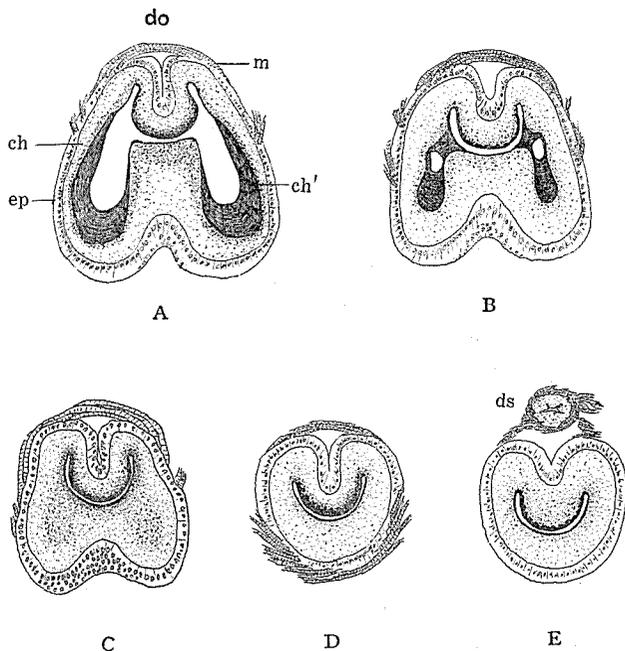
are usually of a length sufficient to contain one or two eggs. The oviductus gemini unite into a duct, the oviductus communis, which is of the same length as the oviductus geminis, covered with a more developed musculature and lined with chitin intima. Its caudal end opens at the cephalo-ventral side of the vestibulum at an obtuse angle. The vestibulum which has a muscle layer thicker than that of the oviductus communis and a cavity just large enough to contain a single egg opens directly into the vagina with a cavity having a capacity of two or three eggs. In the vaginal wall the muscle and the chitinous lining are highly developed.

In the sternite of the eighth abdominal segment, ventral to the orifice of the vagina, lies the orificium bursae, whence begins the ductus bursae leading to the bursa copulatrix. From the dorsal side of the neck of the bursa starts the ductus seminalis which opens into the vestibulum, at a point on the left side of it somewhat ventral, near the region of crossing of the vestibulum and oviductus. From the tip of the vestibulum starts the ductus tortuosus which coils and ends in the receptaculum seminis. The receptaculum seminis has a deep constriction near the center and appears to consist of two rooms, to one of which the glandula receptaculi belongs. The glandula mucosa opens on the dorsal side of the anterior portion of the vagina.

Besides the above described organs which are easily detectable macroscopically, there may be seen with the microscope the canaliculus fecundans in the ductus tortuosus and the torus vestibuli in the vestibulum (Text-Figs. 12-16).

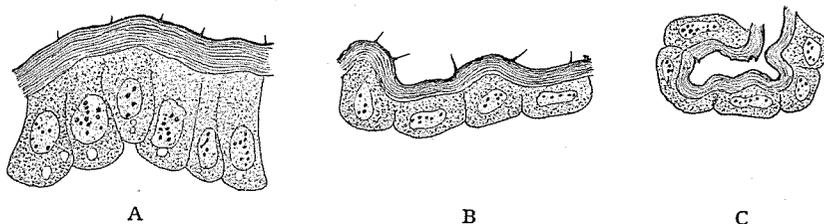
Except the ovaries and the oviductus gemini all organs described above are lined with chitin intima.

2. *Ductus bursae*. The ductus bursae is a stout chitinous duct which holds the penis in the course of copulation. Though it has a cavity large enough to contain a penis at mating, in the resting time the dorsal wall is folded down as is shown in Text-Fig. 2, assuming a horse-shoe shape with a narrow cavity being effected by muscles furnished on the duct (Text-Fig. 2). When stained with haematoxylin, there are found numerous minute granules in the outer layer of the chitin wall of the dorsal side (Fig. 3), and also setalike patterns in the chitin layer of the dorso-cephalic end (Fig. 2). On the cephalic end of the dorsal side a small portion of the epidermis is found transformed into a glandular appearance (Fig. 1), but it has not been determined yet whether this portion has secretory ability.



Text-Fig. 2. A series of cross sections of the ductus bursae of a non-mated female. A...most distal, E...most proximal, B, C, D...intermediate parts to the bursa copulatrix. ch...chitin layer, ch'...surface of the chitin wall which becomes narrow towards bursa copulatrix, do...dorsal side, ds...ductus seminalis, ep...epidermis, m...muscle. GARNOY, HEID.-haem.  $\times 60$ .

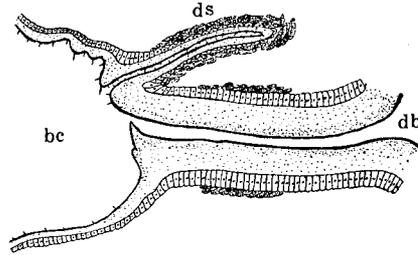
3. *Bursa copulatrix*. The bursa copulatrix is a sack of simple chitinous construction, the wall of which becomes thinner to the bottom (Text-Fig. 3). Denticules exist scattered all over the inner surface.



Text-Fig. 3. Wall of the bursa copulatrix. A...neck, B...middle, C...basal portion. CARNOY, HEID.-haem.  $\times 350$ .

4. *Ductus seminalis*. The ductus seminalis starts from a dorsal

point at the proximal end of the bursa copulatrix (Text-Fig. 4) and ends at the vestibulum (Fig. 5) in a very acute angle (Text-Fig. 1). The inside of the duct is composed of denticulated chitin, surrounded with a well developed musculature (Text-Figs. 10, 11). At the portion distal to the vestibulum the chitin intima is thicker and the cavity is smaller than at the proximal portion (Text-Figs. 10, 11).



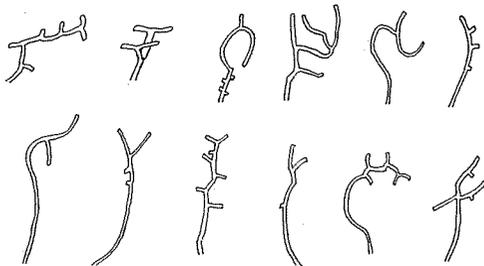
Text-Fig. 4. A longitudinal section of the neck part of the bursa copulatrix with the ductus bursae. Somewhat schematic. bc...bursa copulatrix, db...ductus bursae, ds...ductus seminalis. CARNOY, HEID.-haem.  $\times 50$ .

5. *Vestibulum, oviductus communis and vagina.* There is no structural difference among the oviductus communis, vestibulum and vagina. They are all simple tubes of chitin surrounded with a muscle layer. The vestibulum, the central point of the efferential system at which the oviductus communis, vagina, ductus seminalis and ductus tortuosus gather, is the portion where the fertilization—strictly speaking, the entering of the spermatozoa into the egg—occurs. At the inner surface of the tip of the vestibulum the torus vestibuli protrudes. It is composed of highly developed epidermis being lined with the chitin intima just like the proper part of the vestibulum. It is twisted and transforms into the ductus tortuosus taking a conical shape. (Figs. 9–13). When an egg is brought into the vestibulum the torus vestibuli is pressed by the egg, changing the original protruded position (Figs. 18–22).

6. *Ductus tortuosus and canaliculus fecundans.* The ductus tortuosus starts from the tip of the vestibulum penetrating through the centre of the torus vestibuli and making a spiral which is directed toward the receptaculum seminis. It is a small chitin tube surrounded with a well developed musculature similar to the ductus seminalis (Figs. 5, 8–14).

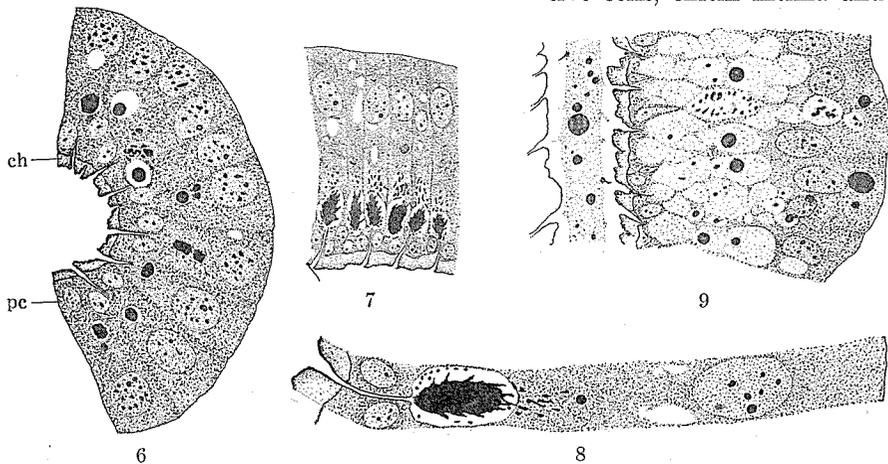
On the outer side of the spiral of the ductus tortuosus, the chitin wall extrudes throughout the proximal half, or more, to the vestibulum to make a minute canal, canaliculus fecundans (Text-Figs. 13–16, Figs. 6–15), which can be detected by vivisection through the muscle layer (Fig. 6). The cross section of the canaliculus shows a thin intima on the inner side (Figs. 8, 14, 15). It is however notable that at the

portion most distal to the vestibulum, the intima appears as a surface layer of the chitin wall (Fig. 8). The diameter of the canaliculus is far smaller at the part proximal to the vestibulum as compared with that at the distal (Figs. 6-8). The canaliculus coils also in the same manner as the ductus (Figs. 6, 7) and ends in the basal part of the torus vestibuli inflowing gradually into its cavity (Text-Figs. 13-16, Figs. 18-22). In its course through the torus vestibuli, the canaliculus spirals remarkably as the torus does, occupying a position in a corner of the cavity proper of the torus (Figs. 9-11). The other end is also indistinct, its cavity uniting gradually with the cavity proper of the ductus tortuosus (Figs. 6-8).



Text-Fig. 5. Forms of the glandula receptaculi of a dozen individuals chosen at random.

7. *Receptaculum seminis*. The receptaculum seminis is a denticulated chitinous sac consisting of two chambers which communicate freely each other (Fig. 1). To one of the chambers belongs an accessory gland, which consists of glandular cells, protective cells, chitin intima and



Text-figs. 6-9. Glandula receptaculi. 6...of a pupa 4 days before emergence (cross section). 7, 8...of a pupa 2 days before emergence, no secretion is emerged into the cavity (longitudinal sections). 9...of a moth just emerged (longitudinal section). ch...chitin intima. pc...protective cell. Champy, Heid.—haem. 6, 7, 9... $\times 350$ . 8... $\times 700$ .

canaliculi passing through the intima as characteristic to the ectodermal gland (Text-Figs. 6-9). The gland has no uniformity in shape. For example, twelve individuals dissected in the present work showed each a different form (Text-Fig. 5). The secretion begins 4 days before the emergence of the imago and reaches the maximum in the oldest pupal stage or youngest imaginal stage (Text-Figs. 6-9). The secretion consists of two sorts of matter, one is haematoxylinophile and the other is haematoxylinophobe (Text-Fig. 9).

### III. Mechanism of the progress of spermatozoa from the bursa copulatrix to the receptaculum seminis

1. *The translocating substance from the bursa copulatrix to the receptaculum seminis.* The spermatophora ejected by a male at the first ejaculation occupies the greater part of the cavity of the bursa copulatrix of the female its orifice occurring near the entrance of the ductus seminalis, not in the ductus seminalis. Immediately after the ejaculation, numerous spermatozoa are found in the spermatophora with the seminal fluid but none in the receptaculum seminis. However, several hours after, a great majority of the spermatozoa disappear from the spermatophora, while most of the seminal fluid is left. At this time the receptaculum seminis is found filled with spermatozoa which crowd in an extremely compact mass seemingly mixing with little of the seminal fluid (Fig. 17). Some spermatozoa are found also in a compact mass one or two hours after ejaculation in the ductus seminalis and ductus tortuosus (Text-Fig. 10, Fig. 14). This indicates that the translocation of the spermatozoa from the bursa copulatrix to the receptaculum seminis is carried out without being accompanied with the seminal fluid in the spermatophora commencing after the finish of the ejaculation.

2. *The progress of the spermatozoa.* The ejaculated spermatozoa which were moving gently when first emitted become vigorously active in about one hour after ejaculation, and sperm-bundles into which many of the spermatozoa have been united disappear one by one being separated into individual ones. It is sure that the opening of the spermatophora is in communication with the ductus seminalis by a trace of the semen which has escaped from the opening of the spermatophora. Therefore in reference to the fact that the seminal fluid does not follow the spermatozoa to the receptaculum seminis but remains in the spermatophora, it is quite sure that the active spermatozoa in the

spermatophora progress into the ductus seminalis by their own movement, without the assistance of any pumping or sucking action by any efferential organ (cf. Fig. 16).

Now, the manner by which the spermatozoa in the spermatophora enter into the ductus seminalis is to be considered. Supposing that they move in all directions within the spermatophora and flow into the ductus seminalis rather by chance, they may consume much time to get into the ductus seminalis and many of them may remain in the spermatophora being unable to enter into the ductus. But, in reality, a great majority of the spermatozoa in the spermatophora are found to have translocated to the receptaculum seminis some five hours after the ejaculation. Therefore, the conclusion follows that they choose their course of progression in some manner—possibly being induced by some chemical or mechanical factors.

In this connection, as the possible inductor of a chemical nature which might cause the chemotaxis of the spermatozoa, some fluid locating near the orifice of the spermatophora has been examined. There are the pellucidan and lactean fluids from the male, which have been proved in the previous paper to have nothing to do with the fertilization (cf. ÔMURA 1938). In addition to these a fluid from the female side is brought there, namely the fluid found in the bursa copulatrix of non-mated female having similar appearance to that in the receptaculum seminis (Fig. 4). In an old non-mated female, sometimes the bursa copulatrix is found swollen to some degree with this fluid. Whatever it may be, however, there is little reason to consider it as the inductor of the chemotaxis of the spermatozoa in the spermatophora.<sup>1)</sup> The concentration of the substance which causes the positive chemotaxis of the spermatozoa must be higher on the side of the destination of the progression than at the starting point. In the present case, such a condition of the concentration seems not to occur, for the ductus seminalis, which is the one which may carry down some fluid to the spermatophora, performs a peristaltic movement towards the vestibulum while the progression of the spermatozoa takes place. (See following (p. 119)).

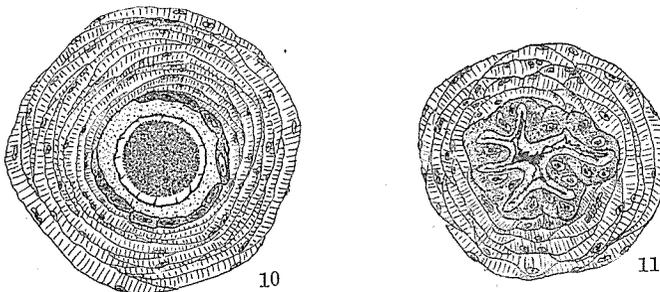
Then we turn to the mechanical factor which may induce the spermatozoa into the ductus seminalis. Microscopical observation on

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1) In his preliminary report (1935) the present writer stated that it might be inferred to originate from the glandula receptaculi and to play the rôle of the inductor. That opinion is not correct.

the bursae copulatricae vivisectioned out from mated females with the basal part of the ductus seminalis, sometimes shows a rhythmical forward and backward movement of a fluid near the entrance of the ductus seminalis. In this flow, there are found active spermatozoa in the same movement as the flow keeping their tail parallel with the direction of the flow. This movement of the fluid is in harmony with a regular periodical centripetal movement of a part of the wall of the bursa copulatrix adjoining the ductus seminalis, caused evidently by the sphincteral movement of muscles on the ductus seminalis. This fact suggests the mechanism of the entry of the spermatozoa into the ductus seminalis: the sphincteral movement of the ductus seminalis will cause the fluid, which occupies the part adjacent to the entrance of the ductus seminalis, to make a flow which will extend to some inner part of the spermatophora, and the spermatozoa which come within the range of the influence of this flow will begin their progress to the ductus seminalis by rheotaxis. Thus the great majority of them can transfer to the ductus seminalis in a few hours.

The progress of the spermatozoa thus entered into the ductus seminalis is illustrated in Text-Figs. 10 and 11, both of which are cross

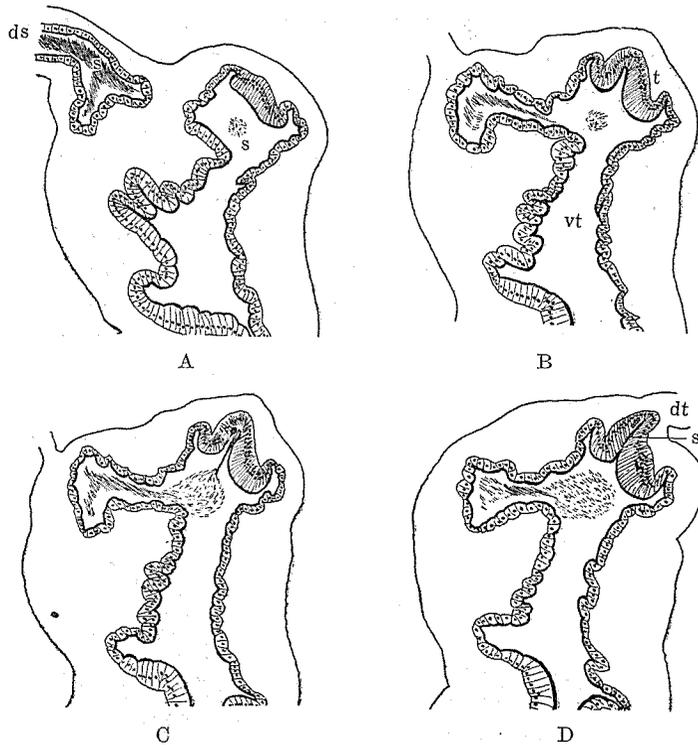


Text-figs. 10, 11. Cross sections of the ductus seminalis of a female 2 hours after a 60 minutes mating. 10...Proximal portion to the bursa copulatrix, containing a large quantity of spermatozoa. 11...middle portion containing a small quantity of spermatozoa. PETRUNKEWITSCH, DEL.-haem.  $\times 150$ .

sections of the ductus seminalis of one and the same individual two hours after a 60 minutes mating; in Fig. 11 there are found a few spermatozoa while in Fig. 10 there are many. This indicates that the spermatozoa are carried up through the duct by the peristaltic movement of the duct.

The progression of the spermatozoa in the vestibulum is illustrated

in Text-Fig. 12 and Figs. 12 and 13. They progress along the wall of the left side of the vestibulum through the shortest way to the torus vestibuli. When they reach a point under the torus vestibuli, they strangely appear as if they were about to be sucked up into the cavity



Text-Fig. 12. A series of sections of the vestibulum of a female 2 hours after a 60 minutes mating. ds...ductus seminalis, dt...ductus tortuosus, s...spermatozoa, t...torus vestibuli, v...vagina, vt...vestibulum. C and D are the same as Figs. 12 and 13 in Plate II respectively. PETRUNKEWITSCH, DEL.-haem.  $\times 35$ .

of the torus vestibuli by some power (Text-Fig. 12 C, Fig. 12). As only a few spermatozoa migrate out from their course into the cavity of the vestibulum proper despite the lack of a special canal for them, and no interruption is found in their progression, the spermatozoa seem to progress there in a different manner from that in the ductus seminalis. They must progress there through a definite course being allured by some inductor. However, in the present work no decisive explanation could be found for this matter.

The progression in the ductus tortuosus seems to occur in a similar manner as in the ductus seminalis, though it was not so finely demonstrated as in the latter. The spermatozoa always take their course in the cavity proper of the ductus tortuosus and never in the canaliculus fecundans (Fig. 14).

By vivisection, the spermatozoa arrived in the receptaculum seminis are visible *in situ* through the wall of it. When there were only a few spermatozoa, they were found to move about in both chambers of it and some of them were seen to go up to the head of the accessory gland. Then they seem to accumulate gradually into a so compact mass in both chambers that no movement is allowed them (Fig. 17). In the receptaculum seminis at two to five hours after the ejaculation, when most of spermatozoa have been translocated there, moving spermatozoa are visible only on the periphery of the mass.

The spermatozoa first arrived in the receptaculum seminis, though they are few in number, can be detected by vivisection without difficulty, as they are moving about near the entrance of the ductus tortuosus. Therefore the speed of the progression of the spermatozoa from the bursa copulatrix to the receptaculum seminis can be measured by observing the time of their arrival at the destination. The receptaculum seminis of individuals which were mated for 40 minutes were examined after various intervals of time (Table 1). Twenty minutes after the mating no spermatozoa were found in the receptaculum seminis of any of the moths while 30 minutes after more than half the number of moths had some spermatozoa there. After 40 minutes they were present in the receptaculum seminis of all moths.

TABLE 1. Time consumed for the progression of spermatozoa from the bursa copulatrix to the receptaculum seminis.

Number of individuals	Duration of mating (minutes)	Time from mating to examination (minutes)	Number of indivs. which have received sperm. in their recep. sem.	Number of indivs. which have received no sperm. in their recep. sem.
5	40	20	0	5
5	40	30	4	1
5	40	40	5	0

Ejaculation is usually accomplished by some 40 minutes mating (cf. ÔMURA, 1938) and the progression of the spermatozoa does not com-

mence until ejaculation is finished. Therefore, supposing that the progression is commenced immediately after the separation of the mating, the time used for the journey of a spermatozoon from the bursa copulatrix to the receptaculum seminis may be estimated roughly from the table, to be about 30 minutes.

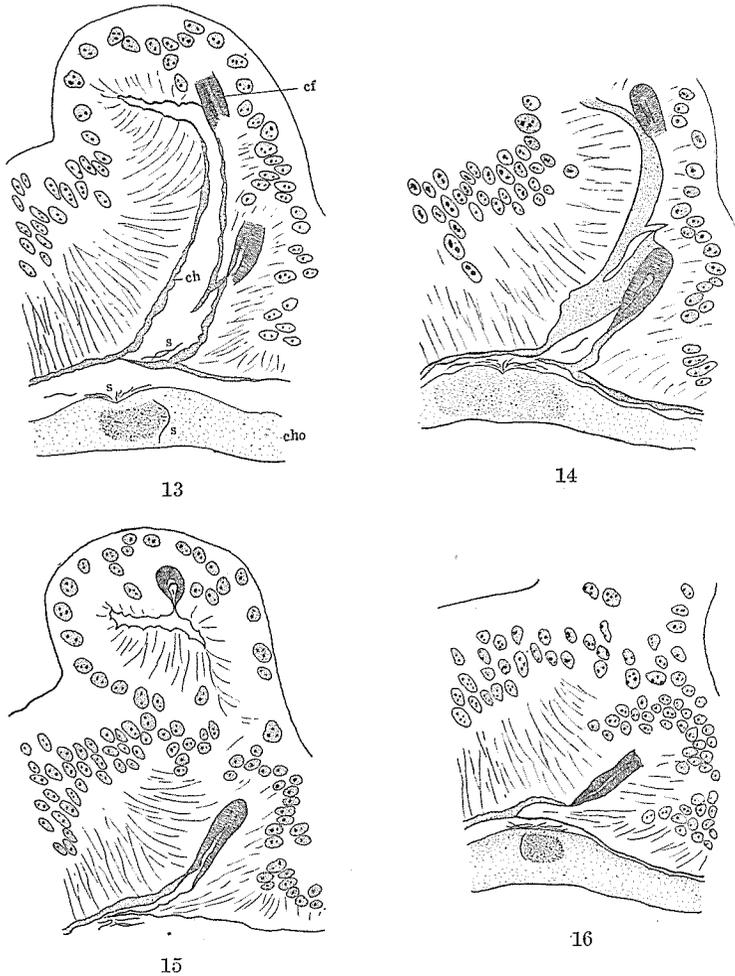
#### IV. Process of entering of the spermatozoa into the egg

The ductus tortuosus of the females in the process of oviposition were examined by sectioned preparations 3 hours after a 60 minutes mating. In the cavity proper of the ductus no spermatozoa were found while some were in the canaliculus fecundans (Figs. 23-25). This fact shows that the spermatozoa ascend to the receptaculum seminis through the cavity proper but they flow down to the vestibulum through the canaliculus fecundans. As the wall of the canaliculus fecundans is too thick in proportion to its diameter to assume a peristaltic movement, presumably the spermatozoa pass down to the vestibulum by their own movement.

By cutting the egg in the vestibulum of an ovipositing mated female *in situ* with the wall of the organ, fortunately the writer could obtain one series of sections which shows the process of the entrance of the spermatozoa into the egg (Text-Figs. 13-16, Figs. 18-22). The egg is embraced by the torus vestibuli setting its micropyle close to the outlet of the canaliculus fecundans. There are found some spermatozoa flowing down the canaliculus fecundans, ten or more individual ones lying near the micropyle, several with heads just inserted into the micropyle and a few passing through it.

The precise mechanism by which the spermatozoa approach the micropyle and pass through it has not as yet been ascertained, but the fact that the region surrounding the micropyle is of a different nature from chorion proper (Text-Figs. 13, 14, 16, Figs. 19, 20, 22) seems very suggestive.

In order to learn the speed with which the spermatozoa enter into the egg, the speed of oviposition at a temperature of 25°C in a dark thermostat was observed, employing a hybrid between a Japanese and a Chinese race as the material. The number of fertilized eggs laid by females after a 30 minutes mating was counted every 30 minutes commencing at 1 p.m. In every moth the period of the highest speed of oviposition occurs between 2 and 3 hours after mating. The number of fertilized eggs laid by every individual during the period and the



Text-Figs. 13-16. A series of longitudinal sections of the torus vestibuli of an ovipositing female. Same as Figs. 19-22 in Plate III respectively. ch...chitin intima, cho...chorion of egg, cf...canaliculus fecundans, s...spermatozoa. There are found one or two spermatozoa in the canaliculus fecundans of upper side of Text-Figs. 13, 14 and 15, and one spermatozoon passing through the micropyle in Text-Figs. 13 and 16. PETRUNKEWITSCH, DEL-haem.  $\times 220$ .

speed of the oviposition are to be seen in Table 2.

The table shows 269 as the average, ranging from 175 to 340 eggs per period. The time used for laying per egg is 6.7 seconds on the

TABLE 2. The highest number of fertilized eggs laid within a period (every 30 minutes)

Material: a hybrid between a Japanese and a Chinese race.

Number of individual	1	2	3	4	5	6	average
Maximum number of fert. eggs laid in a period	250	340	324	270	255	175	269
Time used for laying per egg (seconds)	7.2	5.3	5.6	6.7	7.1	10.0	6.7

average, the shortest being 5.3 seconds in individual No. 2. As a moth does not continue oviposition at a constant speed, the time used for laying one fertilized egg will be less than 5.3 seconds in an extremely rapid case. The time used for laying per egg may be regarded as equal to the time within which the egg passes into the vestibulum from the oviduct and receives spermatozoa. Consequently it may be stated that spermatozoa are able to enter into egg within 5 seconds in an extremely rapid case.

## V. Discussion

The ring muscle of the ductus bursae of *Bombyx mori* is presumably of the same nature as the one found by STITZ (1902) on the hals of bursa copulatrix of some Microlepidoptera. Its function may be mechanical to tighten the ductus bursae and at the same time it may play some rôle in mating. The peculiar figures described as appearing in the chitin layer of the ductus bursae and the gland-like cells in the cephalic portion of its epidermis have never been discussed and their significance is yet unknown.

The musculature on the bursa copulatrix in Lymantridae described by KLATT (1920) is not found in *Bombyx mori*. The laminae dentatae found by HAGEN (1882) and PETERSEN (1907) on the bursa copulatrix in some Lepidoptera as well as the bulla seminalis found by STITZ (1902) and PETERSEN (1907) on the ductus seminalis in some Lepidoptera are also not found in *Bombyx mori*.

The organ, so-called "Epitelwulst" after STITZ (1902), in the vestibulum in some Microlepidoptera or the "Papille" found by KLATT (1920) in Lymantridae corresponds probably to the present writer's torus vestibuli, though their description of its form and location does not exactly coincide with the present material. STITZ (1902) assumed it to have secretory ability, but so far as the present research goes there is

no evidence to support his view. Concerning the shape of the glandula receptaculi the present observations support CORNALIA's description (1855).

The canaliculus fecundans which was discovered by the present writer in *Bombyx mori* in 1935 (ÔMURA, 1935) was found also in *Antheraea yamamai*, *Antheraea pernyi* and some other Lepidopteran insects. Perhaps this organ is one of the general characters of Lepidoptera. However a question still remains whether a homologous organ exists in insects belonging to other orders.

The progression of the spermatozoa in the female genital system has been treated only fragmentally. As to the starting of the spermatozoa from the spermatophora, an opinion was offered by HAGEN (1882) that it begins after the break of the spermatophora by the laminae dentatae, while PETERSEN (1907) said that spermatozoa are pushed partly by the function of the muscle of the bursa copulatrix and partly by that of the "Bauchmuscultur." KLATT (1920) was of the opinion that spermatozoa break the spermatophora by themselves, making an orifice, and are pushed into the ductus seminalis by the coagulating contraction of the spermatophora as well as by the power of the muscle covering the bursa copulatrix, while MICHAEL (1923) regarded the starting as caused by a negative chemotaxis which originated from the substance constituting the spermatophora. However, none of these opinions was based on experimental researches, and therefore, it is of little value to discuss them.

KLATT (1920) was the only one to give an opinion on the progression of the spermatozoa in the vestibulum in Lymantridae saying that the ductus seminalis and ductus tortuosus are connected directly being pressed by some muscle. But the present study proves that this view is rather imaginary.

On the process of flowing down of the spermatozoa from the receptaculum seminis to the vestibulum, there are many works in Hymenoptera (e.g., BRESSLAU (1906), ADAM (1913), etc.) finding sphincters and a chitin plate in the ductus tortuosus as regulators of the flow of the spermatozoa. However, in Lepidoptera, no special attention has been paid to this point, and the ductus tortuosus is considered by many simply as a single tube through which the spermatozoa advance mechanically toward the receptaculum seminis and flow down to the vestibulum. At present the discovery of the canaliculus fecundans and its probable function proves that the manner of the flowing down of

the spermatozoa in Lepidoptera differs from that of Hymenoptera. Considering the passing down of the long-tailed spermatozoa of *Bombyx mori* through an extremely minute canaliculus, the spiral construction of it seems to have something to do with this point.

ADAM (1913) inferred that the "Ventilwulst," found in Hymenoptera by BRESSLAU (1906) on the ventral side of the vestibulum opposite the opening of the ductus tortuosus, acts as the holder of the egg during the time of entering of the spermatozoa into the egg and named it "Eileiterklappe." Though ADAM did not observe the behaviour of the spermatozoa in the vestibulum, his inference on the function of the "Ventilwulst" is similar to the finding of the present writer on the torus vestibuli.

In insects, the phenomenon of the entering of the spermatozoa into the egg was already observed by MEISSNER (1855) in *Musca vomitria* egg taken out from the vagina but the complexity and the delicacy of the process of the entering have been described first in the present work. The process of fertilization occurring in such loci as the ovarian tube and oviduct other than the vestibulum as informed by MATUNAGA (1934) is out of the question.

The significance of the complex relation of the situation of all organs around the vestibulum has become notably clear since the course of the spermatozoa was traced out.

## VI. Résumé

The female genital system of *Bombyx mori* was studied macroscopically as well as microscopically resulting in the discovery of the canaliculus fecundans and in the elucidation of the significance of the complex location of the organs and in the revelation of the physiological function of all organs except the glandula receptaculi.

The progression of the spermatozoa from the bursa copulatrix to the receptaculum seminis, and from the receptaculum seminis to the vestibulum was traced.

The process of entering of the spermatozoa into the egg was observed.

The speed of the progression of spermatozoa from the bursa copulatrix to the receptaculum seminis as well as the time used for the entering of spermatozoa into the egg was measured experimentally.

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## VIII. Explanation of Plates

## Plate I

- Fig. 1. Gland-like cells in the epidermis of the cephalic portion of the ductus bursae. Pupa, 3 days before emergence. CARNOY, HEID.-haem.  $\times 180$ .
- Fig. 2. Peculiar figure stained with haematoxylin in the chitin layer of the cephalic end of the ductus bursae. One day old moth. CARNOY, HEID.-haem.  $\times 360$ .
- Fig. 3. Granular substance stained with haematoxylin in the chitin layer of the dorsal side of the ductus bursae. One day old moth. CARNOY, HEID.-haem.  $\times 360$ .
- Fig. 4. Neck part of the bursa copulatrix near the entrance of the ductus seminalis, containing some substance. One day old non-mated moth. CHAMPY, HEID.-haem.  $\times 180$ .

- Fig. 5. Chitin intima of vestibulum, ductus seminalis(*ds*) and ductus tortuosus(*dt*). Muscles are dissolved out with NaOH solution. Unstained.  $\times 60$ .
- Fig. 6. Ductus tortuosus of one day old non-mated moth. The canaliculus fecundans is detectable through the muscle layer. Left side is proximal to the vestibulum. Vivisection, unstained.  $\times 180$ .
- Fig. 7. Chitin intima of the ductus tortuosus of moth, showing the thickness of the wall of the canaliculus fecundans. Left side is proximal to the vestibulum. Muscles are dissolved out with NaOH solution. Unstained.  $\times 360$ .
- Fig. 8. Ductus tortuosus of moth. Left side is proximal to the vestibulum. The canaliculus fecundans found at the center is the most distal portion to the vestibulum. PETRUNKEWITSCH, DEL.-haem.  $\times 180$ .

## Plate II

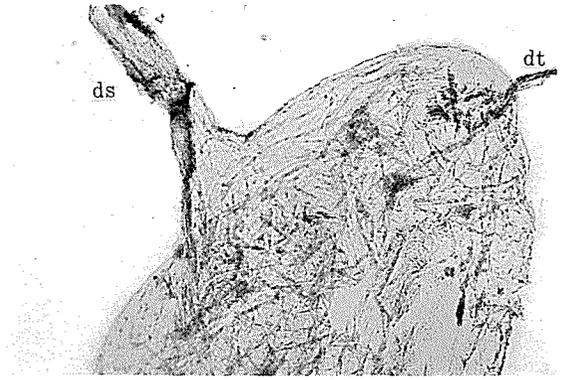
- Figs. 9-11. Continuous sections of torus vestibuli of a pupa 3 days before emergence, showing the situation of the canaliculus fecundans in the torus vestibuli. CARNOY, HEID.-haem.  $\times 80$ .
- Figs. 12, 13. Longitudinal sections of vestibulum of a moth 2 hours after a 60 minutes mating, showing the progression of the spermatozoa from the ductus seminalis to the ductus tortuosus. Same as Text-Fig. 12, C and D respectively. The matter found in the vestibulum besides the spermatozoa, which are shown clearly in Text-Fig. 12, is some substance generally found in the oviduct. PETRUNKEWITSCH, DEL.-haem.  $\times 80$ .
- Fig. 14. Cross section of the ductus tortuosus which is found in Fig. 12, showing a mass of spermatozoa in the cavity proper of the ductus tortuosus and none in the canaliculus fecundans.  $\times 480$ .
- Fig. 15. Cross section of upper part of the torus vestibuli of moth. PETRUNKEWITSCH, DEL.-haem.  $\times 350$ .
- Fig. 16. Longitudinal section of the entrance of the ductus seminalis, showing the transmission of the spermatozoa from spermatophora. Two hours after a 60 minutes mating. PETRUNKEWITSCH, DEL.-haem.  $\times 180$ .
- Fig. 17. Opening of the ductus tortuosus into the receptaculum seminis of an ovipositing female at 5 hours after a 60 minutes mating. CHAMPY, DEL.-haem.  $\times 180$ .

## Plate III

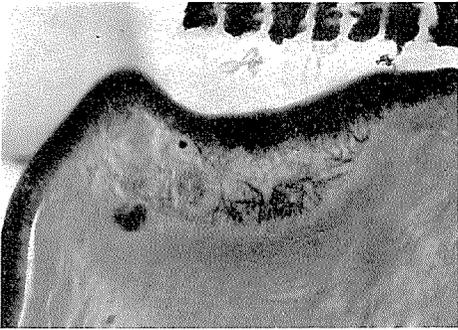
- Fig. 18-22. Continuous sections of torus vestibuli of an ovipositing female holding an egg. Showing the moment of entrance of spermatozoa into the micropyle. PETRUNKEWITSCH, DEL.-haem.  $\times 180$ .
- Figs. 23, 24. Canaliculus fecundans found in Figs. 18 and 19, containing spermatozoa flowing down to the vestibulum.  $\times 350$ .
- Fig. 25. Cross section of the ductus tortuosus of an ovipositing female, showing 3 spermatozoa in the canaliculus fecundans, but none in the cavity proper of the ductus tortuosus. CARNOY, DEL.-haem.  $\times 700$ .
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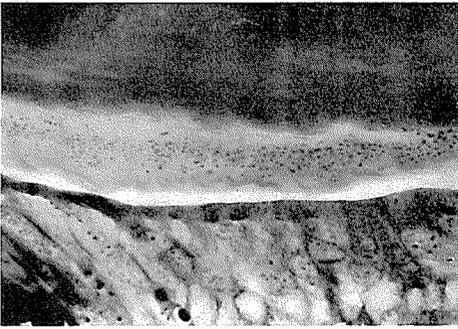
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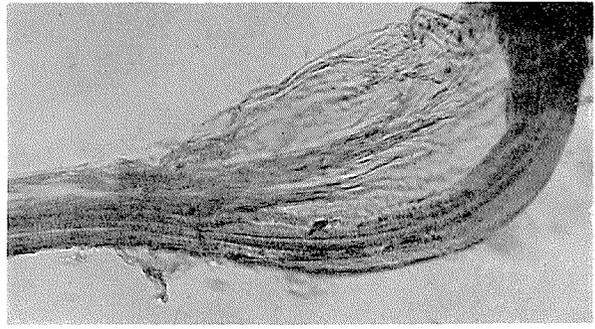
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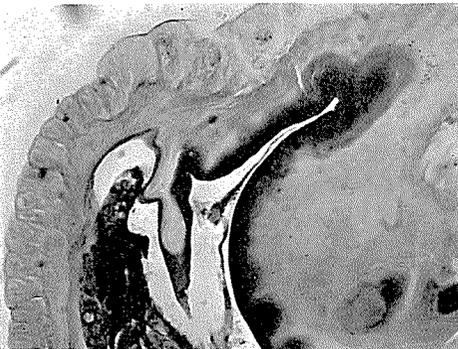
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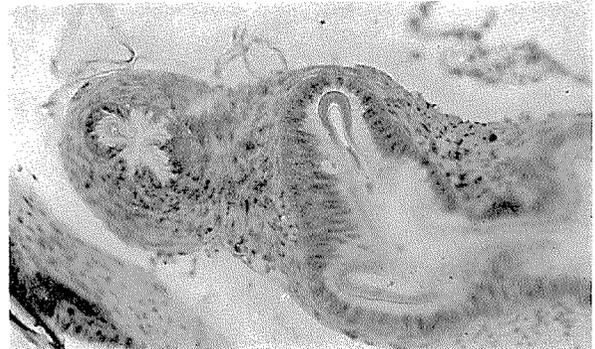
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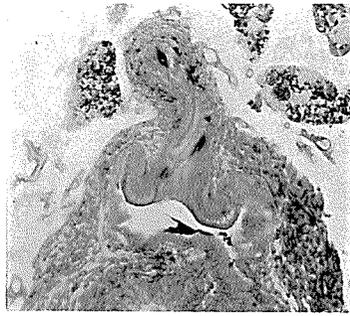
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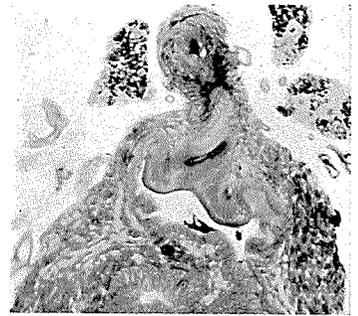
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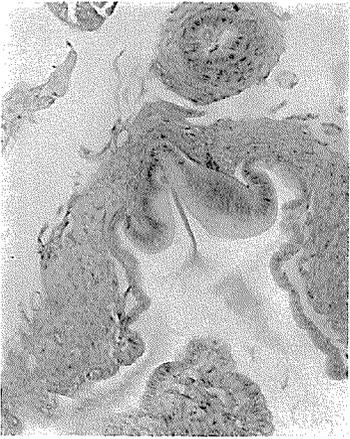
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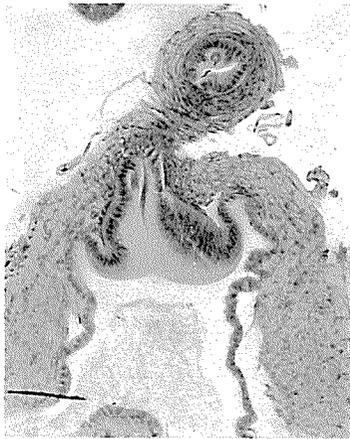
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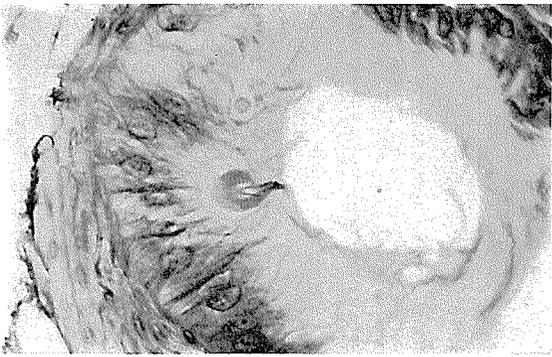
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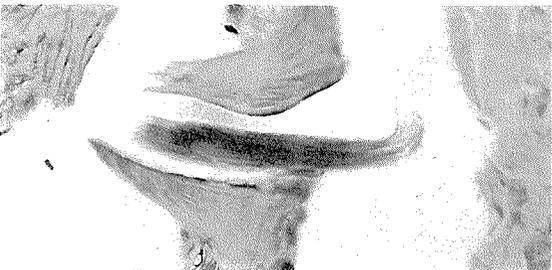
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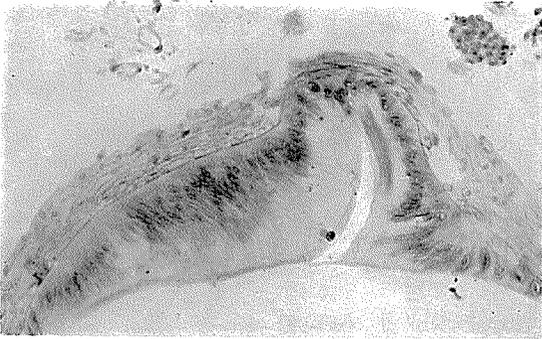
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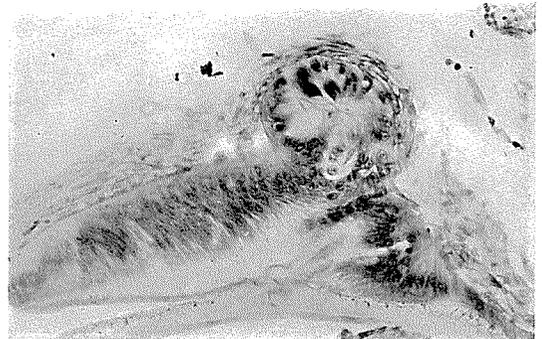
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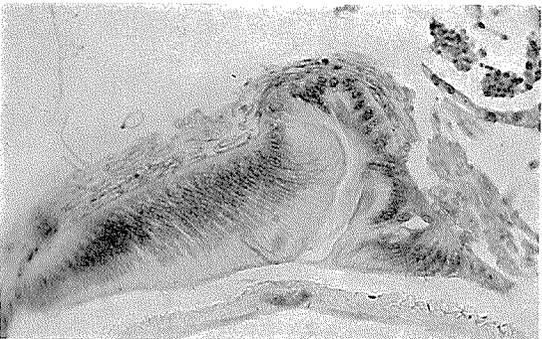
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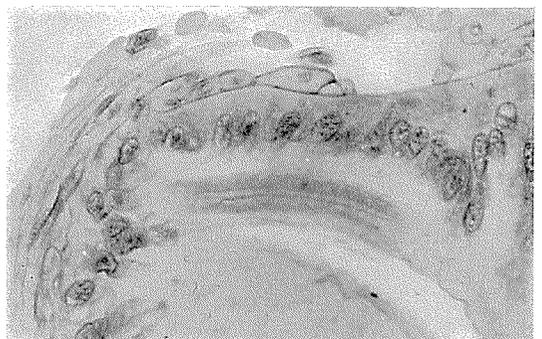
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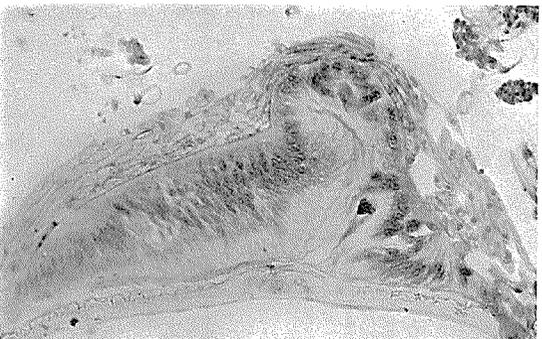
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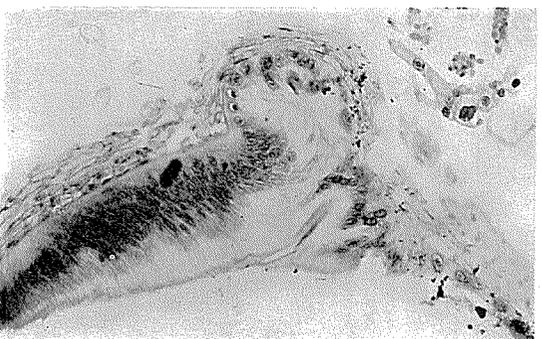
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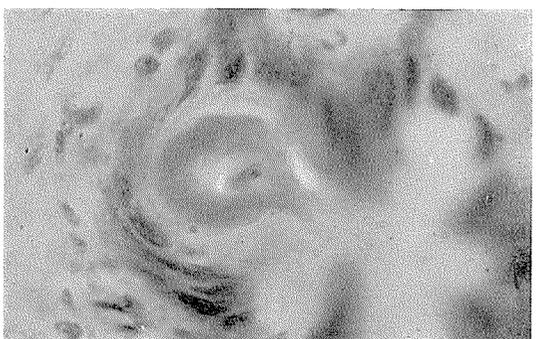
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