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# STUDIES ON THE REPRODUCTIVE SYSTEM OF THE MALE OF *BOMBYX MORI*

## II. Post-Testicular Organs and Post-Testicular Behaviour of the Spermatozoa<sup>1)</sup>

By

**Seinosuke Ômura**

### I. Introduction

Hitherto hundreds of contributions to the topographical anatomy of insects including the reproductive system have been made. However, apart from the pioneer work on the morphology of the male reproductive organs done by ESCHERLICH (1894) in Coleoptera, STITZ (1901) in Lepidoptera, etc., there have been few studies so far published on the detailed microscopical investigation which might be of primary importance in relation to the general morphology of insects. Thus the lack of fundamental knowledge of morphology has recently caused confusion even in the nomenclature of organs and therefore most entomologists who engage in morphological study have been compelled to use ambiguous anatomical terms of their own. Consequently not only in the morphology of each organ but also in the physiology many important problems have been left unsolved.

As to the male reproductive organs of insects matters have remained as obscure as ever in morphology as well as in physiology; particularly, little is known about the intermediate stage between the gametogenesis and the fertilization of the male germ cells. Although some studies have been directed by Japanese authors to the mating, ejaculation and fertilization of the silkworm from the physiological point of view, the essential problem has not yet been touched. Lately a contribution on the structure and physiological function of the testis has been made by the present writer (1936b). In this paper are presented further ob-

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1) The general matter of this paper was lectured at the Sapporo Nôringaku-Kai meeting for the year 1934 (ÔMURA, 1935a) and also at the sixth Nippon Nôgaku-Kai congress (ÔMURA, 1935b).

servations made on the post-testicular male reproductive organs, particular attention being directed to the processes of the transmission of spermatozoa from the testis to the female mating organs. In addition the nomenclature for the reproductive system in general is carefully examined.

As the material, pupae and imago of the silkworm, *Bombyx mori*, were used. Unless otherwise stated the culture as well as the experiments were carried on at a temperature of ca. 25°C. The development of *Bombyx mori* in this temperature has been described in the previous paper (cf. ÔMURA, 1936b).

The writer wishes to express his great indebtedness to Professor E. KAWAGUCHI who has directed the work giving invaluable suggestions and criticisms, and to Professor T. INUKAI who kindly read through the manuscript before publication and offered many criticisms. He is also indebted to Professor K. NAGAI, Professor S. KODAMA and Dr. S. IMAI, under whose guidance the new nomenclature has been drawn up. Thanks are also due to Dr. J. MACHIDA, of Tôkyô Imperial University, who has kindly lent literature indispensable to the work.

## II. General constitution

The testes are located on the dorsal side of the fifth abdominal segment and the other attached organs are located on the ventral side of the hind body, centering about the vesiculae seminales which lie on the ventral side of the seventh and eighth abdominal segments.

All of the representative nomenclatures hitherto employed for the male reproductive system of Lepidoptera are summarized for reference in Table 1.

The present writer has divided the male reproductive system into 9 parts as shown in Text-Fig. 1, using names as follows.

1. Testis(*t*) (includes ductuli efferentes testis)
2. Ductus deferens(*dd*) (includes ampulla ductus deferentis(*add*))
3. Glandula pellucida or glandula accessoria distalis(*gpl*)
4. Glandula lacteola or glandula accessoria proximalis(*gl*)
5. Vesicula seminalis(*vs*)
6. Glandula spermatophorae(*gs*)
7. Glandula alba(*ga*)
8. Glandula prostatica(*gp*)
9. Penis(*p*) (includes ductus ejaculatorius)

TABLE 1. Representative nomenclatures of male reproductive system of Lepidoptera

Author	Material	1 (t)	2 <sup>1)</sup>	2-3 (dd)	3 (add)	4 (vs)	5 (gp)	6 (gl)	7 (gsp)	8 (ga)	9 (gp)	10 (ms)	11 (3)	12 (dje)	13 (2)	14 (1)
CORNALIA(1855)	<i>Bombyx mori</i>	Testicolo	Tubo deferente circonvoluto	Condotto deferente	Allargamento genicolato del tubo deferente	Vesicolo seminale	—	—	—	—	—	—	—	—	—	—
							Ghiandola accessoria (5, 6)		Condotto ejaculatore (7, 8, 9)							Penis(13, 14)
TOYAMA and ISHIWATA(1896)	<i>Bombyx mori</i>	Testis	—	Vas deferens	—	Sperm vesicle	—	—	—	—	—	—	—	—	—	—
							Accessory gland (5, 6)		Ductus ejaculatorius (7, 8, 9)							Penis(13, 14)
STITZ(1901)	Micro-lepidoptera	Hoden	Vas deferens		Vesicula seminalis	Paariger Drüsenschlauch	—	—	Erster unpaarige Drüsenschlauch	Zweiter unpaarige Drüsenschlauch	Dritter unpaarige Drüsenschlauch	—	Ductus ejaculatorius	—	—	—
							Accessorischer Drüsenschlauch (5, 6)									Penis(13, 14)
ZANDER(1903)	Lepidoptera	—	—	—	—	—	—	—	—	—	—	—	—	Ductus ejaculatorius	—	—
									Vas deferens (7, 8, 9)					Penis(10, 11, 13, 14)		
IKEDA(1913) <sup>2)</sup>	<i>Bombyx mori</i>	Testis	—	Vas deferens	—	Vesicula seminalis	—	—	—	—	—	—	—	—	—	—
							Glandula mucosa (5, 6)		Ductus ejaculatorius (7, 8, 9, 10)			ampulla ductus ejaculatorii				Penis(13, 14)
ÔMURA	<i>Bombyx mori</i>	Testis	(Pars proprius)	Ductus deferens	Ampulla ductus deferentis	Vesicula seminalis	Glandula pellucida (Glandula accessoria distalis)	Glandula lacteola (Glandula accessoria proximalis)	Glandula spermato-phorae	Glandula alba	Glandula prostatica	Musculus sphincter penis	Radix penis	Ductus ejaculatorius	Crus penis	Corpus penis
																Penis(10, 11, 12, 13, 14)

1) Portion 2 indicates the part of the ductus deferens other than ampulla ductus deferentis (Text-Fig. 1).

2) Original names are given in Japanese.

Note: The abbreviations under portions 1-9 are as found in Text-Fig. 1, and those under portions 10-14 are in Text-Fig. 24.

In fact, testis is the only term which is assigned identically by all authors, although no definition of its extent had been given until the present writer made it clear (ÔMURA, 1936b). Naturally the nomenclature proposed by the author has its proper reason and significance as explained later.

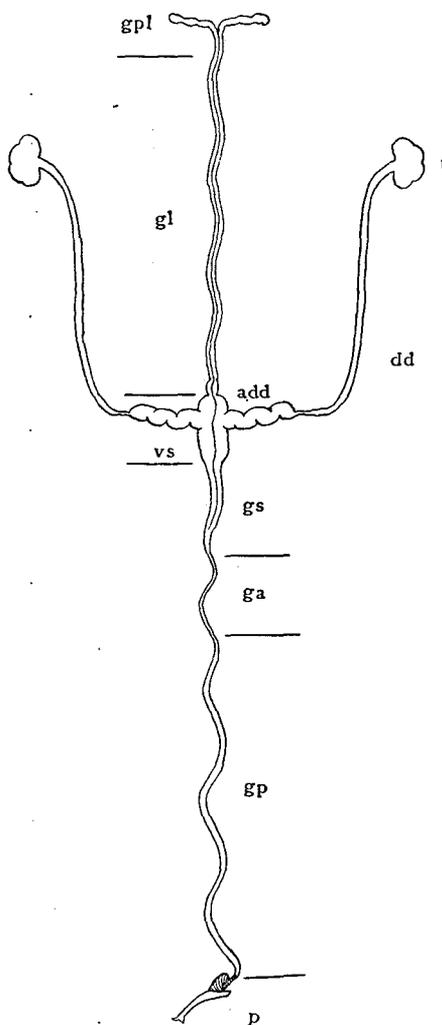
The dimensions of each organ of the system vary with age and also in individuals (Table 2). The approximate dimensions and their tendency to change with age are explained in the table. The testis, ampulla ductus deferentis and vesicula seminalis change in size remarkably with age. Text-Fig. 1 is a schematic figure of the male reproductive system drawn from the pupa one or two days before emergence, making the relative dimensions to agree with the values of Table 2 as nearly as possible.

### III. Structure of each organ

#### 1. *Ductus deferens*

(Text-Figs. 1(*dd*, *add*), 2-4.  
Figs. 1-4)

The ductus deferens is separable into two portions; the pars proprius and the pars ampullaris. The former scarcely changes dimensions with age. It is a long tube of ca. 20 mm length with the diameter varying at the proximal, middle and distal parts



Text-Fig. 1. Schematic figure of male reproductive system of pupa one or two days before emergence. *add*...ampulla ductus deferentis, *dd*...ductus deferens, *ga*...*gl.* alba, *gl*...*gl.* lacteola, *gp*...*gl.* prostatica, *gpl*...*gl.* pellucida, *gs*...*gl.* spermatophorae, *p*...penis, *t*...testis, *vs*...vesicula seminalis.  $\times 2$ .

TABLE 2. Dimensions of male sexual organs

Material: Akajyuku (a Japanese race). Unit: millimetre.

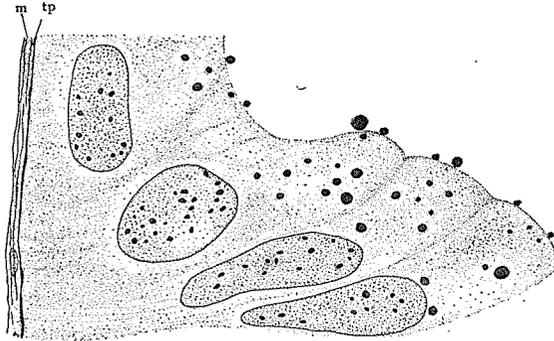
Stage of individual	Testis	Ductus deferens (pars proprius)	Ductus deferens (Pars ampullaris)	Vesicula seminalis	Glandula lacteola	Glandula pellucida	Glandula spermatophorae	Glandula alba	Glandula prostatica	Penis <sup>1)</sup>
Pupa, 4-5 days before emergence	3.94 × 2.05 × 1.9	—	4.9 × 0.47	3.0 × 0.53	—	—	—	—	—	—
Pupa, 1-2 days before emergence	3.1 × 1.73 × 1.4	19.7 × 0.47 <sup>3)</sup> 0.21 <sup>4)</sup>	5.5 × 0.83	3.7 × 0.87	26.0 × 0.24	5.7 × 0.31	7.8 × 0.3	5.9 × 0.2	28.0 × 0.3	3.7
Adult, ca. 12 hours old	2.5 × 1.3 × 1.4	20.0 × 0.40 0.20	5.1 × 0.9	3.3 × 1.0	23.9 × 0.27	6.3 × 0.38	7.2 × 0.34	6.4 × 0.25	26.5 × 0.36	—
Adult, 3 days old	1.8 × 1.1 × 0.9	18.5 × 0.30 0.22	5.5 × 1.1	3.4 × 1.1	25.0 × 0.26	6.1 × 0.36	6.7 × 0.38	5.5 × 0.25	28.5 × 0.38	—

1) The length of the penis was measured from the top to the middle point of the sphincter.

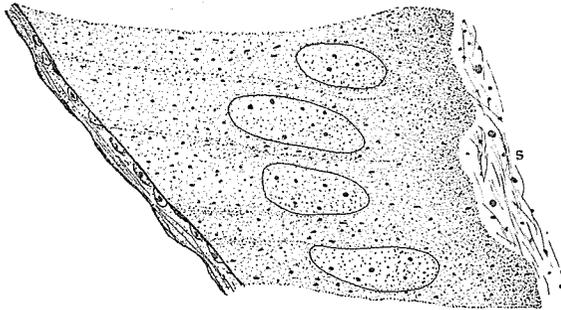
2), 3), 4) Indicate the diameters of most proximal, middle and most distal part to the testis respectively.

Note: Values are average of 4-5 individuals. Strict measurement is difficult, as many of the organs are spiral and there is some degree of shrinkage or elongation when they are stretched under the scale, so that the value shows approximate dimensions. Somewhat large individual deviations are found on glandula spermatophorae, glandula prostatica, glandula lacteola and glandula pellucida.

(cf. Text-Fig. 1 and Table 2). On the middle part, there is often found an outer protuberance, which is sometimes prolonged to meet the main tube again making a ring canal (Fig. 4). The writer has made some observations in which more than 50% of the individuals have



Text-Fig. 2. Longitudinal section of pars proprius of ductus deferens of pupa 4 days before emergence. m...muscle, tp...tunica propria. CHAMPY, HEID.-haem.  $\times 800$ .



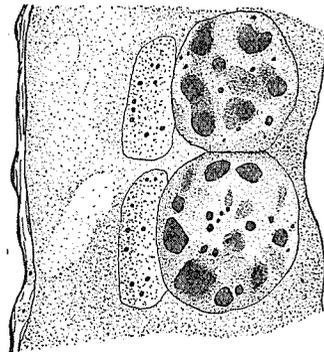
Text-Fig. 3. Longitudinal section of ampulla ductus deferentis of ca. 24 hours old moth. s... spermatozoa. CHAMPY, HEID.-haem.  $\times 1100$ .

proprius having several protuberances on it. But in the imaginal stage, when the semen is accumulated, the protuberances disappear and the diameter of the duct increases to more than twice the original one (Text-Fig. 14. Table 2), its length remaining almost the same, measuring about 5 mm. The inner surface which has taken an undulating form before as pars proprius does (Fig. 1), becomes flat swollen with semen (Fig. 3).

The histological construction of the ductus deferens is the same throughout the whole length. The outermost layer is

such malformation. The cause or character of the malformation has not been determined.

The pars ampullaris varies in diameter with age. When it contains no semen, its diameter is about the same as that of the middle part of the pars



Text-Fig. 4. Cross section of ampulla ductus deferentis, showing the giant vacuoles. Pupa, the day before emergence. CHAMPY, HEID.-haem.  $\times 800$ .

composed of a thin layer of longitudinal and ring muscles and the inner is made of glandular cells, the tunica propria lying between them (Text-Figs. 2-4. Fig. 1).

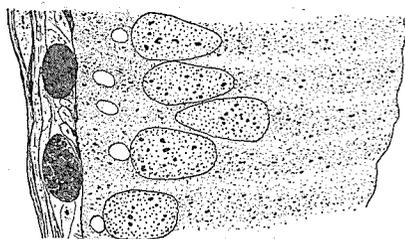
The secretion of the glandular cells begins to appear in the pupal stage 4 days before emergence, and this is immediately before the beginning of the escape of the spermatozoa from the testis (Text-Fig. 2) (cf. ÔMURA, 1936b). The secretion continues into the imaginal stage declining markedly with age (Text-Fig. 3). In the cells at secretion, there are found granules of various sizes (Text-Figs. 2, 3. Fig. 2). They show no fatty reaction to osmic acid. The secretion is mixed with the spermatozoa which flow down through the ductus (Figs. 1, 2). In the glandular layer a secretion occurs making here and there a mass like a vacuole (Text-Fig. 4. Figs. 1, 2). The character of this secretion has not yet been studied.

Mating exerts no effect upon the activity of the glandular cells.

## 2. *Glandula lacteola* (*Glandula accessoria proximalis*)

(Text-Figs. 1(*gl*), 5-9. Figs. 5-13)

The glandula lacteola is a long, paired, coiling, tube starting from above the vesiculae seminales and continuing to the glandula pellucida at the distal part. It measures ca. 25 mm in length with a diameter of ca. 0.25 mm swelling a little at the basal part. It is milky white in colour at first due to the secretion contained but becomes transparent when empty. The tube consists of inner glandular cells, the tunica propria and an outer thin layer of longitudinal and ring muscles. Notable secretion has not yet occurred in the pupa 4 days before emergence but only a small number of minute granules are found (Text-



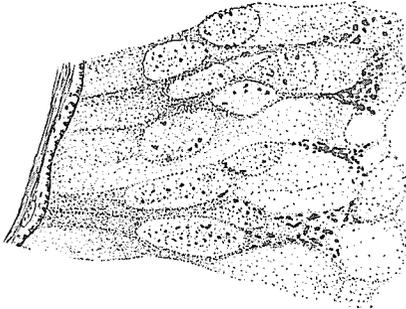
Text-Fig. 5. Longitudinal section of glandula lacteola of pupa 4 days before emergence. Fatty globules are found in muscle layer. CHAMPY, HEID.-haem.  $\times 500$ .

Fig. 5). In the muscle layer, there are found some ellipsoidal masses of fatty substances, as in the muscle layer of the other parts in the same stage. Incidentally, it may be remarked that these masses are never found in the imaginal stage.

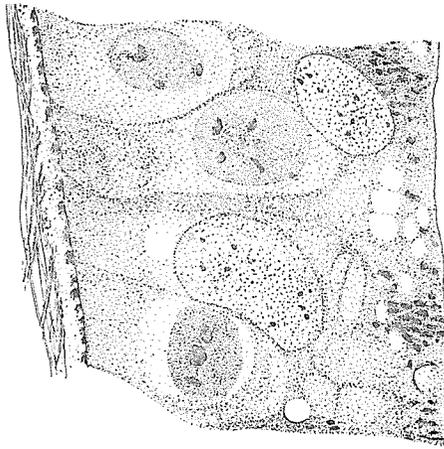
In the pupa 1-2 days before emergence, there appear many secreted granules in the lumen of the tube taking a radial arrange-

ment increasing their size towards the centre (Fig. 5). This suggests that the secretion does not flow down yet in the tube but remains stagnant a while, thus increasing the size inwards by union with the neighbouring granule.

In the pupa one day before emergence, the secretion declines (Text-Figs. 6, 7) and some cells show a degenerating feature which is demonstrated more clearly with age (Text-Figs. 6, 9, Figs. 11-13). The nucleus of the degenerating cell is sometimes located on the outer side of the cell (Text-Fig. 6), sometimes on the inner side (Text-Fig. 9, Figs. 11-13). In some cases the contents of the nucleus appears coagulating (Text-Fig. 6, Figs. 11, 12) and in other, on the contrary, dissolving (Text-Fig. 9, Fig. 13). Immediately after the ejaculation no secretion is found in the cavity of the tube (Figs. 9, 11-13). After 2-3 hours however, a new secretion occurs, but it is not so vigorous as the first, the degenerating cells having not recovered (Text-Figs. 8, 9).



Text-Fig. 7. Longitudinal section of glandula lacteola of pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 500$ .

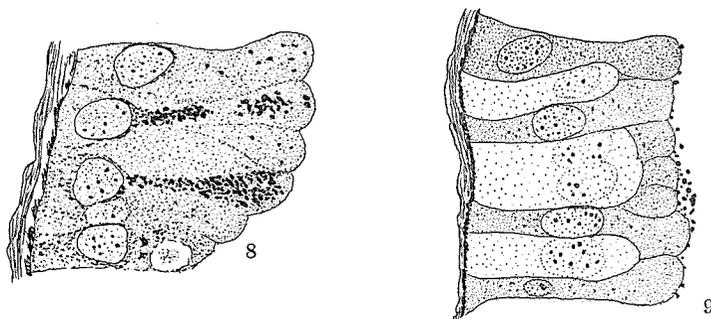


Text-Fig. 6. Longitudinal section of glandula lacteola of pupa 2 days before emergence. CHAMPY, HEID.-haem.  $\times 800$ .

The giant vacuole-like masses of secreted matters of different staining (haematoxilinophile or eosinophile) similar to those in the ductus deferens, are also found in this part.

These matters never extrude into the cavity of the duct even after the ejaculation (Fig. 9).

The connection between the glandula lacteola and the vesicula seminalis is very narrow and at that part many giant vacuole-like masses are found in the glandular layer (Fig. 6). At the distal part, the ductus passes into the glandula pellucida without any constriction.



Text-Figs. 8, 9. Longitudinal sections of glandula lacteola of moths, the day of emergence and 2 hours after a 50 minutes mating. One shows recovered secretion without degenerated cells, the other shows a small quantity of secretion and degenerated cells. CHAMPY, HEID.-haem.  $\times 500$ .

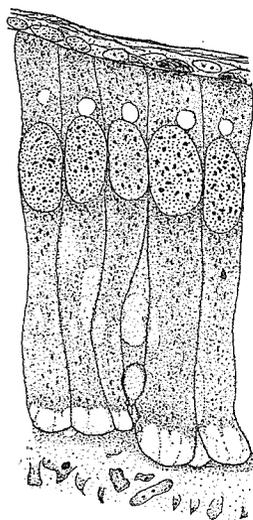
A peculiar valve-like appendix, which consists of glandular cells, is found on the inner surface of the glandula lacteola at the connecting part to the glandula pellucida (Figs. 7, 8). In the basal part of the glandula lacteola, there is often found a rather large mass of spermatozoa (Fig. 10), which seems to have been translocated from the vesicula seminalis by the movement of muscles.

### 3. *Glandula pellucida (Glandula accessoria distalis)*

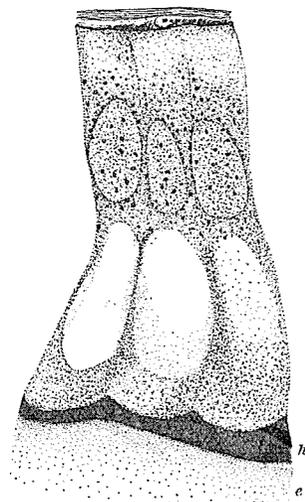
(Text-Figs. 1 (*gpl*), 10-13. Figs. 14, 15)

The glandulae pellucidae are paired ducts of which the distal parts bifurcate, making an approximate right angle (Text-Fig. 1). The total length is ca. 6 mm with a diameter which increases to the end, the maximum being about 0.35 mm. Generally several small swellings are found on the distal part. The inner structure of the duct is quite the same as that of other parts, consisting of muscles, tunica propria and glandular cells. The contents of the duct is transparent and viscous.

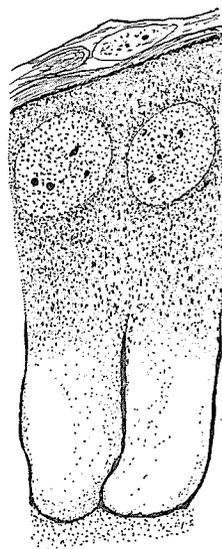
The actual secretion begins, as in the glandula lacteola, 4 days before emergence. At first, some granules appear in the cytoplasm of the glandular cells and then a slight secretion in the cavity of the duct (Text-Fig. 10). It is noted that at the beginning of secretion vacuoles of various size are found in the inner side of the secreting cells, increasing in size with the advance of the secretion. In the pupa one day before emergence, the vacuoles become larger than the nucleus (Text-Fig. 11), and in some parts they disturb the contour of the cells. In the



Text-Fig. 10. Longitudinal section of glandula pellucida of a pupa 4 days before emergence, showing small vacuoles and slight quantity of secreted matter. CHAMPY, HEID.-haem.  $\times 500$ .



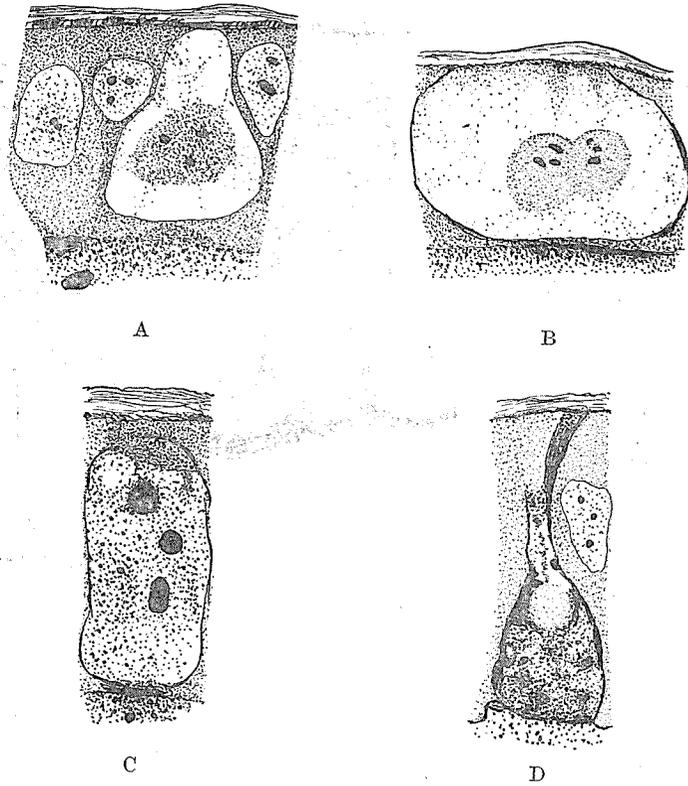
Text-Fig. 11. Longitudinal section of glandula pellucida of a pupa the day before emergence, showing large vacuoles and two layers of secreted matter. h...the layer stained with haematoxylin, e...the layer stained with eosin. CHAMPY, HEID.-haem. and eosin.  $\times 800$ .



Text-Fig. 12. Glandula pellucida of one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 1100$ .

pupa 1-2 days before emergence, the cavity of the duct is filled with the secreted substance, which makes two layers, a thin black layer adhering to the cells and a thick red layer, when counterstained with haematoxylin and eosin (Text-Fig. 11). This condition is maintained until the imaginal stage.

At the time of ejaculation, the entire contents is emptied out. However, soon after this, the glandular cells recover their activity becoming far more vigorous than ever. They have abundant mitochondria with growing vacuoles in them (Text-Fig. 12. Fig. 14). In this case the secreted matter can be counterstained just as in the primary, but instead of making two distinct layers it appears as a suspension of the haematoxylinophile substance in an eosinophile matrix. Sometimes there are found in the secretion large globules of the haematoxylinophile substance making a network cemented with the eosinophile one (Fig. 15). Sometimes an abnormal feature occurs in the glandular cells, for example,



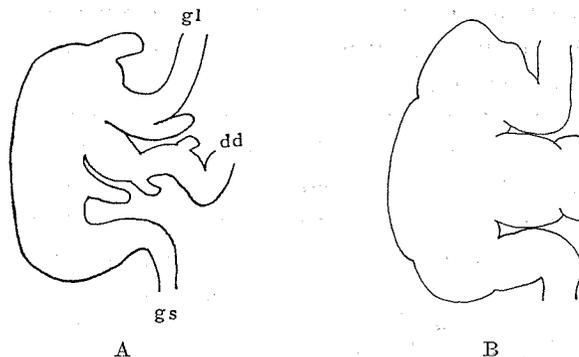
Text-Fig. 13. Glandula pellucida of one day old moth at 3 hours after a 50 minutes mating. Enlarged from Fig. 15 of Plate II, showing degenerating phases of the cells. CHAMPY, HEID.-haem. and eosin.  $\times 1100$ .

in some the nucleus is hypertrophic and vacuolized, in some the nuclear substance shows an appearance of plasmolysis (Text-Fig. 13, A, C) and in one case the union of two nuclei is indicated (Text-Fig. 13, B). These cells are destined to degeneration as illustrated in Text-Fig. 13, D.

#### 4. *Vesicula seminalis*

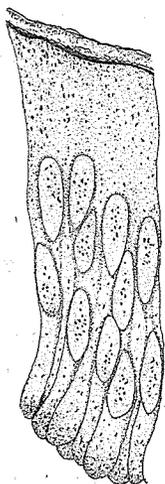
(Text-Figs. 1(*vs*), 14-17. Figs. 16-20)

The vesiculae seminales are short paired tubes making a half circle in profile (Text-Fig. 14). Generally three small appendages are found on each tube. The tubes are originally ca. 3.5 mm in length with a diameter of 0.5 mm, but when the cavity is full of semen the diameter widens to some 1 mm with a slight change of the length (cf. Table 2),

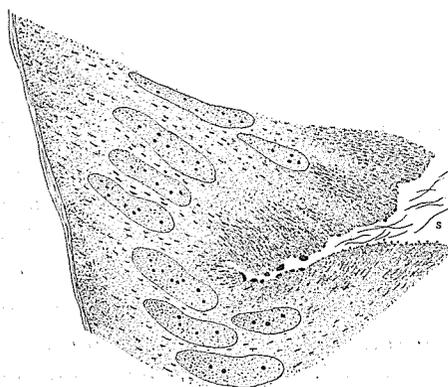


Text-Fig. 14. A...Vesicula seminalis of a pupa 4 days before emergence, showing three appendages on the vesicula seminalis and some on the ampulla ductus deferentis. B...Vesicula seminalis of a 2 days old moth. dd...ampulla ductus deferentis, gl...glandula lacteola, gs...glandula spermatophorae. Vivisected specimen.

the appendages disappearing (Text-Fig. 14), as in the case of the ampulla ductus deferentis. No distinct demarkation exists either between the vesicula and the ampulla ductus deferentis, or between the vesicula and the glandula spermatophorae. The constituents of the vesicula are muscules, tunica propria and glandular cell layer which projects inwardly when the contents of the vesicula is scanty.

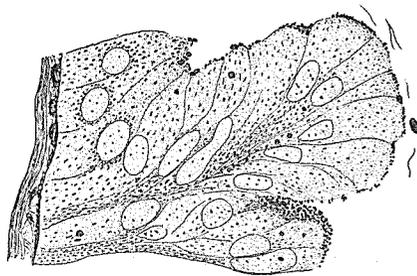


Text-Fig. 15. Longitudinal section of vesicula seminalis of a pupa 4 days before emergence. Champy, Heid-haem. x 350.



Text-fig. 16. Vesicula seminalis of pupa the day before emergence. s...spermatozoa. CHAMPY, HEID-haem. x 550.

In the pupa 4 days before emergence, no remarkable secretion occurs in the vesicula seminalis, to which no spermatozoa have been transferred, and only a few mitochondria are found in the cytoplasm of the glandular cells (Text-Fig. 15). Soon later when the in-flooding of the sperm begins, the secretion becomes notably active, but it continues for a short time declining gradually in the oldest pupa and in the young imago (Text-Fig. 16. Fig. 16). The secretion is made of a haematoxylinophile matter in the form of granules of various sizes and an eosinophile viscous



Text-Fig. 17. Longitudinal section of vesicula seminalis of a one day old moth at 3 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 350$ .

one appearing generally in the upper part of the connecting portion with the ductus deferens (Text-Figs. 16, 17. Figs. 19, 20). In the glandular cell layer there are found some giant vacuoles the significance of which is not clear.

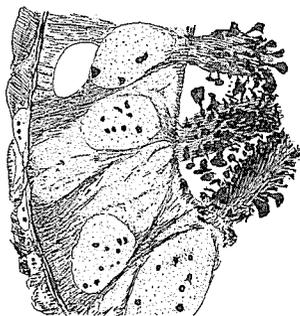
Immediately after ejaculation, the vesicula is completely empty. The boundary between the vesicula and the ampulla ductus deferentis is distinct, for the former becomes transparent while the latter still retains milky white contents with sperm. Then after 30–60 minutes some quantity of spermatozoa from the ampulla ductus deferentis is found in the vesicula and the glandular cells begin secretion anew (Text-Fig. 17. Figs. 17–20).

##### 5. *Glandula spermatophorae*

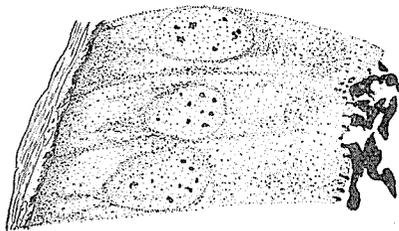
(Text-Figs. 1(*gs*), 18, 19. Figs. 21–24)

The glandula spermatophorae is a Y-shaped tube connecting the paired vesiculae seminales with the single glandula alba. It measures ca. 7–8 mm in length and ca. 0.3–0.35 mm in diameter. A thin layer of muscle, tunica propria and glandular cells build up the tube. The inner cavity which is narrow runs spirally sending off many irregular branches (Fig. 21). When the tube is filled with the secretion, its extent can be easily distinguished from the adjoining glands as the contents is transparent while it is white or milky white in the others.

The secretion commences in the pupa 4 days before emergence and declines toward the imaginal stage as in the case of the vesicula seminalis. In the fresh state the secreted substance is extremely viscous as detected



Text-Fig. 18. Cross section of glandula spermatophorae of a pupa the day before emergence, showing decreased secretion and viscous secreted matter. CHAMPY, HEID.-haem.  $\times 800$ .



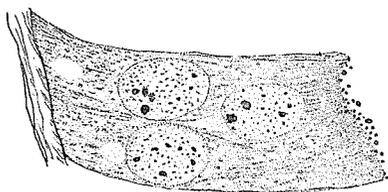
Text-Fig. 19. Glandula spermatophorae of a one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 800$ .

in the sectioned preparations in which the secretion from each cell appears as an isolated radial stream (Text-Fig. 18. Figs. 23, 24). By the ejaculation the contents is ejected out and some hours thereafter a slight recovery of secretion is seen in the glandular cells (Text-Fig. 19. Fig. 22).

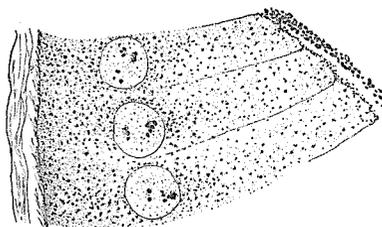
#### 6. *Glandula alba*

(Text-Figs. 1(*ga*), 20, 21. Figs. 25-28)

The glandula alba is the most slender duct of the system, measuring ca. 6 mm in length and 0.2-0.25 mm in diameter. The histological structure is the same as the other glands. In the lower part of the tube is found an inner protuberance consisting of glandular cells as in the case of the glandula lacteola (Fig. 26). The inner cavity is similar in shape to that of the glandula spermatophorae (Figs. 25, 27).



Text-Fig. 20. Longitudinal section of glandula alba of a pupa 4 days before emergence, showing little secretion. CHAMPY, HEID.-haem.  $\times 800$ .



Text-Fig. 21. Longitudinal section of glandula alba of a one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 800$ .

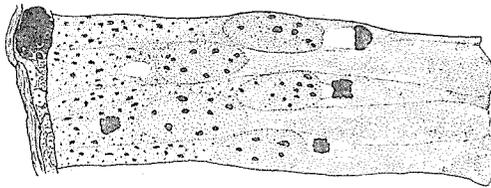
The secretion begins 4 days before emergence and declines toward the oldest pupa. The secreted substance is at first highly refractive being compactly composed of fine granules, but it becomes somewhat transparent at the part proximal to the glandula prostatica probably because the granules change their nature, concentration, size etc.

By the ejaculation the tube becomes completely empty and the glandular cells recover their activity to some degree, though in a few of them the nucleus is degenerated (Text-Fig. 21. Figs. 27, 28).

### 7. *Glandula prostatica*

(Text-Figs. 1(*gp*), 22, 23. Figs. 29-33)

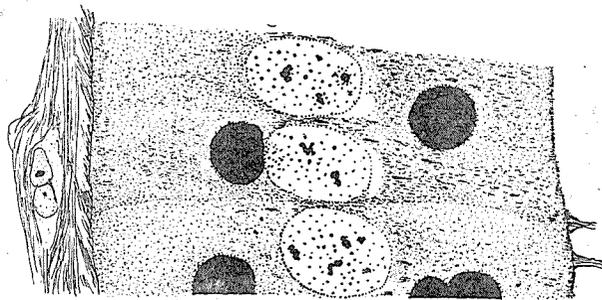
The glandula alba passes to the long glandula prostatica which measures 26-30 mm in length and 0.3-0.35 mm in diameter differing



Text-Fig. 22. Glandula prostatica of a pupa 4 days before emergence. CHAMPY, HEID.-haem.  $\times 800$ .

more or less in parts and with age. There is no clear structural demarkation between the glandula alba and the glandula prostatica, but they are easily distinguished by the thickness of the tube and by the content which is transparent and of low viscosity

in the latter. This gland leads to the penis with a clear demarkation line as the proximal part of the penis is tightened with a thick layer of muscles showing a smaller diameter (Text-Fig. 24. Fig. 34). The structural constituents are the same as the other glands.



Text-Fig. 23. Glandula prostatica of a pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 800$ .

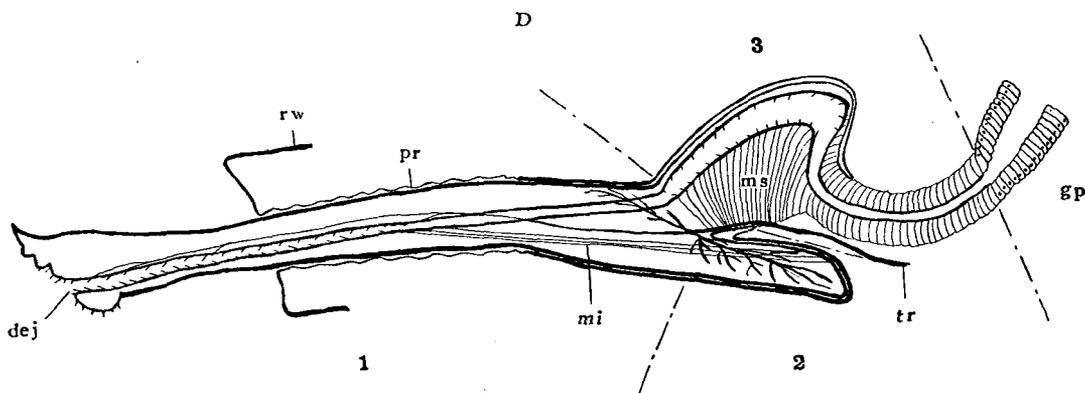
The secretion begins 3–4 days before emergence (Text-Fig. 22), and continues until one day before emergence (Text-Fig. 23. Fig. 29). The secreted matter is extruded into the cavity of the tube in the form of a drop or a minute granule (Text-Fig. 23. Figs. 29–33).

In the imago the secretion declines remarkably and is in abeyance during the ejaculation. But immediately thereafter it recommences (Fig. 32) and becomes far more vigorous than the primary in some 30 minutes, then continuing for some hours (Figs. 31, 33).

### 8. Penis

(Text-Figs. 1(p), 24. Figs. 34, 35, 37, 38)

The penis is of a complex construction made of chitin and muscle without glandular cells. For the sake of convenience, it is divided into three sections: corpus penis, crus penis and radix penis (1, 2 and 3 in Text-Fig. 24). As is shown in the longitudinal section, the chitin wall



Text-Fig. 24. Longitudinal section of penis. A schematic figure. The epidermis under the chitin is omitted. 1...corpus penis, 2...crus penis, 3...radix penis, D...dorsal side, dej...ductus ejaculatorius, gp...glandula prostatica, mi...musculus internus penis, ms...musculus sphincter penis, pr...praeputium, rw...ringwall, tr...trachea.

of the penis consists of three layers; praeputium, the outermost layer which is invaginated from the ringwall<sup>1)</sup> and extends to the outer wall of the corpus penis, crus penis and radix penis, the middle layer comprising the proper wall of the corpus penis and the inner wall of the crus and radix penis, and lastly the ductus ejaculatorius, the innermost

1) The term "ringwall" is from Zander (1903).

layer. Of these, the praeputium, the ductus ejaculatorius and the double wall of the radix penis are comparatively thin, while the walls of the corpus penis, crus penis, etc. are thick and strong. There is a well developed musculature in the radix penis around the ductus ejaculatorius, named "musculus sphincter penis" by the present writer (*ms* in Text-Fig. 24). The distal half of the muscle develops highly taking a bulbous shape (Text-Fig. 1), but the dorsal edge of the ductus ejaculatorius is free of the muscle (Text-Fig. 24). The proximal half of the sphincter is not covered with the chitin membrane (Text-Fig. 24). In the corpus penis there exists a musculature connecting a ventral point of the middle part of the ductus ejaculatorius with the inner wall of the crus penis. This is called "musculus internus penis" by the writer (*mi* in Text-Fig. 24).

The ventral side of the bulbous sphincter is covered incompletely with the chitin membrane, leaving an opening which the tracheae enter. The tracheae make a large tracheal bush in the crus penis filling its cavity and a small one at the dorso-proximal part of the corpus penis, probably strengthening the construction and serving to support the locomotory musculature of the penis.<sup>1)</sup> Some tracheae run through the corpus penis along the ductus ejaculatorius (Text-Fig. 24).

At the proximal part of the corpus penis and at the crus penis, the outer and the inner chitinous layers stick fast to each other, but at the other part of the corpus penis the inner layer remains free so as to allow the penis a telescopic movement which is set up by the locomotory muscles on the crus penis and proximal part of the corpus penis, folding and extending the praeputium. The ductus ejaculatorius is of a uniform diameter except an enlarged portion in the distal half of the radix penis, and is denticulated at the distal part and at the enlarged portion as is shown in Text-Fig. 24. The region where the tip of the corpus penis is transformed into the ductus ejaculatorius has several vertical foldings on the dorsal side and a pair of cheek-like pouch swellings on the ventral side (Text-Fig. 24, Fig. 38). These structures are movable forward and backward following the similar movement of the ductus ejaculatorius caused by the musculus internus penis in the course of the ejaculation.

The boundary between the penis and the glandula prostatica makes a zigzag line and hence, a cross section of this region contains the

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1) Details regarding these musculatures are found in UMEYA'S work (1926).

glandular cells of the glandula prostatica and the epidermis of the ductus ejaculatorius alternatively (Fig. 35).

#### IV. Activity of the spermatozoa

Spermatozoa were taken from the ductus deferens, ampulla ductus deferentis and vesicula seminalis respectively in vivisectioning the silkworm in physiological salt solution. Their activity was observed on the slide glass. The examination was made every day with 10 individuals from the stage of the pupa 3 days before emergence to the 3 days old adult. As there was only one if any active spermatozoon per field, it is clear that the fully matured spermatozoa<sup>1)</sup> in the post-testicular organs remain inactive.

Next, the activity of the ejaculated spermatozoa were examined as they were outside or inside the female body, taking out either from the spermatophora made on the tip of the penis<sup>2)</sup> or from that in the bursa copulatrix of the female which had been mated for 40 minutes. In both cases each including more than 10 individuals, the spermatozoa without exception showed a vigorously active movement. Thus it appears that some stimulus is derived from the male organ to activate the inactive spermatozoa in the course of the ejaculation.

A precise study vivisectioning the ejaculating moths showed that the spermatozoa were activated when passed through the glandula prostatica (Table 3). In order to discover the sperm-activator the secretions of the different glandular organs were added one at a time to the sperm

TABLE 3. Activity of the spermatozoa in the course of ejaculation

Spermatozoa		just ejaculated	in median part of duct. ejac.	in basal part of duct. ejac.	in distal part of gl. prost.	in gl. alba
Number of indvl.'s whose sper- matozoa were	active	10	10	10	8	0
	non-active	0	0	0	2	10

1) In *Bombyx mori* spermatozoa attain full maturity in the testis (ÔMURA, 1936b).

2) Such spermatozoa are gathered easily as the male continues the ejaculation even if separated from the female (cf. p. 147).

taken from the vesicula seminalis of young moth. The results are seen in Table 4.

TABLE 4. The nature of secretions of the male organs in relation to the activity of the spermatozoa

Secretion taken from		gl. pel.	gl. lact.	gl. spermatop.	gl. alba.	gl. prost.
Number of indvl's, whose secre- tions was	positive	0	0	0	0	10
	negative	10	10	10	10	0

As in the table only the substance secreted from the glandula prostatica is proved to activate the spermatozoa.

Next is it necessary to see the nature of the prostatic secretion in relation to the sperm activity without the secretions from the vesicula seminalis, ductus deferens and maybe some of the adjoining glands to the vesicula seminalis. For this purpose the sperm taken from the ductus deferens was tested (Table 5). The result was the same as in

TABLE 5. Function of the prostatic secretion in activation of the spermatozoa from the ductus deferens

Sperm taken from		ampulla ductus deferentis	most proximal part of ductus deferens
No. of indvl's, whose spermatozoa were	activated	10	10
	not activated	0	0

the former case. The spermatozoa both from the part of the ductus deferens most proximal to the testis and from the most distal part were activated by the prostatic secretion. This shows that the secretion of the ductus deferens has no connection with activation of the spermatozoa. It has become clear again from the above two experiments that only the secretion from the prostatic gland can activate the spermatozoa.

All examinations in relation to the activation of the spermatozoa carried out with different combinations of the secretions excluding the prostatic one have proven negative. It has also been shown by experiments that none of the secretions acts to neutralize that from the prostatic gland.

## V. Ejaculation

### 1. Relation between copulation and ejaculation

The fact that in insects some duration of copulation is necessary and sufficient to stimulate the male to ejaculation has been determined first by MACHIDA and WATANABE (1927). According to them *Bombyx mori* needs only 2-5 minutes copulation to make ejaculation—the temperatures have not been described—, as the male moth performs normal ejaculation, even if separated from the female after having made a copulation lasting for such a period.

Experiments in the same line were repeated with similar results to those of the above authors. In this case a copulation for about 5 minutes at 25°C caused the male moth to ejaculate. It is very interesting to note that the cutting off of the anterior part of the body after the copulation exerts no influence upon the ejaculation. In the male, cutting off up to about the 4th abdominal segment and in the female up to about the 7th abdominal segment after 1-2 minutes copulation still has no effect on the ejaculation. Table 6 shows the speed of the ejaculation

TABLE 6. Effect of the cutting of the male at 3rd abdominal segment one minute after the beginning of the mating on the speed of ejaculation

No. of indvl.	Duration of mating (minute)	Degree of ejaculation	
		Experimented individual	Normal individual
1	30	90%	90%
2	"	95	95
3	"	80	95
4	"	80	99
5	"	90	90
6	40	100	100
7	"	98	100
8	"	100	98
9	"	100	100
10	"	100	100
11	50	100	100
12	"	100	100

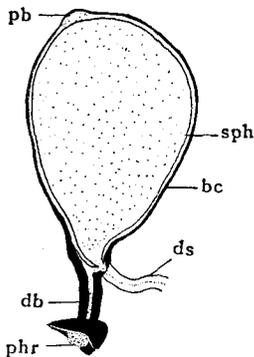
Note: Degree of ejaculation is represented roughly in % by measuring with naked eye the volume of the matter ejaculated into the bursa copulatrix of the female during the mating, taking the volume of the matter ejaculated after the separation of the mating into account. For example, 90% shows that the volume of the ejaculated matter found in the bursa copulatrix is some 9 times as large as that of the matter ejaculated after separation, thus meaning some 90% of semen has been ejaculated during the mating. 100% means the completion of the ejaculation.

in the case of the cutting of the male at the 3rd abdominal segment after one minute copulation. The operated male ejaculated too if separated from the female after 5 or more minutes mating. If both are cut, the female at the 7th abdominal segment and the male at the 3rd abdominal segment, 2 minutes after the beginning of the mating, the ejaculation occurs normally.

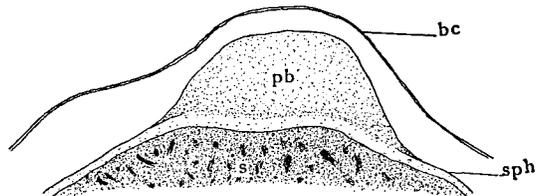
## 2. *Bursa copulatrix of mated female*

In the virgin female the bursa copulatrix which receives the spermatophora from the male is a shrivelled chitinous sac, and in a mated female the shape is changed according to the form of the spermatophora, generally being oval. The shape and size of the spermatophora are affected by the condition of the ejaculation. There are also some racial or individual variations. The bursa copulatrix measures 4–5 mm × 2–3 mm in the female which has mated for 60 minutes with some 24 hours old virgin male moth of the race hybrid between “Kokusan-Si-No. 106” and “Kokusan-Niti-No. 7” (average of 30 specimens).

The spermatophora made by the first ejaculation of a male is always possessed of a small pearl-like body on its top (Text-Figs.



Text-Fig. 25. Bursa copulatrix of the female moth mated for some 60 minutes. A schematic figure. bc...bursa copulatrix, db...ductus bursae, ds...ductus seminalis, pb...pearly body, phr...spermatophragma, sph...spermatophora.



Text-Fig. 26. The top of the bursa copulatrix of mated female moth, showing pearly-body (pb). s...semen. CARNOY, DEL.-haem. × 70.

25, 26). When the ejaculation is accomplished, the opening of the spermatophora is found in the neck part of the bursa copulatrix, and the ductus bursae is quite filled with a fluid, which coagulates into a plug (Text-Fig. 25) named “spermatophragma” by PETERSEN (1928) or “sphragis” by ELTRINGHAM (1925) etc., in other species of Lepidoptera.

From this feature it is shown that the pearly body precedes the spermatophora and that the spermatophragma is made after the spermatophora formation.

### 3. Duration of ejaculation

It is known that the male of *Bombyx mori* does not begin ejaculation immediately after the beginning of copulation and does not separate from the female until the second or third ejaculation is finished continuing the mating for several hours. So the duration of the copulation does not indicate the duration of ejaculation.

Immediately after the beginning of copulation, a female moth was cut at the 7th abdominal segment and the bursa copulatrix was observed from headward removing the contents of the body cavity. No ejaculation was effected by the mating male at least for 5 minutes. Then the penes of male moths were observed in order to learn the instant of the ejaculation after a 5 minutes mating. From this observation it was indicated that the ejaculation begins 5-10 minutes after the beginning of the copulation.

In order to ascertain the time of the finish of the ejaculation, three groups of male moths which had mated respectively for 30 minutes, 40 minutes and 50 minutes, were kept quiet for 60 minutes. At this time those which had some secretion on their penes tops show that they had not completed the ejaculation during the mating, while the others which had nothing on the penes indicate that they had finished it. The results of these examinations are summarized in Table 7. In 30 minutes mating no moth has finished the ejaculation. In 40 minutes mating a majority of them and in 50 minutes mating all of them were found to have completed it.

TABLE 7. Time of finish of ejaculation

Material: Kokusan-Niti-No. 106. 1 day old moths.

Duration of mating		30 min.	40 min.	50 min.
No. of indivl's. whose ejaculation have been	finished	0	8	10
	not finished	10	2	0

As the time of the beginning and also the time of the finish differ according to the individual, it is difficult to determine precisely the duration of the ejaculation. Nevertheless the average duration is presumably for 25-30 minutes.

4. *Duration of spermatozoa ejaculation*

In the ejaculation above discussed the general semen is emitted, which does not necessarily contain spermatozoa. To ascertain the time of the ejaculation of spermatozoa, the contents of the bursae copulatrices of the females, which had been mated for various durations of time, were observed microscopically (Table 8). The data show that the emission of the spermatozoa occurs most frequently about 15 minutes after copulation.

TABLE 8. Time of beginning of ejaculation of spermatozoa

Material: Kokusan-Si-No. 106 × Kokusan-Ô-No. 18. 1 day old moths.

Number of indivl's.	Duration of mating (minutes)	No. of indivl's. who ejaculated	
		spermatozoa	no spermatozoa
10	10	0	10
10	12	0	10
10	13	0	10
10	14	3	7
10	15	5	5
10	16	8	2
10	17	10	0
10	18	9	1
10	19	10	0
10	20	10	0

Next, to know the time of the finish of ejaculation, male moths were mated for various durations of time and their penes were examined after separation. When the semen on the penis contained some spermatozoa the moth was regarded not to have finished the spermatozoa

TABLE 9. Time of finish of ejaculation of spermatozoa

Material: Same as that in Table 8.

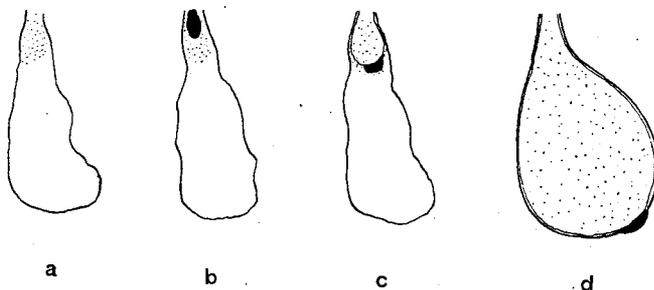
Number of indivl's.	Duration of mating (minutes)	No. of indivl's. whose ejacl. of sp. has been	
		accomplished	not accomplished
10	20	0	10
10	25	6	4
10	30	9	1
10	35	10	0

ejaculation during the mating while the semen without spermatozoa showed that they had finished it (Table 9).

It has become clear that the ejaculation of spermatozoa is generally finished some 25 minutes after the beginning of the copulation. Likewise, it is ascertained that the semen without spermatozoa is ejaculated for 5–10 minutes and then follows the ejaculation of spermatozoa which lasts for some 10 minutes. Thereafter the emission of the semen without spermatozoa occurs again lasting for some 10 minutes (cf. Table 7).

##### 5. Process of spermatophora formation

The process of the spermatophora formation in the bursa copulatrix was observed *in situ* in the mating female which was cut at the 6th or 7th abdominal segment 5 minutes after the beginning of the mating. Text-Fig. 27 shows the process schematically. First, a small quantity of a transparent fluid is found (a), next follows a small pearly white mass (b), and then the formation of the spermatophora begins (c) growing continuously until the contour is made up (d). The time of appearance of each stage is shown roughly in the following table in which one may see wide individual variations.



Text-Fig. 27. The process of spermatophora formation.  
A diagrammatic figure.

Stage	(a)	(b)	(c)	(d)
Time from the beginning of the copulation (min.)	6–12	8–15	12–18	25–30

(Material: Kokusan-Si-No. 106, one day old moth).

As the beginning of the true ejaculation occurs at some 15 minutes after the beginning of the copulation and lasts for some 10 minutes, the formation of a spermatophora does not precede the ejaculation of spermatozoa but the two are of simultaneous occurrence.

As to the transparent fluid and the pearly body, they were tested for any function in the formation of a spermatophora, interrupting copulation after the pearly body was ejaculated. The separated male moth made up a normal spermatophora outside of the bursa copulatrix, showing that the spermatophora is made of only spermatophora-forming substance from the male without any mechanical assistance from the female side.

#### 6. *Character of ejaculated fluids and their sources*

From the above observations on the process of ejaculation and spermatophora-formation, the ejaculated substance from the male can be divided into five parts: transparent fluid, pearly mass, sperm in the spermatophora, the substance forming a spermatophora and transparent fluid ejaculated finally without spermatozoa. The sources of these substances were sought.

For this purpose, the reaction of the transparent fluid first ejaculated to the spermatozoa from the vesicula seminalis was tested. The result was always positive; the fluid activated the spermatozoa, so it was proved to be the prostatic secretion. Judging from the character and quantity the pearly mass was inferred to be originated from the glandula alba. The transparent fluid finally ejaculated without spermatozoa was proven not to be prostatic secretion. As the ejaculation of this substance is known to occur approximately in the last 10 minutes of copulation (cf. p. 151), it is inferred to be the contents of the glandula pellucida and the glandula lacteola. This leads to the secondary inference that these contents are not mixed with that of the vesicula seminalis before the ejaculation. It has not yet been determined whether these two substances are mixed up in the course of ejaculation.

In sectioned preparations two sorts of substances were detected in the bursa copulatrix immediately after the ejaculation. One is a mass of granular substance in the spermatophora near the orifice containing no spermatozoa. The other is the spermatophragma which is post-positional to the spermatophora occupying the cavity of the ductus bursae. The former is supposed to be the secretion of the glandula lacteola and the latter that of the glandula pellucida. (Few hours after the ejaculation the former could not be detected, probably being mixed with semen).

The spermatophora-forming matter is considered necessarily to have originated from the glandula spermatophorae. The prostatic secretion

first ejaculated before the formation of the spermatophora is too small in quantity to fill the cavity of the prostatic gland but the greater part must be emitted with spermatozoa, activating them (cf. p. 145).

Now the order of emission of the contents of the male sexual organs has become clear. First, a part of the prostatic secretion emerges, second, the content of the glandula alba, third the contents of the vesicula seminalis and glandula prostatica with that of the glandula spermatophorae forming spermatophora, then the lacteal fluid and last the pellucid fluid from glandula pellucida.

#### 7. *Mechanism of ejaculation and spermatophora-formation*

The glandulae prostaticae were observed by sectioned preparations immediately after a 20 minutes mating, that is, approximately at the mid-point of the ejaculation of spermatozoa. The distal half of the organ was found filled with a substance containing numerous spermatozoa from the vesicula seminalis and a number of masses of viscous looking material from the glandula spermatophorae (Fig. 36). There some of the prostatic secretion must also be present, as the spermatozoa taken from this portion show an active movement (cf. p. 145).

The ductus ejaculatorii of the same individuals were also investigated. In the radix penis, the cavity of the duct had been reduced by the contraction of the musculus sphincter penis and so only a small amount of ejaculating matter was found. In the corpus penis, a spermatophora—strictly speaking, a proto-spermatophora—was found filled with seminal fluid and in contact with the wall of the ductus ejaculatorius (Fig. 37). Therefore, it is inferred that the viscous mass found in the glandula prostatica must be transformed, in its course through the proximal half of the ductus ejaculatorius, into a tubular proto-spermatophora including semen in it. For the formation of proto-spermatophora the musculus sphincter penis and the construction of the ductus ejaculatorius, that is, the broad cavity and denticulous inner surface are probably instrumental.

During the ejaculation, the distal end of the penis moves back and forth slowly but regularly, thus causing a slow outflow of the viscous contents of the ductus ejaculatorius. Probably this movement is effected mainly by the musculus internus penis, and partly by the musculus sphincter penis. The denticules on the distal part of the ductus ejaculatorius seem to serve to prevent a retrogressive flow.

The tubular proto-spermatophora in the ductus ejaculatorius is

ejected as an ovoid spermatophora but not as a tubular one. The quantity of the spermatophora-forming matter found in the prostatic gland in the course of the ejaculation is very small as compared with that of the semen (Fig. 36). The same relation in quantity of the two matters is seen also in the completed spermatophora (Text-Fig. 26 suggests it). But in the proto-spermatophora the relation is quite reversed the semen being smaller in quantity (Fig. 37). These facts suggest that the proto-spermatophora is pushed out slowly, probably owing to the high viscosity and the close contact with the wall of the ductus ejaculatorius, while the contents flows out comparatively quickly, pushing and expanding the ejected spermatophora. Thus from the tubular proto-spermatophora an ovoid spermatophora results.

The question arises naturally how the three kinds of contents from the glandula prostatica, glandula spermatophorae and vesicula seminalis mix together in the course of the ejaculation, instead of being ejaculated separately in the order of position. It is certain that the significant difference in the shape and size of the cavity among the glandula spermatophorae, glandula alba and glandula prostatica, the inner protuberance of the glandula alba, and again the physical characters of each fluid, etc. all play some part in the mixing up of these fluids.

The translocation of the contents of each organ to the ductus ejaculatorius may be brought about by the peristaltic movement caused by the muscle of the organ. Such movement is frequently seen in vivisected individuals.

#### 8. *Reproductive organs after ejaculation*

Sexual organs vivisected out of a virgin male moth are filled with their own contents. The contents of the glandula pellucida is transparent and somewhat viscous, that of the glandula lacteola is milky white, that of the vesicula seminalis and that of the ampulla ductus deferentis are also milky white (both containing spermatozoa), that of the glandula spermatophorae is transparent and very viscous, that of the glandula alba is highly refractive and lastly, that of the glandula prostatica is transparent and lightly viscous.

Immediately after the ejaculation, all glandular organs, except the ductus deferens, are empty, becoming transparent and somewhat shrunken. Some of these organs are observed to be filled again with the contents which are detected in fresh state. In so far as the secretion from the prostatic organ is concerned, which activates the sperma-

tozoa, this is clearly shown experimentally. The vesicula seminalis is re-filled most quickly. Though the secretion of its own glandular cells could not be detected macroscopically, a milky white inflow of the semen from the ampulla ductus deferentis appears some 20 minutes after the finish of the ejaculation and occupies almost the whole cavity in 30–60 minutes. The recovery of the secreting activity of the glandula prostatica is also quick. Some individuals showed the presence of the secretion 30–40 minutes after the ejaculation, and most of them had it 90 minutes after. In the glandula alba within 5 hours after ejaculation and in the glandula lacteola within 10 hours, the secretion did not reach an amount detectable with the naked eye. Moreover, these glands never attained the same quantity of contents as before, no matter how long the individuals were kept from mating. The recovery of the glandula spermatophorae occurred but the wall of the secondly ejected spermatophora was very thin as compared with that of the first, even when a very long interval (some 50 hours) had passed between the first and the second ejaculation. The glandula pellucida was not studied precisely, though there appeared some secondary content which is transparent and without any special character.

The secretion is repeated as many times as the ejaculation occurs.

9. *Translocation of sperm from ampulla ductus deferentis to vesicula seminalis*

The inflow of the semen from the ampulla ductus deferentis to the vesicula seminalis does not seem to occur during the ejaculation, as there is found no inflow immediately after the ejaculation. But it remained to be determined whether the inflow continues in the imaginal stage when the secretion of the glandular organs has almost ceased. For that purpose the amount of the contents of the vesicula seminalis at various stages was measured indirectly by weighing the ejaculated spermatophora. The increase of the contents of the spermatophora indicates approximately the increase of the semen which entered the vesiculae seminales, as all glandular secretions scarcely occur in adult stage until the ejaculation is executed.

The results have been summarized in Table 10 showing that the semen in the vesiculae seminales increases continuously independent of the ejaculation. It is seen from the table that the speed of the inflow seems to be controlled by the quantity of the contents of the vesicula seminalis. The most speedy inflow is seen when the vesicula seminalis

TABLE 10. Weight of the spermatophorae ejected by the males at various stages

Material: Kokusan-Si-No. 106 × Kokusan-Niti-No. 7.

Series	Mating	Time from preceded mating (hour)	Number of indiv'l's.	Weight per spermatophora (gr.)	Ratio	Condition of the males used
1	1st	—	30	0.0085	100	About 20 hours old.
2	2nd	5	8	0.0031	36	Once used in series 1.
3	2nd	10	8	0.0030	35	Ditto.
4	2nd	24	8	0.0049	58	Ditto.
5	3rd	24	6	0.0036	42	Twice used in series 1 and 2.
6	1st	—	9	0.0132	156	12 days old in some 15° C.

Note: All matings were continued for 60 minutes at some 25°C. The weighing was undertaken 10 minutes after the mating with the bursa copulatrix, of which the weight (0.0005 gr.) was then deducted.

is empty.

The flowing down of the sperm through the ductus deferens occurs throughout the entire adult stage, because the escape of the spermatozoa from the lobulus testis into the ductulus efferens testis does not cease until the death of the moth (ÔMURA, 1936b).

## VI. Aging of the spermatozoa in the post-testicular organs

As the spermatozoa, which have attained full maturity in the testis (ÔMURA, 1936b), rest for a long while in the post-testicular organs, it is worth while to ascertain whether they age during that resting period. First, the aging of the spermatozoa in the pars proprius of the ductus deferens was examined. Spermatozoa taken from both the most proximal and the distal part of the pars proprius and from the pars ampullaris showed positive reactions to prostatic secretion (Table 5), suggesting that they undergo no aging in this organ.

Next, the aging of the spermatozoa in the ampulla ductus deferentis and the vesicula seminalis was tested. Two lots of spermatozoa taken from the vesicula seminalis of the imago and the pupa two days before emergence were inseminated artificially with prostatic secretion and their fertility and the character of the progenies if any were examined.

Though the former spermatozoa had remained for a far longer time in these organs than the latter had, there was found no difference in the fertility and the progenies remained entirely the same, as has been shown in a previous paper (Tables 1, 2 and 3 of ÔMURA, 1936a). Therefore there is no sign of aging in this case.

That there is no aging of the spermatozoa in the vesicula seminalis, is shown from the following fact. Ejaculation takes place periodically in a long or in successive matings at intervals of 60-90 minutes (SUDA, 1913. MACHIDA and WATANABE, 1927), and each ejaculation finishes in the course of some 40 minutes mating as shown in the present paper. Therefore, it is possible to fertilize a female by the first ejaculation, mating with a virgin male for one hour, and another female by the second, mating with the same male for the next two hours. In the first mating, most of the ejaculated spermatozoa have remained in the vesicula seminalis for longer than 50 hours, for example from 2 days before emergence, and in the second, they had been there for some one hour or less. In this connection MATUI's paper (1933) appears to present significant data, and they are summarized by the present writer in Table 11, which shows that no aging of spermatozoa is found in the vesicula seminalis.

TABLE 11. Some characters of the spermatozoa ejaculated by two successive matings

Material: Kokusan-Niti-No. 1.

Mating	Number of female moths	Number of eggs per batch	Fertility (%)	Hatchability (%)	Ratio of reared to full grown larvae (%)
1st 1 hour	5	561	98.3	86.2	70.1
2nd 2 hours	5	555	98.1	85.8	72.9

## VII. Fate of the spermatozoa left in the dead male

The spermatozoa in the vesicula seminalis and ampulla ductus deferentis of dead male moths at some 50 hours after the death were tested using the prostatic secretion from a living moth. No spermatozoa were found alive. It is interesting to note that these dead spermatozoa curl up and are isolated from each other, instead of making bundles or of

having smooth wavy tails if separated as the normal spermatozoa. A question may arise whether they were exhausted by vivid movement before death. In this regard the semen from the vesicula seminalis of various individuals including just dead moths was observed in pursuit of moving spermatozoa and as was expected the spermatozoa were proved to show a violent activity which may cause their rapid death shortly before or after the death of the moth (Table 12). In some just dead as well as old moths most of the spermatozoa move with abnormal violence, while in others many spermatozoa either show a tardy movement or are almost in a motionless state showing curled tails. The spermatozoa taken from the ampulla ductus deferentis showed the same behaviour as those in the vesicula seminalis.

TABLE 12. Character of the spermatozoa in the vesicula seminalis of the moths of different age

Material: Kokusan-Niti-No. 1 × Kokusan-Si-No. 101

Temperatures in which materials were held was ca. 25°C.

Age of moth		2 day old	5 day old <sup>1)</sup>	5 day old <sup>2)</sup>	Remarks
Number of indivl's. whose sperm. in the ves. sem. were	inactive	10	1	0	Contained no or a few active spermatozoa in a field, and no dead ones.
	active	0	7	5	Contained numerous active ones mixed with some inactive alive or dead ones.
	dead	0	2	5	Some individuals contained a few active ones.

1) Oldest, 2) Just dead moth. Both of the moths were taken from the same strain, the great majority of which (80-90%) were dead at the time of the experiment.

Note: The distinction between the dead and alive was based upon the features shown on p. 157.

Then the contents of the glands adjoining the vesicula seminalis were taken from the old or just dead moths whose spermatozoa were active, and tested whether they activate the normal inactive spermatozoa. Results are shown in Table 13.

Thus it is shown that the secretion of the glandula lacteola in such cases is effective for the activation of the spermatozoa. Probably this secretion had undergone some changes induced by unhealthiness of the

TABLE 13. Tests to find the substance which has activated the spermatozoa in the vesicula seminalis as well as in the ampulla ductus deferentis of oldest or dead moths

Secretion taken from	glandula pel.	glandula lact.	glandula spermatop.	glandula alba	
Number of sound indiv's. whose spermatozoa were	activated	0	5	0	0
	not activated	5	0	5	5

individual. Such a change is found to occur also in pupae which have been kept for a long time at a low temperature and failed to emerge. The prostatic secretion has nothing to do with such abnormal movement of spermatozoa, as it does not translocate into the vesicula seminalis.

The fertilizing ability of these abnormal spermatozoa and of normal ones activated by such deteriorated secretion of the glandula lacteola was tested by artificial insemination (Table 14).

TABLE 14. Fertilizing ability of abnormally activated spermatozoa

Material: Eikô × Mangetu

Spermatozoa used	Active spermatozoa taken from ves. sem. or amp. duct. def. of very old moth	Normal spermatozoa taken from ves. sem. of young moth and activated by secretion of gl. lact. of very old moth	Normal spermatozoa taken from ves. sem. of young moth and activated by its own prostatic secretion
Number of fertilized females	0	0	4
Number of non-fertilized females	8	5	1

It is proven that none of the tested spermatozoa has the power of fertilization, contrary to the high fertility of normal spermatozoa activated by the prostatic secretion.

### VIII. Substance necessary to the fertilization of the spermatozoa

As the spermatozoa from the ampulla ductus deferentis of the pupa have fertilizing ability when activated by the prostatic secretion (cf. ÔMURA, 1936 a), the secretions of the glandula pellucida, glandula lacteola, glandula spermatophorae, glandula alba and vesicula seminalis

are not essential for the fertilization. The secretion of the ductus deferens seems also unnecessary, as the spermatozoa taken from the most proximal part of it have been inferred to be similar, in character to those in the ampulla ductus deferentis (cf. p. 146). Therefore, in the post-testicular system, there exist no secretions necessary to the fertilization other than the prostatic one which induces the full matured spermatozoa into sound action.

### IX. Water solubility and heat resistance of the prostatic secretion

A series of experiments was carried out in order to ascertain the aspect of the prostatic secretion. When a trace of distilled water or physiological salt solution is added to the prostatic secretion, the effect upon the spermatozoa becomes very quick. When the secretion was diluted with distilled water to ca. 1/5 of the original concentration the reaction of the spermatozoa was not weakened. Next the spermatozoa gathered from 20 mated females at 24 hours after a 60 minutes mating were broken and mashed with glass rod and mixed with about the same amount of distilled water. The mixture was centrifuged for 20 minutes (about 3000 rotations per minute with the diameter of 16 cm) and divided into two layers, upper transparent and underlying sedimental ones. The upper layer activates the spermatozoa. Therefore it may be said that the effective elements show a high solubility in water.

TABLE 15. Heat resistance of prostatic secretion

		With fresh prost. secret. without treatment	With prost. secret. heated for 2 minutes at the temp. of					With salt sol. (0.75%)	Sperm only	Remarks
			60°	70°	80°	90°	100°			
No. of activated spermatozoa	Exp. 1	100	50-60	30-40	10			0	0	Secretion was gathered after being mixed with same volume of water.
	Exp. 2	100		40	10-15	5-10		1	0	Without water.
	Exp. 3	100			20-30	10	3-5	0	0	Without water.

Note: The number of the activated spermatozoa in the table is by no means the actual one but shows the relative number; that is, 100 shows the case in which most of the spermatozoa are activated; 50, about a half of them are active; 10, several; 5, a few, while 1 shows that one spermatozoon in two or three field is active.

Some of the above transparent fluid was taken into capillary glass tubes with the outer diameter of 1/4–1/5 mm and heated for 2 minutes in hot water at different temperatures. Some part of the fluid probably of proteinic nature was coagulated. The heated was tested as to the function to the spermatozoa. The result is seen in Table 15, in which the effectiveness of the secretion is indicated by the number of the activated spermatozoa.

The data show that the substance (sperm-activator) is stable in some degree under heat treatment.

### X. Discussion

In Lepitoptera, STITZ (1901) and ZANDER (1903), the latter dealing only with the copulatory organs, have proposed a trustworthy nomenclature based upon their minute morphological studies. However, certain of STITZ's terms, such as "paariger," "unpaariger" or "erster unpaariger" etc. (see Table 1) seem extremely provisional. The present nomenclature in *Bombyx mori* is derived from precise morphological and physiological studies taking into consideration the above authors' opinion. In the following, explanations will be given on some points so far as necessary.

Instead of STITZ's "vesicula seminalis" the present writer gives the name "ampulla ductus deferentis," as the organ is nothing but a transformed portion of the ductus deferens. The "ductus ejaculatorius," as employed by all authors other than STITZ and ZANDER (who made a notable error in calling it "ductus deferens") (cf. Table 1), is divided into three parts: glandula spermatophorae, glandula alba and glandula prostatica. This coincides well with STITZ's division. As they are of glandular nature the old name is not correct. It is uncertain whether the "ghiandole prostatiche" described by MIALL and DENENY in Orthoptera (BERLESE, 1909) without ascertaining its function, is the same organ as the present writer's glandula prostatica. The penis and ductus ejaculatorius are exactly the same as ZANDER's. The ductus ejaculatorius is clearly a portion of the penis, not independent of the penis as ZANDER considered. ZANDER was the first to dissect the radix penis minutely and to show it to be a part of the penis. The opinion that the radix penis is a part of the "ductus ejaculatorius" of the other authors is not tenable. SHARP and MUIR (1912) who worked extensively on the Coleopteran genitalia regarded the name penis as unreasonable and applied the name "median lobe" to the present writer's corpus penis,

and "tegmen" to the lateral lobes and basal piece. The median lobe and the tegmen together are called by them as "aedeagus." According to them the ductus ejaculatorius is divided into "ductus ejaculatorius" and "internal sac." In Coleoptera there perhaps exists a portion analogous to the present writer's radix penis, judging from the macroscopical figures of SHARP and MUIR, but they did not discuss its minute construction. In his textbook, IMMS (1929) has adopted "aedeagus" after SHARP and MUIR and regarded the term "penis" as incorrect without any adequate reason.

The author has proposed in the present work to use the names glandula pellucida (glandula accessoria distalis), glandula lacteola (glandula accessoria proximalis), glandula spermatophorae, glandula alba, glandula prostatica, musculus sphincter penis, musculus internus penis and radix, crus and corpus penis. Taking these terms as a basis of the nomenclature a more natural and universal one will be drawn up in a future work.

Although in *Bombyx mori*, macroscopical figures of the male reproductive organs have been given many times by various authors, those figures differ very greatly in dimensions from those measured by the present writer. Moreover, sometimes, for example, a set of young pupal testes is erroneously combined with imaginal ampullae ductuum deferentium. As a matter of fact in the literature available to the writer there is found no natural figure at all.

The "Schaltstück" found by STITZ in Microlepidoptera at the boundary between the different glandular constructions has not been found in *Bombyx mori*. In Coleoptera, ESCHERLICH (1894) has found regenerative cells in the ectadeny<sup>1)</sup> probably making an error as to the protective cells, which are common to the ectodermal gland lined with chitin intima. Judging from his figures, his so called "regenerative cells" seem to correspond to glandular cells, and his "glandular cells" to protective cells. In *Bombyx mori*, no regenerative cells are found, as may naturally be imagined from the fact that the adult of Lepidoptera has a short reproductive period.

So far as the construction of the penis is concerned the present author agrees exactly with VERNON and BISSON (1896) who studied the post-embryological stage of the genitalia in *Bombyx mori*. He holds

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1) "Ectadeny" is the term applied to an accessory gland of the male reproductive system which is lined with chitin intima (ESCHERLICH, 1894).

ZANDER'S view too.

As to the nature of the secretions from the ductus deferens and vesicula seminalis, a determinative conclusion is not reached by the present work. Judging from the fact that no aging of the spermatozoa occurs in the post-testicular male organs, the secretions seem to serve for prevention of senile decay of the spermatozoa. The lactean secretion which is mixed into the semen in the bursa copulatrix appears to have a similar function to the above. The function of the pearly body attached to the spermatophora originating from the glandula alba is not certain.

The function of each organ has been made clear for the first time in this work. Especially it was revealed that the glandula prostatica and the penis play an important rôle in the intermediate course between the gametogenesis and ejaculation.

The significance and the function of the substance contained in the giant vacuoles which occur in the glandular cell layer of the ductus deferens, vesicula seminalis and glandula lacteola were not studied.

It is quite natural that each glandular organ begins its secretion parallel with the beginning of the translocation of the spermatozoa from the testis, and that the secretion of each glandular organ except the ductus deferens occurs periodically in connection with the ejaculation. That the glandula prostatica is extremely active in periodical secretion, and that this secretion is the only one necessary to the fertilization, are especially interesting points when one considers the polygamous habit of *Bombyx mori*.

PAYNE (1933) working on the behaviour of the spermatozoa in Orthoptera (*Cortophaga viridifasciata*) showed that spermatozoa are active when they are in the course of emergence into the ductus deferens from the testis, while they remain inactive in post-testicular organs. He failed to activate the spermatozoa by any method. The present author (1936b) found that spermatozoa are active when they pass from the lobulus testis into the ductulus efferens testis but become quiescent in the ductulus efferens. The quiescent spermatozoa are proved to have life when they are activated by the secretion of the glandula prostatica, otherwise there is no way to prove the life of the spermatozoa.

The active spermatozoa are not always capable of fertilisation. Such is seen in an unhealthy moth.

The presence of the glandula prostatica was first noted by the

present writer in 1932 in *Bombyx mori* and this led him to find an easy method of artificial insemination (1936a). The investigation on the nature of the prostatic secretion from the physicochemical point of view in connection with the activity of the spermatozoa will be highly suggestive in the general physiology of the reproduction of insects. A substance of the same nature as the prostatic secretion of *Bombyx mori* exists in the male reproductive system of other Lepidopteran insects. The modes of activation of spermatozoa<sup>1)</sup> differ a little according to the species. As to the prostatic secretion the present writer found positive results of interaction respectively between *Bombyx mori* and *Bombyx mandarina*, *Bombyx mori* and *Lymantria dispar*, *Bombyx mori* and *Colias hyale poliographus*, *Bombyx mori* and *Pieris rapae*. A negative result was obtained between *Bombyx mori* and *Apha tychoona*.<sup>1)</sup>

When the secretion of the glandula lacteola deteriorates the spermatozoa die in the post-testicular system. This is considered to be caused simply by rapid and abnormally violent movement of the spermatozoa but not by the aging.

According to MICHAEL (1923) who worked on ejaculation in *Bombyx mori* by sectioned preparations, the contents of the testis as well as the ductus deferens are ejected by ejaculation. Having observed the process of copulation he said that the penis penetrates into the bursae of the female 3 or 4 minutes after the beginning of the copulation and remains in that position for 30 or 40 minutes. Moreover he observed that the musculus sphincter penis, designating it "Penisbulbus," is elongated during ejaculation and plays the rôle of "das Ueberpumpen des Spermas durch das Penisrohr" and acts as "das Gummihütchen einer Pipette." Then, 45 minutes after the beginning of the copulation, in the vesicula seminalis and in the azygotic duct<sup>2)</sup>, the substance of the accessory gland<sup>3)</sup> is found which was called by him a "Spülmasse" of the male genital tube, considering it as the constituent matter of the "Begattungspfropt,"<sup>4)</sup> while he found "Begattungspfropt" in the female which had mated for 45 minutes. It is almost impossible to obtain the exact natural posture of copulation by means of sectioned preparations, which suffer so many treatments such as fixation, inbedding, cutting etc.

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1) The details will be published in a later paper.

2) "Ductus ejaculatorius" in his nomenclature.

3) The term "accessory gland" indicates the present writer's glandula lacteola and glandula pellucida.

4) This seems to be a synonym of spermatophragma.

Therefore his findings are so far not complete.

On the other hand MACHIDA and WATANABE (1927) observed ejaculation experimentally in the living moths of *Bombyx mori*. They found that the male is stimulated to ejaculate by 2-5 minutes mating and that the ejaculation is finished some 30 minutes after the beginning of the copulation. The present research agrees with them and verifies their finding that the male is stimulated at 25°C by some 5 minutes mating to make an ejaculation which commences at 5-10 minutes after the beginning of the copulation and lasts for 25-30 minutes.

The spermatozoa ejaculation and mechanism of the spermatophora formation have for the first time been treated precisely in the present work. Previous authors have supposed that the spermatophora is formed before spermatozoa ejaculation (PETERSEN, 1908. KLATT, 1920. MICHAEL, 1923). But the present work confutes that idea absolutely, indicating that the two processes occur simultaneously.

MICHAEL considered the spermatophora-forming substance as originating from the azygotic duct and the "Begattungspfropf"-forming substance from the accessory gland. ELTRINGHAM (1925) also came to the same conclusion as MICHAEL's and said that the source of the sphragis (spermatophragma) of *Parnassius apollo* is the fluid of an accessory gland. However, neither of them gave any exact description of their observation.

According to UMEYA (1926), in *Bombyx mori* male sterility is caused by the abnormality of the musculatures controlling the movement of the penis. Judging from the present study any minute disturbance occurring in such complex and delicate organs as those of the male reproductive system of *Bombyx mori* may easily cause a complete sterility.

## XI. Conclusion

The male reproductive system of *Bombyx mori* can be divided morphologically as well as physiologically into nine distinct parts; testis, ductus deferens, glandula pellucida, glandula lacteola, vesicula seminalis, glandula spermatophorae, glandula alba, glandula prostatica and penis. Particular attention was paid to the post-testicular organs which are made of an outer thin layer of circular and longitudinal muscles, tunica propria and inner glandular cell layer except the penis which is composed of chitin membranes and muscles. In the course of the ejaculation the movement caused by the muscles advances the contents

along towards the penis. The function of each post-testicular organ and its secretion has been determined as follows.

Name of organ	Function of organ	Function of secretion
Ductus deferens	Transportation of the sperm from testis to vesicula seminalis.	Seems to prevent the senile decay of the spermatozoa in the post-testicular system, but not necessary to fertilization.
Pars ampullaris	Storage of the sperm which is not stored in the vesicula seminalis.	As above.
Vesicula seminalis	Storage of the sperm which is to be used in an ejaculation.	As above.
Glandula pellucida	No particular function in relation to the spermatozoa.	Spermatophragma-forming material. Not necessary to fertilization.
Glandula lacteola	As above.	May be nutrient of the spermatozoa in female genital system. Not necessary to fertilization.
Glandula spermatophorae	Has some connection with ejaculation especially with spermatophora formation.	Spermatophora-forming material. Not necessary to fertilization.
Glandula alba	As above.	Forms the pearly body attached to the spermatophora. Not necessary to fertilization.
Glandula prostatica	Has some connection with spermatophora-formation.	Activates the spermatozoa fully matured and gives them fertilizing ability. Essential to fertilization.
Penis	Indispensable to mating and ejaculation.	
Ductus ejaculatorius	Indispensable to ejaculation and spermatophora-formation.	
Musculus sphincter penis	Necessary to spermatophora formation.	
Musculus internus penis	Assists ejaculation.	

The secretion of each organ begins at the old pupal stage some 4 days before emergence of the moth nearly when the translocation of spermatozoa from testis to post-testicular organs occurs, but declines remarkably on the day before emergence. However the secretion is recovered as often as ejaculation is repeated except in the ductus deferens.

The prostatic secretion is soluble in water and tolerably stable against heat treatment.

At the temperature of 25°C some 5 minutes copulation is enough to cause the male to ejaculate. Ejaculation of the semen generally begins at 5-10 minutes after the beginning of the copulation and lasts for some 25-30 minutes, but the proper ejaculation of the spermatozoa which commences some 15 minutes after the beginning of the copula-

tion lasts for some 10 minutes.

Ejaculation is pursued under the cooperation of the peristaltic movement of muscles of each organ and the function of the penis which is very complex. The formation of the spermatophora is mainly controlled by the musculus sphincter penis and the ductus ejaculatorius, and occurs simultaneously with spermatozoa ejaculation.

No indication of aging of the spermatozoa is found in the post-testicular system. The spermatozoa remaining unejected in the male sexual system die owing to the sudden extinction of energy by a violent movement which is brought about by a change occurring in the lactean secretion when the moth approaches death.

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### XIII. Explanation of plates

#### Plate I

- Fig. 1. Longitudinal section of the ductus deferens of pupa the day before emergence, showing spermatozoa in the cavity and several giant vacuoles. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 2. Another figure from the same section as Fig. 1. Shows a giant vacuole and glandular cells containing small granules on their innermost side. The cavity contains spermatozoa, which are in bundles or separated, as well as secretion from the glandular cells.  $\times 480$ .
- Fig. 3. Ampulla ductus deferentis of one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 4. Longitudinal section of a malformed middle portion of the ductus deferens. CARNOY, DEL.-haem.  $\times 70$ .
- Fig. 5. Cross section of the glandulae lacteolae of pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 6. Cross section of the boundary portion between glandula lacteola and vesicula seminalis of pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 7. Longitudinal section of the inner process on the glandula lacteola of pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 8. Cross section of the same portion as Fig. 7. One day old moth 3 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 9. Glandula lacteola of a one day old moth immediately after a 50 minutes mating. Shows a giant vacuole, which has not secreted out the contents in the course of ejaculation. CHAMPY, HEID.-haem.  $\times 480$ .
- Fig. 10. Longitudinal section of proximal part of glandula lacteola of pupa the day before emergence, showing the spermatozoa transferred. Large black masses are cross sections of sperm-bundles. CHAMPY, HEID.-haem.  $\times 180$ .
- Figs. 11, 12. Glandula lacteola of a one day old moth, immediately after a 50

- minutes mating. The former shows nuclei stained with eosin, the latter those stained with haematoxylin. CHAMPY, HEID.-haem. and eosin.  $\times 480$ .
- Fig. 13. Longitudinal section of the glandula lacteola of one day old moth immediatly after a 50 minutes mating. Shows degenerated nuclei. CHAMPY, HEID.-haem.  $\times 480$ .

## Plate II

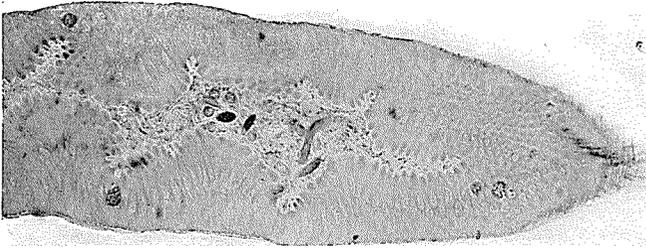
- Fig. 14. Glandula pellucida of a one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem. and eosin.  $\times 480$ .
- Fig. 15. Glandula pellucida of a one day old moth at 3 hours after a 50 minutes mating, showing degenerated cells and secreted matter. CHAMPY, HEID.-haem. and eosin.  $\times 110$ .
- Fig. 16. Longitudinal section of the portion of the vesicula seminalis containing globular secretion. Pupa the day before emergence. CHAMPY, HEID.-haem. and eosin.  $\times 480$ .
- Fig. 17. Longitudinal section of the portion of vesicula seminalis that contains spermatozoa and secretion. One day old moth at 3 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 480$ .
- Fig. 18. Longitudinal section of the portion of vesicula seminalis containing many spermatozoa. A one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 19. Longitudinal section of the portion of vesicula seminalis containing much secretion and some spermatozoa, the latter of which is not manifest in the figure. One day old moth at 3 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 20. Longitudinal section of a portion of the vesicula seminalis containing eosinophile secretion. One day old moth at 2 hours after a 50 minutes mating. Horizontal brush-like traces are spermatozoa. CHAMPY, HEID.-haem. and eosin.  $\times 480$ .
- Fig. 21. Longitudinal section of the azygotic portion of the glandula spermatophorae of the pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 22. Longitudinal section of glandula spermatophorae of a one day old moth at 3 hours after a 50 minutes mating. A rather large quantity of spermatozoa and a slight quantity of viscous secretion are found. CHAMPY, HEID.-haem.  $\times 480$ .
- Figs. 23, 24. Cross sections of zygotie portion of the glandula spermatophorae of a pupa the day before emergence. CHAMPY, HEID.-haem. Fig. 23,  $\times 180$ . Fig. 24,  $\times 480$ .
- Fig. 25. Longitudinal section of glandula alba of a pupa the day before emergence. CHAMPY, HEID.-haem.  $\times 110$ .

## Plate III

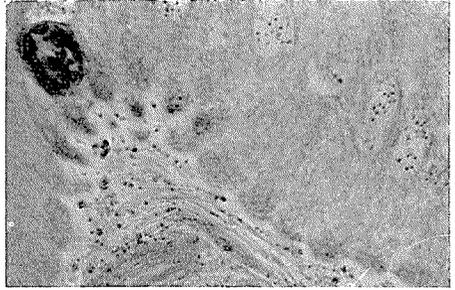
- Fig. 26. Cross section of the portion of glandula alba showing the inner process. One day old moth at 3 hours after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 110$ .
- Fig. 27. Cross section of the glandula alba of a one day old moth at 2 hours after a 50 minutes mating. CHAMPY, HEID.-haem. and eosin.  $\times 180$ .
- Fig. 28. Longitudinal section of glandula alba of a one day old moth at 2 hours

after a 50 minutes mating. CHAMPY, HEID.-haem.  $\times 480$ .

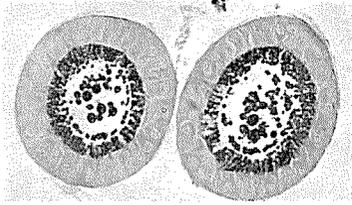
- Fig. 29. Cross section of the glandula prostatica of a pupa the day before emergence. Notice the secreting drops which are emerging into the cavity. CHAMPY, HEID.-haem.  $\times 180$ .
- Fig. 30. Another part of the same preparation as Fig. 29.  $\times 480$ .
- Figs. 31-33. Cross sections of the glandulae prostaticae of one day old moths. CHAMPY, HEID.-haem. Fig. 32 is immediately after, Figs. 31, 33 are 2 hours after a 50 minutes mating. Figs. 31, 32,  $\times 480$ . Fig. 33,  $\times 180$ .
- Figs. 34, 35. Longitudinal and cross sections of the boundary portion between the glandula prostatica and penis of one day old moths, immediately after a 50 minutes mating. CHAMPY, HEID.-haem. Fig. 34,  $\times 180$ . Fig. 35,  $\times 110$ .
- Fig. 36. Longitudinal section of the glandula prostatica of ca. 10 hours old moth. Immediately after a 20 minutes mating. Shows a part of the substance flowed down from the glandula spermatophorae making a large white mass and the semen which contains many sperm-bundles. CARNOY, DEL.-haem.  $\times 110$ .
- Fig. 37. Cross section of the distal portion of corpus penis of the same individual as that of Fig. 36. Shows the ductus ejaculatorius containing a proto-spermatophora and semen. e...ductus ejaculatorius with the epidermis, 1...proto-spermatophora, 2...semen.  $\times 110$ .
- Fig. 38. Penis top of a living individual. Immediately after a 20 minutes mating.  $\times 65$ .
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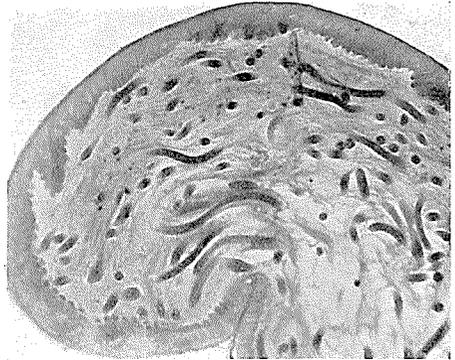
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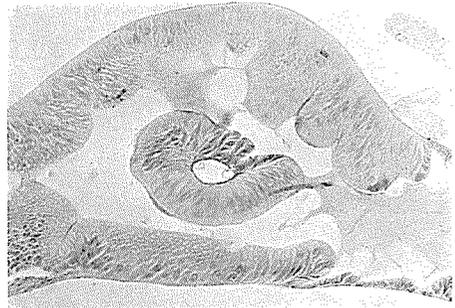
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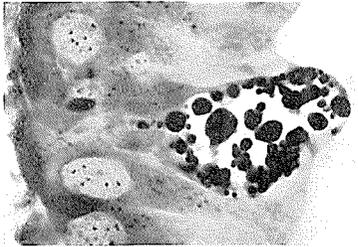
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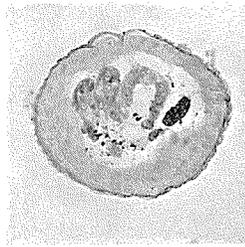
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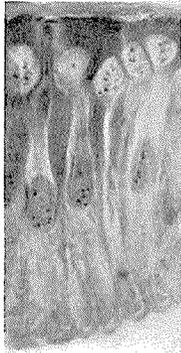
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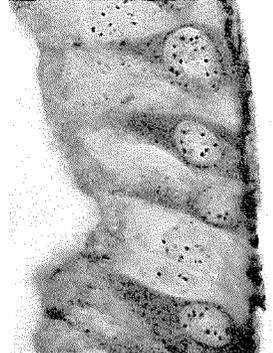
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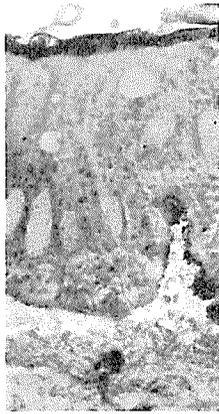
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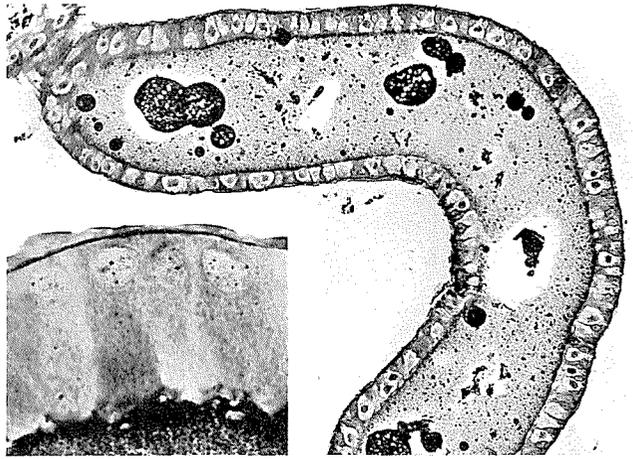
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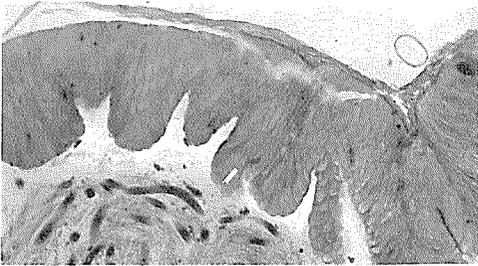


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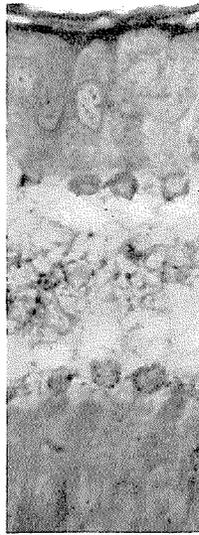


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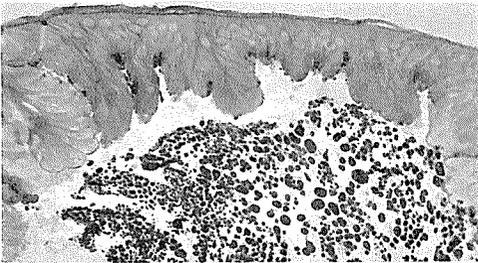
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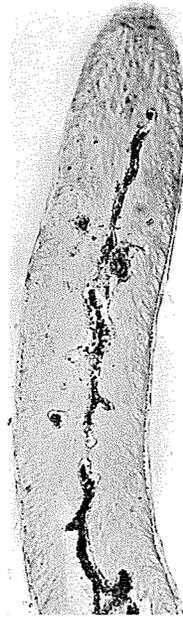
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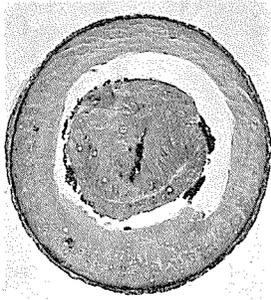
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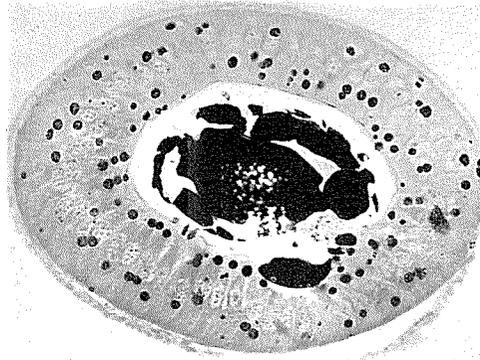
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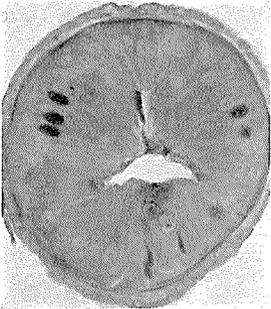
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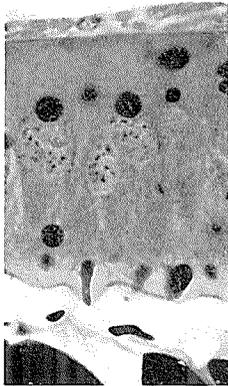
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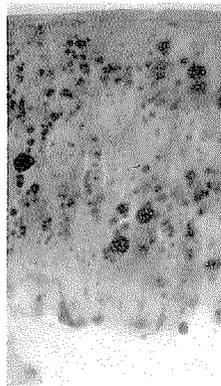
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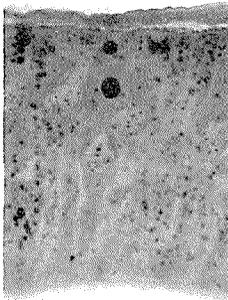
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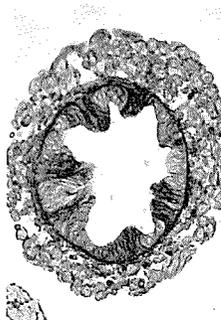
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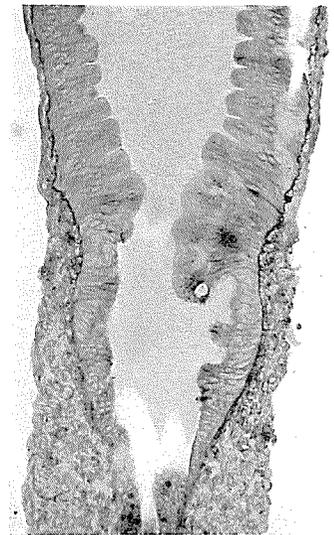
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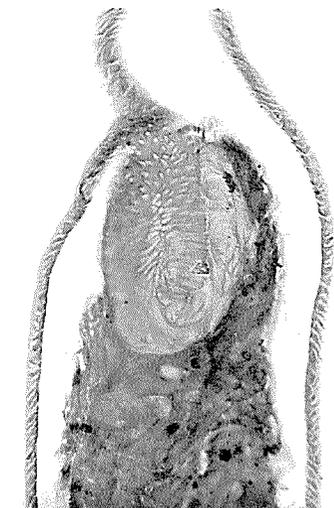
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