



Title	STUDIES ON THE REPRODUCTIVE SYSTEM OF THE MALE OF BOMBYX MORI : . STRUCTURE OF THE TESTIS AND THE INTRATESTICULAR BEHAVIOUR OF THE SPERMATOZOA
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Citation	Journal of the Faculty of Agriculture, Hokkaido Imperial University, 38(2), 151-181
Issue Date	1936-10-25
Doc URL	http://hdl.handle.net/2115/12711
Type	bulletin (article)
File Information	38(2)_p151-181.pdf



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

I. STRUCTURE OF THE TESTIS AND THE INTRATESTICULAR BEHAVIOUR OF THE SPERMATOZOA

By

Seinosuke ÔMURA

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I. Introduction

Since the middle of the last century, many authors have described various forms of testis in insects. But most of the investigations were limited to the macroscopical features of the reproductive

organs. In the microscopical field, the works of CHOLODKOWSKY on Lepidopteran ('86) and Dipteran testes ('05) are representative ones. His works are those of extensive nature, and, for that reason, it is not possible for us to obtain a detailed knowledge of the insect testis.

However, within the last thirty years little addition has been made to the results of CHOLODKOWSKY's work on the structure of the testes in insects. During this period numerous papers on karyology and spermatogenesis have been published, but our knowledge of the minute structure of the testis remains little more than a blank. For this reason, little information is available concerning, for example, the nutrition of testis or the intratesticular behaviour of the spermatozoa. Occasionally opinions have been expressed on the former problem by ST. GEORGE ('98), GRÜNBERG ('03) and CHOLODKOWSKY ('05), all of whom recognized the "Apikalzelle" of *Bombyx mori* to be the source of nutrition in spermatogenesis, by CHOLODKOWSKY ('05) who found the "Nutrientzelle" in Dipteran testis. On the latter problem light has been thrown by PAYNE ('33), who discovered interesting facts concerning the movements of spermatozoa inside the Orthopteran testis, and by IKEDA ('13) who followed the course of the spermatozoa in *Bombyx mori* from the testis to the ductus deferens and found a special path for escape of the sperm in the membrane between the follicle and the ductulus efferens testis.

The writer's efforts were directed towards securing detailed knowledge of the histological structure of the testis and of the intratesticular behaviour of the spermatozoa.

For many valuable suggestions and criticism the writer is indebted to Prof. E. KAWAGUCHI, under whose guidance the work has been carried out. He is also indebted to Prof. T. INUKAI, who kindly read through the manuscript before publication and offered helpful criticism.

II. Material and Method

The material used in these investigations consisted of various races of *Bombyx mori* cultured in the Sericultural Laboratory of the Hokkaido Imperial University. The age of the pupa or imago was calculated under the normal temperature of about 25°C.

The development, after the fifth larval stage, varies in different races and sexes of the silkworm and is affected by the environmental conditions also. Of recent materials (male only) it is shown roughly as follows (in 25°C).

5th larval stage (up to the pupation)	11-12 days
Full grown	7-8th day
Beginning of the spinning	8-9th day
Pupal stage	12-14 days
Adult	5-8 days

The constructive age of the pupa, *e. g.*, five days before emergence etc., was calculated counting backward from the emergence day of the standard pupae which had been selected as a control at the same stage as the experimented pupae.

The estimation of the pupal or the imaginal age was important for this work. In this respect *Bombyx mori* proved to be a suitable material, because material of the same age could easily be procured.

Observations were made *in vitro* as well as upon sectioned preparations. CARNOY, BOUIN and CHAMPY's fluids were used as fixatives.

Experiments were undertaken employing the second glass tubule method of artificial insemination (*cf.* ÔMURA, '36).

III. Structure of the Testis

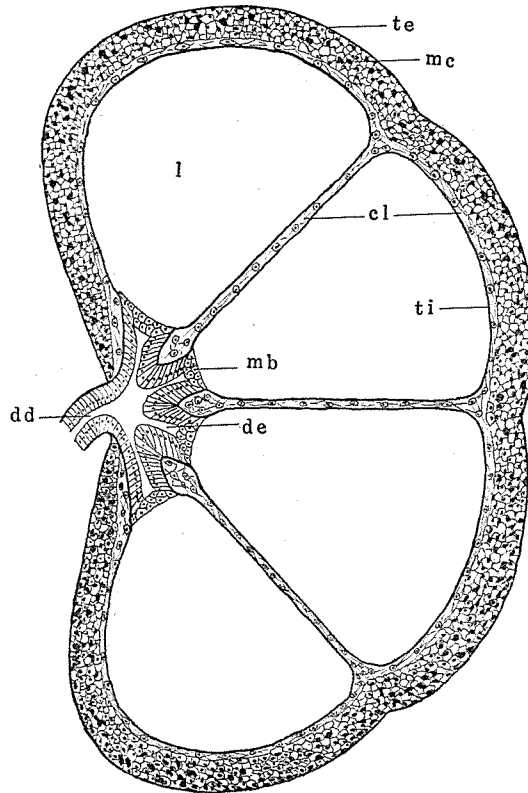
A pair of testes are situated in *Bombyx mori* on either side of the dorsal vessel in the fifth abdominal segment, without being united into a single mass as is the case in some species.

The testis is usually made up of four lobuli testis and ductuli efferentes testis. Each lobulus is bounded by the capsula lobuli and lined internally by the tunica interna, its opening into the ductulus efferens being closed by the membrana basilaris. The four lobuli and their ductuli are covered by the membrana communis and the tunica externa; thus constituting a testis (Text-Fig. 1). The former membrane stops short at the beginning of the ductus deferens (Figs. 8, 11), where the ductuli efferentes unite to form the ductus deferens.

In the pupal stage the lobuli swell out but during the larval stage they have a kidney-shape form. The size of a testis varies with the

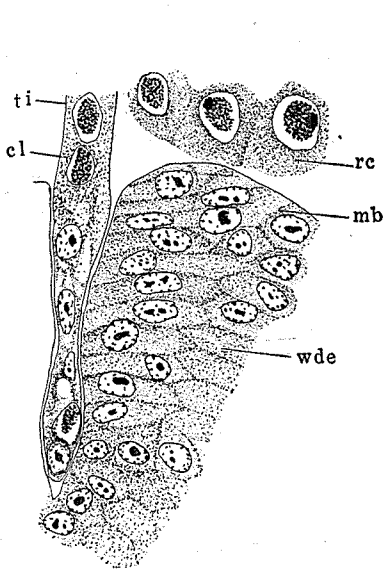
race of the worm and also with its age. The maximum size of a testis in a Chinese race of these worms measured on an average 3.94 mm in length, 2.05 mm in width and 1.9 mm in height.

The tunica externa is a very thin structureless membrane enveloping the testis (Fig. 1). It extends up to the ductus deferens beyond which it extends as its tunica propria (Fig. 11). The tunica externa is not a permanent covering because it breaks down during the pupal stage. Its break-down begins in a two day old pupal testis and becomes noticeable in a seven day old one; in the imaginal testis no tunica externa exists (Figs. 2-4).

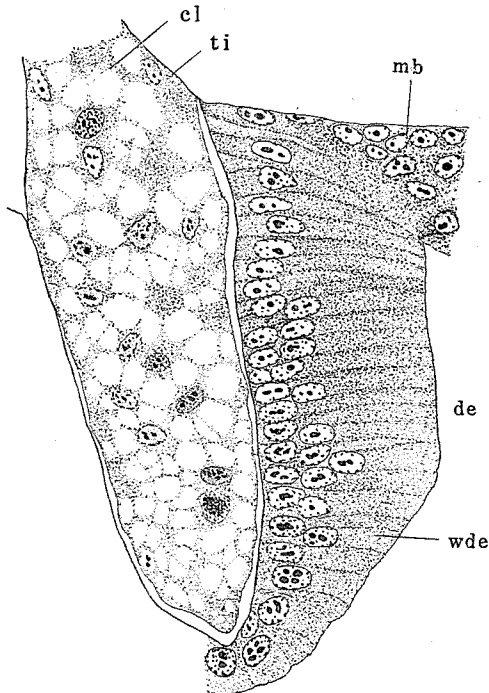


Text-Fig. 1. A schematic figure of testis of a full grown larva. cl...capsula lobuli, dd...ductus deferens, de...ductulus efferens testis, l...lobulus testis, mc...membrana communis, mb...membrana basilaris, te...tunica externa, ti...tunica interna. ca 30×.

The membrana communis lies just under the tunica externa and envelopes the whole testis. It is thick and made up of numerous cells, laden with fatty substances, which lie irregularly. The fatty substances contained in this layer were found to be abundant in the full grown larva, but diminished during the pupal stage (Figs. 1-3). In the adult testis the cells constituting the membrane are completely collapsed (Fig. 4).



Text-Fig. 2. Wall of ductulus efferens testis of 3 day old larva of 4th stage, cl...capsula lobuli, mb...membrana basilaris, rc...germ cell, ti...tunica interna, wde...wall of ductulus efferens. 1200 \times .



Text-Fig. 3. Wall of ductulus efferens testis of 3 day old larva of 5th stage. 1200 \times .

The capsula lobuli is the third layer in succession composed of connective tissue. It has three ramifications and divides the cavity of the testis into four lobuli (Text-Fig. 1, Fig. 8). The end of each ramification is wider, of which a cross section shows a deltaic shape (Figs. 8, 10, 11), and it also provides the basis for the wall of the ductulus efferens (Text-Figs. 2, 3, Figs. 8, 10, 11).

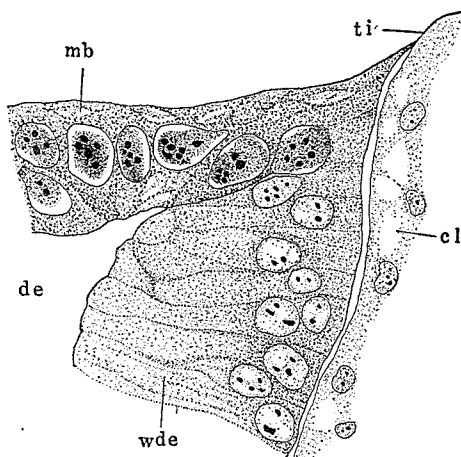
In the fully grown larval testis, numerous fatty granules are found everywhere inside the capsula lobuli which diminish as the

pupa advances in age and in correspondence with the reduction of the fatty substances in the cells of the membrana communis. In the imaginal testis there were found no such granules (Figs. 1-5).

The tunica interna is the innermost membrane. It lines the capsula lobuli, of course the deltaic part of it too, completely without a break. Its extremity envelops the ductuli efferentes and finally unites with the tunica externa, being transformed into the tunica propria of the ductus deferens (Text-Fig. 1, Fig. 11).

The tunica interna is a thin structureless membrane like the tunica externa, but it is easily recognisable in cross sections. It is permanent and never breaks down in the adult (Fig. 4).

The membrana basilaris is the septum between each lobulus and its ductulus efferens. It is independent of the four membranes mentioned above, in its origin as well as function, but is closely related to the ductulus efferens.



Text-Fig. 4. Wall of ductulus efferens testis of 2 day old pupa. 1200 \times .

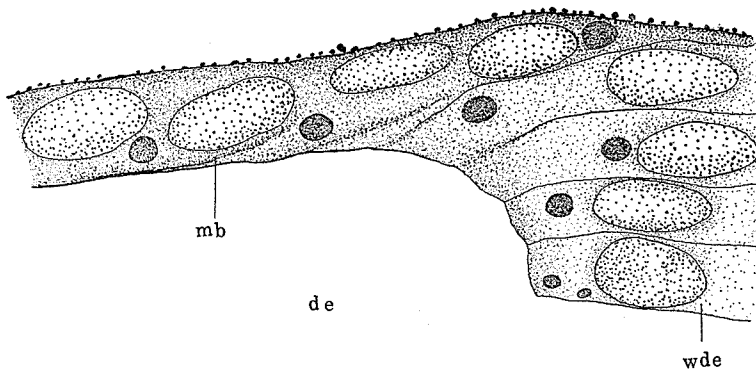
In larval testis, there was found no difference between the membrana basilaris and the ductulus efferens (Text-Figs. 2, 3, Fig. 8). But in a 2-3 day old pupa, a differentiation became noticeable between them: the nucleus of the former appearing to be degenerating with its chromatic substance coagulated, while in the latter no change

occurred (Text-Fig. 4, Fig. 10). In the testis of an eight day old pupa the nuclei of the cells of the membrana basilaris again assume normal appearance and the cells contain some secretory matter akin to that in the cells of the ductulus efferens (Text-Fig. 5). The reversionary process was however not followed.

The membrana basilaris becomes thinner and wider with advancing age and in an old pupa the cells are not disposed in a double layer (Figs. 12, 21, 22).

This membrane shows peculiar characteristics during the passage of the spermatozoa from the lobulus to the ductulus efferens (*v. p.* 161).

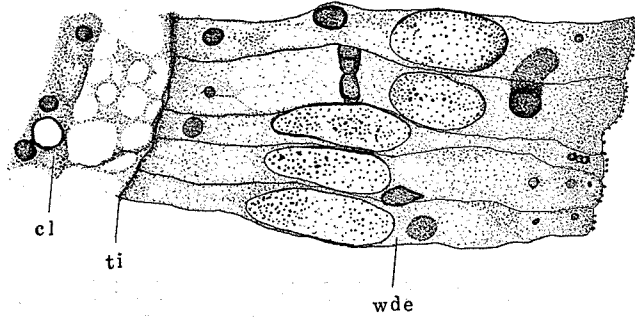
The ductulus efferens testis is the small duct belonging to each lobulus testis. The lumen of the ductulus, which is so narrow in the larval stage as to be hardly recognisable (Fig. 8), widens with age (Figs. 10-14, 22), and in the imaginal testis becomes very much wider. In the old adult (6-7 days old), in which nearly all of the spermatozoa have been discharged the junction of the four ductuli has large dimensions (Fig. 21). In such an adult testis, the ductuli become short and wide flattening out their walls parallel to the membrana basilaris.



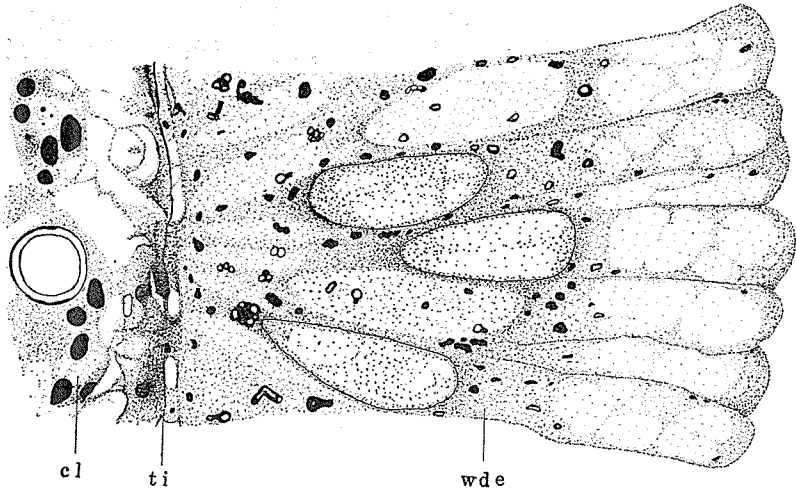
Text-Fig. 5. Wall of ductulus efferens testis of 8 day old pupa. 1200 \times .

The wall of the ductulus is made up of glandular cells, which increase in size during the successive pupal stages (Figs. 8, 10-12). The secretion of the cells begins 6-7 days before the emergence of the imago and reaches its maximum when the passage of the spermatozoa begins, *i. e.*, 3-4 days before the emergence, and then diminishes gradually (Text-Figs. 5-7). In a six day old adult, that

is just before the end of its life, little secretion was found inside these cells (Text-Fig. 8).



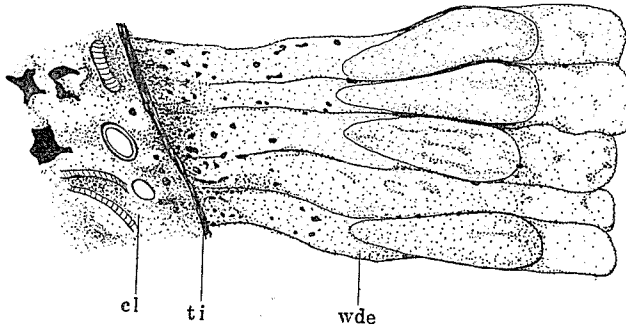
Text-Fig. 6. Wall of ductulus efferens testis of 9 day old pupa. 1200 \times .



Text-Fig. 7. Wall of ductulus efferens testis of pupa 1-2 days before emergence. 1200 \times .

On the whole, though the secretion in the cells of the ductulus efferens begins 2-3 days before the commencement of the passage of the spermatozoa (*cf.* p. 160), the rise and fall of the secretion corresponds roughly with the passage and discharge of the spermatozoa.

The source of the secretion seems to be the fatty substance contained in the capsula lobuli, on which the glandular cells stand. Text-Fig. 7, also Fig. 20, shows the close relation between them. The



Text-Fig. 8. Wall of ductulus efferens testis
of 6 day old imago. 1200 \times .

function of the secretion will be dealt with at a later stage in this paper.

Numerous granules were found in the cavity of the lobuli of old pupae from whose capsula lobuli the fatty granules had disappeared (Figs. 12 and 22). Such granules were also found in the lobuli of the young pupa as well as of the full grown larva in whose capsula lobuli they are contained abundantly (Figs. 3, 6, 7). On the inner surface of the tunica interna also numerous such granules are found (Figs. 3, 5-7). Some of them may be those which have adhered to the tunica at the moment of fixation, but some are probably those just secreted through the tunica interna. The fatty reaction of this substance to osmic acid was negative. It is also notable that in 7 day old pupae, that is 6-7 days before the emergence of the imago, abundant fluid was found in the lobuli testis near the membrana basilaris (Fig. 9). It was not of fatty nature. Its origin as well as its relation to the secretion found in the membrana basilaris in young pupa is unknown to the writer (*cf.* Text-Fig. 5).

IV. Intratesticular Behaviours of the Spermatozoa

1. Mechanism of the passing out of the spermatozoa from the lobulus testis to the ductulus efferens testis through the membrana basilaris

The time of the passage of the spermatozoa from the lobulus testis to its ductulus efferens was determined by means of *in vitro* observation as well as by the study of sections. In the former

investigation, the existence of the spermatozoa in the ductus deferens was examined, because it is difficult to take out a ductulus efferens alone. The results are summarized in Table 1, where it is shown that the spermatozoa commenced coming out from the lobulus in the pre-imaginal stage: to be more precise, spermatozoa begin their exit in the pupae 3-4 days before the emergence of the imagos. The results obtained from sections confirm this view (Figs. 12, 14, Table 2).

TABLE 1.

Beginning of the translocation of the spermatozoa from the testis to the ductus deferens. (Observation *in vitro*).

Material: Kokusan-Si-101

Stage	Number of indvl's	Number of indvl's, in whose duct. def. there are found	
		sperm	no sperm
Pupa, 5 days before emergence	5	0	5
" , 4 "	5	2	3
" , 3 "	5	5	0
" , 2 "	5	5	0

TABLE 2.

Beginning of the translocation of the spermatozoa from the lobulus to the ductulus efferens testis.
(Observation in sections).

Material: Kokusan-Si-101

Stage	Number of indvl's	Number of indvl's, in whose ductul. eff. there are found	
		sperm	no speam
Pupa, 5 days before emergence	5	0	5
" , 4 "	5	3	2
" , 3 "	5	5	0

The mechanism of the escape of the spermatozoa into the ductulus efferens was investigated only from sections.

In a pupa, six days before emergence the spermatozoa lie scattered in the cavity of the lobuli testis in the form of bundles. In a two days older pupa (*i. e.*, four days before emergence) abundant sperm bundles with the heads of the spermatozoa turned towards the membrana basilaris are noticed (Fig. 12). The passage of the spermatozoa into the ductulus efferens is now imminent.

In a testis of a pupa a day older than the previous one, many sperm bundles are found to be passing out through the membrana basilaris (Fig. 14). These spermatozoa look as if they were hanging into the ductulus efferens from the membrane by their heads with tails streaming behind them (Figs. 13 and 16).

Such appearances are presented by the testes of subsequent stages also, although the number of sperm bundles declines with the age of the pupa. And even in a 6-7 day old imago, which is about to die, a few sperm bundles may be found passing out in this manner (Fig. 21).

No special pathway for the sperm bundles was found in the membrana basilaris. In the oldest imaginal testis, from which most of the spermatozoa had emerged and which contained very few sperm bundles, resulting in a significant shrinkage of the lobulus, perfect membranae basiliares were found, though they had been stretched nearly to the fullest extent, and no pathway was found in any case. Fig. 21 shows one of such testes.

The question arises as to how the spermatozoa pass through the membrana basilaris, causing no rupture in its structure.

The figures in Plate II and III solve the question clearly.

In these figures, one can see the sperm bundles perforating respectively at many points on the membrana basilaris and slipping out through these into the ductulus efferens. One will notice a peculiarity on comparing Fig. 12 with Fig. 14; in that the direction of the head of the sperm bundle is quite opposite in the beginning and at the end of the passing out. The sperm bundles are in descending posture at the beginning of their emergence, with their heads turned towards the membrana basilaris, but in the end the tails of the spermatozoa slip out into the ductulus efferens, while the heads remain in the cavity of the lobulus. That is, the spermatozoa reverse their direction in the course of passing out.

The writer has traced the process of the reversion in a series of figures following. The sperm bundles which have collected at the membrana basilaris keep their heads close but not attached to it (Fig. 12). Then the tail is bent round 180° and over (Fig. 28). The bending extends to the anterior part, until at last the direction of the head is turned ('a' in Fig. 22). During that time the head scarcely changes position. That is, a bundle makes a vibration, the head playing the part of the centre. Thus in a moment of vibration the middle part of the tail takes the lead ('a' in Fig. 22). And, at last, the tail touches the membrana basilaris ('b' in Figs. 15, 16, 18, 19).

The tail of the spermatozoa must have no rigidity, therefore, though they tie up into bundles, it will be scarcely possible for them to perforate their own pathway through the membrane in such a manner as a person would stick a needle into cloth, no matter how tender the membrane may be.

The mechanism of the perforation was followed as shown in other figures. The part which at first reaches the membrana basilaris is not the tail end but the portion a little anterior to the end, as is suggested by Fig. 22 ('a'). In fact, the tail part makes an arc at the moment when it touches the membrane. Many of these figures are found in 'b' of Figs. 13, 15, 16, 18 and 19. The very elastic motion of the stretching moment of the arc will make the perforation through the cells of the membrana basilaris. 'b' in the figures indicated above shows the imminent stretching motion of the arc. The moment just next to it must be 'c' in Fig. 19, at which a part of the tail is pushed out keeping the bending posture, and followed up by the successive postures in Fig. 16 ('d'), 17 ('e'), 18 ('f') and 19 ('g'). The moment when the arc is stretched is shown in Fig. 23. Therefore, the part which slips out first is not the tail end, but a point slightly anterior to it. In fact, the writer has neither found a tail part which has slipped out into the ductulus efferens in a stretched form shorter than that as seen in Fig. 23, nor has he caught any figure showing that only the tail end had just slipped out.

Apyrene spermatozoa were generally not found outside the testes, but an exceptional case was met with in which an ampulla ductus deferentis contained a mass of the apyrene spermatozoa and residue of the sheaths of the sperm bundles (Fig. 25). A view of an escaping apyrene sperm bundle was caught in Fig. 24.

As regards the sheath of the sperm bundle, the following facts were observed. The sperm bundles lying inside the lobulus have a complete sheath (Fig. 28), but those that are lying in the ductulus efferens do not possess it (Fig. 16). (Some sperm bundles found on the inner side of the membrana basilaris of Figs. 15-19 appear to have no sheath, owing to the pellucidity of the sheath. In general the sheath is recognized by its nuclei). On the inner side of the membrana basilaris the sheaths accumulate as more and more of the spermatozoa slip out of it (Fig. 16). These three figures lead one to suggest that the sheath is cast off in the course of the passing out of the spermatozoa through the membrana basilaris.

The process of the casting off of the sheath was traced as follows. Fig. 23 shows a sperm bundle, of which a part has slipped out without the sheath, while a part remains inside the lobulus covered with the sheath, which has a somewhat shrunken shape having been partially thrust back towards the head. As the spermatozoa escape out, the sheath shrinks until it is no more than a structureless mass (Figs. 24 and 27). The casting off of the sheath begins at the tail end of the spermatozoa and extends up to its head. The cast off sheaths are left behind inside the lobulus on the membrana basilaris. Fig. 26 shows the sheath enveloping the sperm bundles and also cast off sheaths in which no spermatozoa can be detected.

The position where the perforation is made, that is whether in the cytoplasm or on the boundary of cells, could not be determined accurately in the present work. That no nucleus is ever affected by the passage of the spermatozoa is clear from Figs. 15 and 23.

The fate of the foramina could not be clearly ascertained. But the writer supposes from Figs. 16, 24 and 27 that they may be plugged or covered with the sheaths which have been left behind after the escape of the sperm bundles.

As mentioned above, spermatozoa begin to pass out of the testis in the advanced pupal stage and continue to do so till the death of the individual. But during that time the formation of the spermatozoa does not accompany the process. Thus the contents of the testis diminish with age, resulting in the reduction of the size of it.

The writer has measured the testis by weight. As it is hard to gain the real weight accurately *in vitro* because of the difficulty in removing the lymph from the testis surface, the relative weights were measured after a fixation during thirty minutes by CARNOY'S

fixative. Results are summarized in Table 3. There it is shown that the ratio of the weight of the testis of the pupa four days before emergence (of which no spermatozoa has passed out) to that of the moth just emerged to that of the one four days old is 100: 53: 15. In the testis of a four day old moth, there are found few normal spermatozoa so that its value expresses roughly the relative weight of the testis alone. Consequently the ratio of the sperm quantity contained in the above three testes is shown roughly as (100-15): (53-15): (15-15) = 85:38:1. That is to say, the majority of the spermatozoa have already slipped out when the moth emerges. Also it is shown that copulation has no connection with the speed of the passing out of the spermatozoa.

TABLE 3.
Weight of the testis.¹⁾

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

Stage of the individuals	Date	No. of testes	Weight (gr.)	Weight per testis (gr.)	Ratio	Remark
Pupa, 4 days before emergence	24/X, '34	20	0.212	0.0106	100	
„ , 1 day „	27/X, „	20	0.178	0.0089	83	
Moth, 1 day old	28/X, „	20	0.112	0.0056	53	non-mated
„ , 2 days old	29/X, „	20	0.076	0.0038	36	„
„ , 3 days old	30/X, „	20	0.062	0.0031	29	„
„ , 3 days old	„	20	0.059	0.0030	28	mated 40 minutes on 29/X
„ , 4 days old	3/XI, „	20	0.031	0.0016	15	non-mated

1) The weighing was done after 30 minutes fixation by CARNOY's fluid.

2. Activity of the spermatozoa in the testis

The above mentioned facts enable us to conclude that the passing out of the spermatozoa is due to their own movements and not to any other agency. It seems, therefore, doubtless that the spermatozoa are active at least during the course of the passing out.

Hence the writer has attempted to trace the time of the beginning of the movement and to catch the moment of the end of it. For this

purpose spermatozoa in the testis of different stages were observed *in vitro*. The results are summarized in Table 4. There was found no motile spermatozoon in the testis of young pupae, but some motile ones were found in the old pupal testis where the passing out is about to take place. They showed a weak, yet distinct vibratile movement of the tail.

TABLE 4.

The activity of the spermatozoa in the lobulus testis of different stages.

Stage	No. of indvl's	No. of indvl's, whose spermatozoa are		
		-	+	++
Full grown larva	10	10	0	0
2 day old pupa	10	10	0	0
6 day old pupa	10	10	0	0
10 day old pupa (4 days before emergence)	10	4	6	0

-....no motile spermatozoa, +....several motile spermatozoa per field,
++....many motile spermatozoa per field.

Next, the fate of the activity which is shown in the course of the passing out was studied. In sectioned preparations the spermatozoa which had passed out into the ductulus efferens no longer appear active but take a quiescent attitude (Fig. 22), contrary to the violently active appearance of the ones passing out through the membrana basilaris (Figs. 13, 14).

Examination was pursued *in vitro*, employing the spermatozoa taken from the ductus deferens nearest to the testis, because it was scarcely possible to gather them only from the ductulus efferens without the inclusion of any from another part. These spermatozoa also have shown no activity.

These facts indicate that the spermatozoa must have been inactivated in their course from the membrana basilaris to the ductus deferens. Probably, the activity is lost when they slip out into the ductulus efferens.

The above data justify the supposition that spermatozoa gain activity just before the beginning of the passing out, presumably, at the

start of their gathering at the membrana basilaris, and that they lose it when their whole body has passed out into the ductulus efferens.

3. Experiments to ascertain the time when the full maturity of the spermatozoa is attained

To ascertain when the full maturity of the spermatozoa is attained, certain experiments were made (Table 5).

TABLE 5.
Experiments concerned with the character
of the spermatozoa in testis.

No. of exp.	No. of indivl's.	Spermatozoa researched	Method of experiment	Result
1a	10	Taken from lobuli of pupa 4 days before emergence	Observation of the activity of spermatozoa	Some spermatozoa moved gently
1b	10	Taken from duct. def. of pupa 1 day before emergence	"	No spermatozoa moved
2a	10	Taken from lobuli of pupa 4 days before emergence	Reaction of spermatozoa to the secretion of gl. prostatica	Negative
2b	10	Taken from prox. part of duct. def. of pupa 2 days before emergence	"	Positive
2c	10	Taken from middle part of duct. def. of the same pupa employed in 2b	"	"
2d	10	Taken from amp. duct. def. of the same pupa employed in 2b	"	"
3a	10	Taken from lobuli of pupa 4 days before emergence	Reaction of spermatozoa to the liquid in burs. cop. of the female 2 days after a mating	Negative
3b	10	Taken from duct. def. of pupa 3 days before emergence	"	Positive
4a	20	Taken from lobuli of pupa 4 days before emergence	Art. insemination by the 2nd glass tubule method	No females were fertilized
4b	4	Taken from amp. duct. def. of moth a day old	"	2 females were fertilized

First, the activity of the spermatozoa was examined by observing the ones taken from the lobuli testium of old pupae of four days before emergence, and from the ductus deferentes of pupae of the day before emergence. Some of the former showed gentle activity, while the latter showed none at all (Table 4, Table 5, '1a, 1b').

Second, the reaction of the spermatozoa to the secretion of the glandula prostatica¹⁾ of a fresh moth was examined. Since the sperm bundle in the lobulus is enveloped in a sheath, it was necessary to break the sheath in order to cause the spermatozoa to make contact directly with the secretion of the glandula prostatica. In practice, some of the bundles had lost the sheath in the course of the preparation *in vitro*, probably by the pressure of the cover glass, and these spermatozoa were examined for their activity. But no tendency was found for them to be activated by the secretion. On the contrary, the spermatozoa taken from the ductus deferens all became violently active at the moment when they came into contact with the secretion. In this case there was no difference of the activity in comparison with the spermatozoa which were taken from a different part of the ductus deferens. The spermatozoa taken from the part nearest to the testis showed the same violent activity as those from the most distal part, *i. e.*, from the ampulla ductus deferentis (Table 5, '2a, 2b, 2c').

The above two experiments suggest that the character of the spermatozoa differs in the lobulus testis and in the ductus deferens. Therefore, it is recognized that the spermatozoa are subjected to some influences in their course from the lobulus testis to the ductus deferens but to none in the ductus deferens.

But the second experiment could not determine whether the non-susceptibility of the spermatozoa in the lobulus testis to the secretion of the glandula prostatica is due to their inherent character or to their having not all been supplied with the secretion that had been supplied to those of the ductus deferens. In fact, the spermatozoa in the lobulus testis have received no influence of the secretion of the ductulus efferens at least. The third experiment was made, therefore, intending to keep the spermatozoa from both the

1). The glandula prostatica is a gland included in the male sexual system. The function of the secretion from it is activation of the spermatozoa in the post-testicular male sexual organs. The details will be found in the succeeding paper.

ductus deferens and from the lobulus testis under as nearly as possible similar conditions.

Third, the liquid contained in the bursa copulatrix of the female moth two days after copulation was applied to the spermatozoa from the lobulus testis. This liquid consisted of every secretion of the male sexual organs and so small a number of spermatozoa as not to disturb the examination of the character of those to which the liquid had been added, for most of the spermatozoa ejaculated into the bursa copulatrix had been translocated to the receptaculum seminis. So that, if the spermatozoa in the lobulus testis had the same character as those in the ductus deferens they should show the same reaction as that of the latter ones. But the result was the same as that of the second experiment (Table 5, '3a, 3b').

The last experiment was made in order to research the fertilizing ability of these spermatozoa. They were injected artificially into the bursae copulatrices of virgin females with the secretion of the glandula prostatica by the second glass tubule method of artificial insemination.

Of the twenty females who were injected with the semen containing some active spermatozoa taken from the lobuli testium of the pupae four days before emergence, sixteen individuals received the semen in their bursae copulatrices. But none of them had spermatozoa in their receptaculi seminis, probably since these did not have the ability to progress there (Table 5, '4a', Table 6, '4a').

TABLE 6.

Results of the artificial insemination employing intratesticular and post-testicular spermatozoa by the second glass tubule method.

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

No. of exp.	Employed sperm	Indvl's. experimented	Indvl's. received semen	Indvl's. fertilized	Fertility
4a	Taken from lobuli of pupa 4 days before emergence	20	16	0	—
4b	Taken from amp. duct. def. of 1 day old moth	4	4	2	$\frac{24}{291}$, $\frac{52}{135}$ (8.2%, 39.3%)

In the ductus deferens, at the proximal part, there were found too few spermatozoa to use in the artificial insemination, therefore, some in the ampullae ductum deferentium were used, for they have the same character as those in the proximal part. All of the four females who were injected with the sperm from the ductus deferentes received the semen in their bursae copulatrices, and two of them were fertilized (Table 5, '4b', Table 6, '4b'). In this case the fertility was very low. This is probably due to the lesser quantity of the sperm than is received in natural copulation, but not to the character of the spermatozoa. For, the spermatozoa of *Bombyx mori* are considered to receive no aging influence in the post-testicular male organs¹⁾.

This experiment proved that the spermatozoa in the lobulus testis do not possess fertilizing ability, while those in the ductus deferens have it.

V. Consideration and Discussion

1. Extent of the testis

The present writer has determined the extent of the testis in the region covered by the membrana communis. No precise description was made as to the extent of the testis either by CHOLODKOWSKY, who has studied the testis of insects minutely, or by IKEDA who has researched the construction of the testis of *Bombyx mori*. The present writer's view on this point is based on the following reasoning.

The testis of *Bombyx mori* is not divided into lobuli in the embryological stage of the creature. The ramification of the capsula lobuli begins in the 2-3 day old larva (TOYAMA, '94). A little later the membranae basillares and the ductuli efferentes are formed by the cells, which differ from the spermatogonia and are scattered in the testis (GRÜNBERG, '03). Both the membrana basillaris and the ductulus efferens should be, therefore, included in one region, either in the testis or in the ductus deferens or in a special region.

The membrana communis covers the lobuli testis, membranae basillares and ductuli efferentes, drawing a clear boundary to the

1). The details will be given in the succeeding paper.

ductus deferens. The ductuli efferentes have close relation to the capsula lobuli (*cf.* p. 155), to which the ductus deferens has no relation. Consequently, if no special category is assigned for the membrana basilaris and the ductulus efferens, it will be rather natural to include them in the region of the testis. In the research on the character or behaviour of the spermatozoa, it was not inconvenient to regard a lobulus and a ductulus efferens as a combined organ and not as separated organs. Thus, the present writer has included the membrana basilaris and the ductulus efferens in the region of the testis, giving no special name to them.

2. Structure of the testis

The tunica externa has been described first in Lepidoptera by CHOLODKOWSKY ('86)¹⁾ as "tracheale Hülle". It was considered by him as being made up of transformed tracheae. But no tracheal constitution was found on it by the present writer. It will be more natural to look upon it as the transformed margin of the membrana communis. Its function may be the protection of the membrana communis. This view may be supported by the fact that the breaking down of the tunica externa accompanies the decline of the excretion of the granules from the membrana communis.

The membrana communis has been described as "Fettkörperschicht" by CHOLODKOWSKY ('86), who found the fatty granules in it, and as "Kyôtû-Himaku" (common membrane) by IKEDA ('13). The present writer has chosen the latter name, simply owing to his intention to base the nomenclature not upon the physiological character but upon the morphological one. No particular description has ever been made of the cells of this membrane. Further, the writer's observation has not been sufficient to warrant a conclusive statement on its function, whether it is nothing but the storage of fatty substance or whether it has assimilating and dissimilating ability. But it is notable that this membrane has a very similar appearance to the fat body. The writer considers this membrane as a source of the nutrients of the testis (*cf.* p. 173).

The capsula lobuli is called "Seinô-Himaku" (follicle membrane) by IKEDA, "chitinöse Hülle" by CHOLODKOWSKY, who believed it to

1). The original paper was not at hand, and the résumé was read in his other paper ('05).

have a chitinous character. No discussion will be demanded to deny this view. This membrane consists of connective tissue. The fatty granules herein found may not be found to be the secretion of the cells constituting this membrane, but the substance permeated from the membrana communis. Thus, this membrane may play the rôle of a canal which transports the granules.

The colour of the testis, which is found in some species of Lepidoptera, is said to be due to the granules contained in this membrane (CHOLODKOWSKY, '86). The relation between the granules found by the present writer and the colouring granules could not be made clear, as the testis of *Bombyx mori* has no other colouration than that of the haemolymph. (Further description of the membrane is found on p. 173).

The tunica interna is called "membrana propria" by CHOLODKOWSKY. It may be the transformed margin of the adjoining membrane similar to the tunica externa, serving to protect the capsula lobuli.

CHOLODKOWSKY ('05) has stated after his studies on Lepidopteran and Dipteran testis that even in a family all species do not always have the same constitution of testis, and some of them want one of the membranes. But his view may not be fully warranted, as his results are obtained from a few specimens of various species. Each membrane varies in appearance with the age of the individuals, as shown by the present writer. Therefore, CHOLODKOWSKY'S view will need further proof from the observation of many specimens of various ages in each species.

The existence of the trachea in the lobulus testis is demonstrated by old authors (VERSON, '96, TICHOMIROW, '98), and supported by the present writer's observation (Fig. 21). But CHOLODKOWSKY strongly insisted upon the absence of it ('05). It seems strange that there should be such differences in these easy observations.

It was demonstrated by the present writer that the size of the testis varies remarkably with the age of the individual. The investigations treating the size of insect testis—recently some of these investigations have been pursued in *Drosophila*, e. g., by DOBZHANSKY and STURTEVANT ('36)—will require full attention to this point.

The membrana basilaris is very unusual in its character. It has the same origin as the ductulus efferens. In a three day old pupa, its differentiation to the membrane begins, with the coagula-

tion of the chromatin of the nucleus. But a few days later the coagulation is not found and the cell contains a vacuole enclosing a similar substance to that in the vacuoles of the cells of the ductulus efferens which has just developed as a gland and begun secretion.

Until the spermatozoa mature, the membrana basilaris plays the rôle of a partition wall between the lobulus and the ductulus efferens, but in old pupal stage when the spermatozoa have grown fully, it becomes a unique sort of screen which lets the spermatozoa pass out and through by the movement of the spermatozoa themselves. It is so tender as to be perforated by the tail of the sperm bundle, but so tough as not to be torn by the numerous perforations by the sperm bundles.

MACHIDA ('29 b) has shown that a testis of *Bombyx mori* contains some 3000–4000 bundles of sound spermatozoa, that is, 800–1000 bundles per lobulus. Therefore, 800–1000 perforations, which have nearly the same size as the nucleus of the membrane cell, cannot tear this membrane. If it be assumed that the membrane makes a circle, of which the diameter consists of thirty cells—in actual fact, some thirty cells are numbered in the cross section of the membrane—then the number of the cells constituting the membrane may be calculated roughly as follows; $\pi \times 15^2 = 706$. That is, approximately one perforation is made in each cell and yet does not break the membrane.

On the structure of the ductulus efferens, there has been no study hitherto, just as in the case of the membrana basilaris. The present writer has revealed its glandular constitution and researched the stage of its secretion. Further, on the function of the secretion, an inference has been made (*v. p.* 175).

3. Granular substance in the lobulus testis

Hitherto, scarcely any attention has been paid to the granular substance in the lobulus. Though the present writer made no researches upon the stage when it comes out into the lobulus nor studies of its origin, he has found that the substance appears abundant at the full grown larva and diminishes in the imago. The disappearance of the granules in the capsula lobuli, the appearance of the granules of the lobulus of having been secreted from the capsula lobuli and the close relation of the period of existence of both kinds

of granules in the capsula lobuli and in the lobulus causes one to regard them to have intimate relation, though they differ in character, as the former indicates fatty reaction while the latter does not. If the granules of the lobulus have their origin in the fatty substance of the capsula lobuli, then the capsula lobuli must have some function to change the character of the granules which come out from the membrana communis. The coloured granules in the capsula lobuli of some species of *Lepidoptera* such as are never found in the membrana communis have given a suggestion to the above supposition, for the creation of these granules must be a function of the capsula lobuli. It is to be regretted that the data gained by the present writer are not enough to warrant a conclusion on this point.

GRÜNGERG ('03) regarded the "Apikalzelle" as the source of the nutrition of the spermatogenesis and stated that they have assimilating and dissimilating ability, though no details on the nutritive substance were given. CHOLODKOWSKY ('05) described the "Nahrungszelle" in a Dipteran testis, but he gave no data to indicate its function. He also found large granules transmitting themselves from the capsula lobuli to the lobulus in a species of *Diptera*, but he stated nothing either on their function or on their character. The present writer has no data for a criticism of these opinions, but at least in *Bombyx mori* there was found neither the "Nahrungszelle" nor the "large granules". Further, the "Apikalzelle" has been regarded as becoming functionless in full grown larval stage or early pupal stage (GRÜNGERG ('03)). In the pupal stage, accordingly, there is considered to be no nutritive substance other than the haemolymph and the granules found in the lobulus.

The stage when the granules are found abundantly in the lobulus coincides with the stage when the spermatogenesis enters the latter half of its course when much nutrient will be needed. The present writer considers that these granules serve as a nutrient of the testis, and because they are found in the pupal testis in which most of the spermatozoa have nearly finished maturing, that a part of them are employed as energy sources consumable by mature spermatozoa.

4. Passing out of the spermatozoa and the function of the secretion of the ductulus efferens testis

In Orthoptera, PAYNE ('33) has made an interesting observation *in vitro* on the getting out of the spermatozoa from testis. He has

discovered the fact that the spermatozoa move rather regularly within the follicle of the testis ascending, crossing and descending, then get out into the ductus deferens. PAYNE's observation was mainly directed to the movement of the spermatozoa and no notion is given on the mechanism of the getting out of the spermatozoa from the follicle into the ductus deferens nor even on the presence or absence of a membrane analogous to the membrana basilaris of *Bombyx mori*. IKEDA ('13) has observed the mechanism of the getting out of the spermatozoa in *Bombyx mori* using fixed preparations, and come to the conclusion that spermatozoa flow out from the lobulus through a pathway which appears naturally at some certain position in the membrana basilaris.

The particular mechanism of the passing out of the spermatozoa from the lobulus has been described above in the present paper. It denies the theory of IKEDA.

Whether this is a common mechanism occurring in all insect testes or only in Lepidoptera, is not yet determined.

The main process of the mechanism has been cleared fully, but a few questions are left, which need some consideration.

How do the spermatozoa gather toward the membrana basilaris? PAYNE has explained it by the assertion that the descending posture is due to the function of gravity. But he neither examined the geotaxis of the sperm bundles nor determined the natural position of the follicle. In *Bombyx mori*, though it is unknown what direction is taken by the testis in natural position; no matter in what posture the pupa was placed, no effect was noted on the passing out of the spermatozoa. Therefore, gravity has no effect on the descending posture of the sperm bundles.

MACHIDA ('29a) has stated that in an old pupa the apyrene spermatozoa dissolve in the basal portion of the lobulus, probably due to the character of the haemolymph, which has changed only in the basal part of the follicle under some unknown influence. The present writer supposes that this change of the character of the haemolymph may be due to the substance found near the membrana basilaris shortly before the time when spermatozoa begin to gather towards the membrane, and that this substance attracts the spermatozoa to the membrane by chemotaxis. Although the origin and character of this substance could not be determined, the stage of the appearance of it has led him to make such a supposition. The relation between

this substance and the secreted spheres found in the cytoplasm of the membrana basilaris could not be cleared up.

Other questions arise as to the perforation by the spermatozoa: where it occurs, whether on the boundary of cells or in the cytoplasm, or whether the position is determined to each cell; also whether each bundle makes its own foramen respectively, or whether multiple passing out is allowed through one foramen. It is to be regretted that the present data make no contribution to these problems.

MACHIDA ('29a) stated that apyrene spermatozoa are dissolved in the testis and are never found in the post-testicular organs. The present work also suggests that it is too complicated for the apyrene spermatozoa, which have irregular shape and size and probably have no activity, to pass through the membrana basilaris. In fact, in this work generally no apyrene spermatozoon was found in the post-testicular organs. But one exceptional case was found at the membrana basilaris. That case may not have been caused by the activity of the apyrene spermatozoa themselves but accidentally by the eupyrene spermatozoa, which had pushed out the former by chance. But the other exception, in which a mass of apyrene spermatozoa and the residue of the cystmembranes was found among the spermatozoa in the ampulla ductus deferentis, cannot be explained by the above assertion. Perhaps it has flowed out through a breakage of the membrana basilaris, which might be accidentally made in the course of the passing out of the spermatozoa. Therefore, it cannot be concluded that the membrana basilaris may not be absolutely broken by the perforations of the sperm bundles.

Since the spermatozoa pass out through the membrana basilaris in such a complex manner as mentioned above and some active spermatozoa are found *in vitro*, they must have activity at least during the course of their passing out. Further, it was proved *in vitro* that the spermatozoa are motionless in the most proximal part of the ductus deferens. Therefore, the spermatozoa will gain activity probably when they begin the descending movement and lose it when they have passed out into the ductulus efferens. These facts suggest that the secretion of the ductulus efferens has some function to negative the activity of the spermatozoa.

In addition, it may be well to assume the possibility of the presence of a motor centre in the spermatozoon from the view point of the mechanism of the passing out. If it is an established fact

that the secretion of the ductulus efferens has the above function, spermatozoa cannot slip out from the lobulus unless having a motor centre in their head or middle piece, because, if the centre were in the tail part, the spermatozoa would lose activity in the course of the passing out as soon as the tail has pushed out into the ductulus efferens which is surely filled with the secretion. Therefore, the tail-preceding-progression of the spermatozoa may be a necessary condition for the completion of the slipping out, protecting the head or middle piece, where the motor centre is located, from the anaesthetic solution.

5. Do the spermatozoa attain full maturity in the testis?

In the former paper (ÔMURA, '36) the writer has noted that in the artificial insemination, there was found no difference in the character of the offspring of the spermatozoa of pupa and imago. This furnishes a datum to support the opinion that the spermatozoa are subject to no maturing influence in the post-testicular organs. One of the present experiments has shown that the spermatozoa from different levels of the ductus deferens have the same reaction to the secretion of the glandula prostatica. This shows that the spermatozoa in the proximal part of the ductus deferens have the same character as those in the ampulla. Another experiment has shown that the spermatozoa do not have fertilizing ability in the lobulus but attain it in the ductus deferens.

These results indicate that the spermatozoa attain full maturity not in the lobulus but in the proximal part of the ductus deferens, and justify the inference that the completion of the maturing must take place either while the spermatozoa are passing out through the membrana basilaris or while on their way through the ductulus efferens or in both of the courses. To ascertain this point an attempt was made to add the secretion of the ductulus efferens to the spermatozoa in the lobulus. The result was negative. But it is not clearly known whether or not the function of the secretion is a necessary condition for the full maturity. It will be concluded, however, that the function of the secretion of the ductulus efferens is not a sufficient condition for the attainment of full maturity, and that the full maturity cannot be attained unless the spermatozoa pass out through the membrana basilaris. The particular movement of the

spermatozoa in the strange secretion near the membrana basilaris accompanying the casting off of the sheath of the bundle must be a necessary condition for the accomplishment of maturity. The full maturity of the spermatozoa may depend, perhaps, upon a change in their colloidal state or upon a transformation of their protoplasm.

6. Break-up of the sperm bundle

Judging from the figure of the shrinkage of the sheath in the course of the passing out, the sheath may stick to the sperm bundle only at the top of the head, enveloping the other portion and keeping the spermatozoa in a bundle, but so that they can dishevel their tails in the bundle.

In *Bombyx mori*, as indicated by IKEDA ('13), in the case of some of the spermatozoa which have passed out from the testis, the bundle is dissolved while in others the bundle is kept. Such a dissolution of the bundle appears to occur by chance. This may perhaps be due, the present writer infers, to the manner of the unclothing of the sheath; when the adhered part of the sheath is not taken off at all, the bundle may not disintegrate; when no sheath adheres to the head, all individual spermatozoa separate from the bundle; when a part of it is cast away, some of them fall apart from the bundle.

PAYNE stated that in *Chortophaga* the sperm bundle has a hyaline cap and the bundle is dissolved in the female sexual organ, owing to the dissolution of the hyaline cap by the action of an enzyme. But in *Bombyx mori* the bundles are broken when they are touched by the secretion of the glandula prostatica. This may not be owing to the action of the enzyme but to the result of the movement of the spermatozoa which is brought about by the secretion. The individual movement of the spermatozoa may break the bundle mechanically.

VI. Conclusion

The testis consists of four sets of lobuli testis and ductuli efferentes testis, which are enveloped in the tunica externa and the membrana communis. Each lobulus is covered with the capsula lobuli and lined with the tunica interna. A lobulus is partitioned from its ductulus efferens by the membrana basilaris.

The fatty substance found in the membrana communis is secreted out into the capsula lobuli and is transported through it.

A granular substance was found in the lobulus. There were found some data to lead to the inference that it has its origin in the fatty granules in the capsula lobuli and that it serves as a nutrient of the spermatogenesis.

The ductulus efferens was revealed to consist of glandular cells, of which the secretion seems to have a function to inactivate the activity of the spermatozoa.

In the lobulus the spermatozoa group in bundles. Each bundle passes out into the ductulus efferens through the membrana basilaris in a particular and complex manner each perforating its own pathway by its own action.

In the course of the passing out, the sheath of the bundle is cast away and left in the lobulus covering each foramen.

The passing out of the spermatozoa begins in the old pupal stage 3-4 days before emergence, and the majority of the spermatozoa translocate into the post-testicular organs in the pupal stage.

No formation of new spermatozoa in the lobulus accompanies this translocation, consequently the testis is reduced in size with age.

Spermatozoa gain activity only in the course of the passing out.

The motor centre of the spermatozoa was inferred to be in their head or middle piece from the mechanism of the passing out and the function of the secretion of the ductulus efferens.

The full maturity of the spermatozoa is attained during the passage of the spermatozoa from the lobulus to the ductus deferens, that is, in the testis.

VII. Résumé

1. The construction of the testis was studied histologically and the function of each constituent part of the testis was researched.

2. A definition of the extent of the testis was given.

3. The source of the granular substance found in the lobulus was considered, and its function was discussed.

4. The intratesticular behaviour of the spermatozoa was studied, especially the characteristic manner of the passing out through the membrana basilaris was fully observed.

5. An experimental investigation was made on the attainment of full maturity of the spermatozoa.

VIII. Literature

The literature marked* was not actually accessible to the writer.

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IX. Explanation of Plates

Abbreviations

a, b, c, d, e, f, g progressive stages in the course of the passing out of spermatozoa.

ap	apyrene sperm bundle.
cl	capsula lobuli.
de	ductulus efferens testis.
mb	membrana basilaris.
mc	membrana communis.
sh	sheath of sperm bundle.
sh ₁	cross section of sperm bundle with sheath.
sh ₂	cast off sheath of sperm bundle.
ti	tunica interna.

Plate I

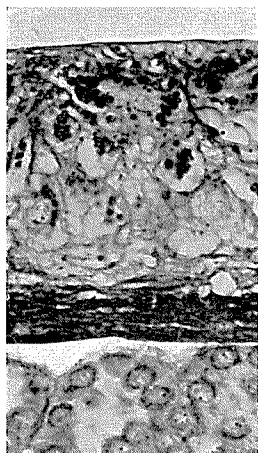
- Fig. 1. Wall of testis. Full grown larva. (CHAMPY, HEID. haem.). 450 ×
 Fig. 2. " 2 day old pupa. (" "). "
 Fig. 3. " 7 day old pupa. (" "). "
 Fig. 4. " 4 day old imago. (" "). "
 Fig. 5. " 7 day old pupa. (" "). "
 Fig. 6. " Oblique section. 2 day old pupa. (BOUIN, HEID. haem.).
 Tunica interna is transparent. 450 ×.
 Fig. 7. Partition between lobuli. 7 day old pupa. (CHAMPY, HEID. haem.). 450 ×.
 Fig. 8. Ductuli efferentes testis. 2 day old larva of 5th stage. (CARNOY, HEID.
 haem.). 100 ×.
 Fig. 9. " 7 day old pupa. (BOUIN, HEID. haem.). 100 ×.
 Fig. 10. " 2 day old pupa. (CARNOY, HEID. haem.). 180 ×.
 Fig. 11. " " (BOUIN, HEID. haem.). 100 ×.

Plate II

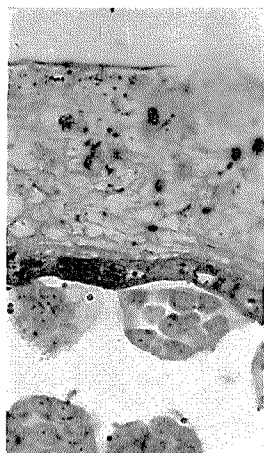
- Fig. 12. Basal part of lobulus. 9 day old pupa (4 days before emergence).
 Many granules are found on upper part. (CHAMPY, HEID. haem.).
 100 ×.
 Fig. 13. Horizontal section of membrana basilaris. Pupa, 2 days before emer-
 gence. (CHAMPY, HEID. haem.). 100 ×.
 Fig. 14. Basal part of lobulus. Pupa, 3 days before emergence. (CHAMPY, HEID.
 haem.). 100 ×.
 Fig. 15. A part of Fig. 14. 450 ×.
 Fig. 16. " " . Many cast off sheaths are accumulated on the membrana
 basilaris. 450 ×.
 Fig. 17. Another figure on the same slide as Fig. 14. 450 ×.
 Fig. 18. " " "
 Fig. 19. " " "
 Fig. 20. Wall of ductulus efferens testis. Pupa, 1-2 days before emergence
 From the same slide as Text-Fig. 7. (CHAMPY, HEID. haem.). 900 ×.

Plate III

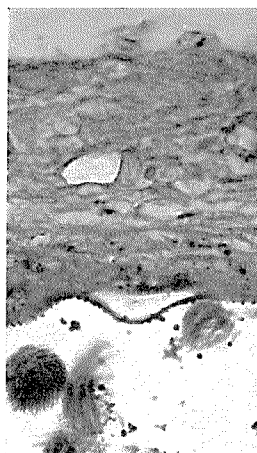
- Fig. 21. Ductuli efferentes testis. 6 day old imago. Many tracheae are shown clearly. (BOUIN, DEL. haem.). 100 \times .
- Fig. 22. Basal part of lobulus and its ductulus efferens. Pupa, 2 days before emergence. Numerous granules are found on upper portion. (CHAMPY, HEID. haem.). 100 \times .
- Fig. 23. A part of Fig. 22. 450 \times .
- Fig. 24. Vertical section of membrana basilaris. 6 day old imago. (BOUIN, DEL. haem.). 450 \times .
- Fig. 25. Ampulla ductus deferentis. Imago of the day just emerged. (CHAMPY, HEID. haem.). 100 \times .
- Fig. 26. Horizontal section of membrana basilaris. Pupa, 2 days before emergence. (CHAMPY, HEID. haem.). 450 \times .
- Fig. 27. Vertical section of membrana basilaris. Pupa, 2 days before emergence. (BOUIN, DEL. haem.). 450 \times .
- Fig. 28. Sperm bundles with bent tails near membrana basilaris. Pupa, 3 days before emergence. Membrana basilaris locates under side. (CARNOY, HEID. haem.). 450 \times .
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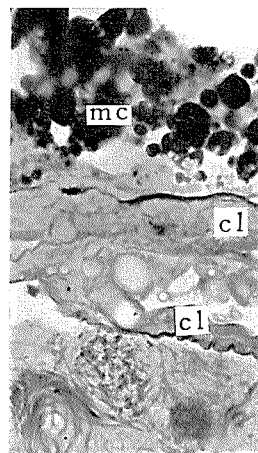
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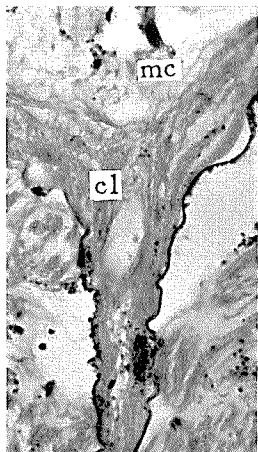
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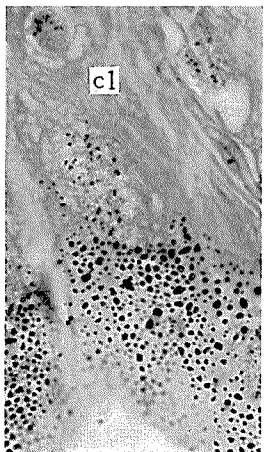
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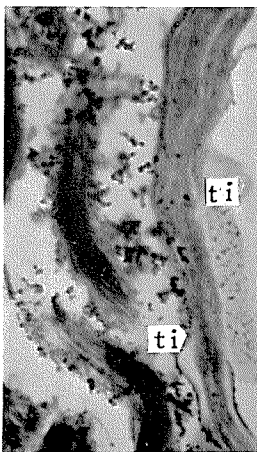
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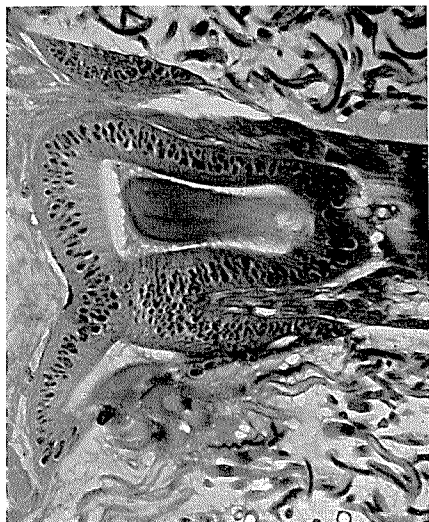
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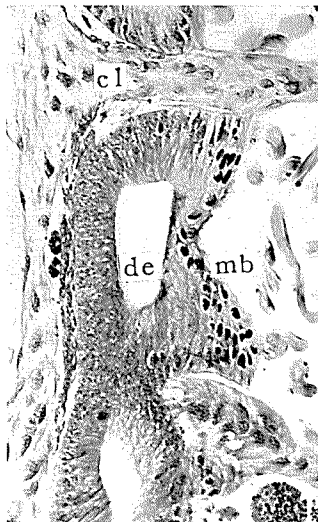
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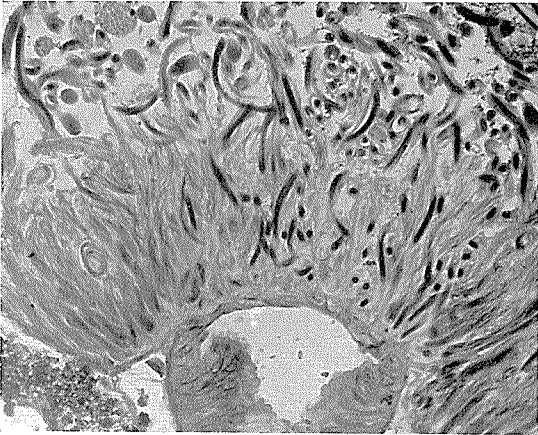
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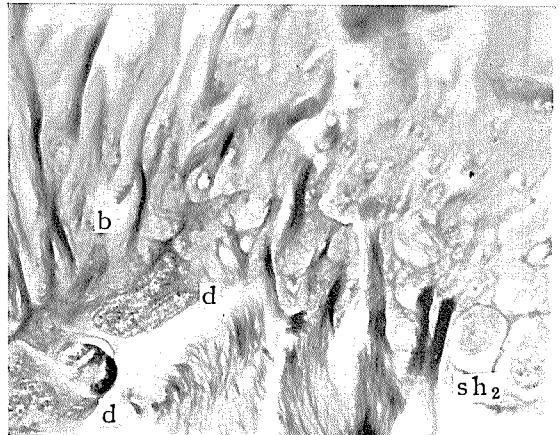
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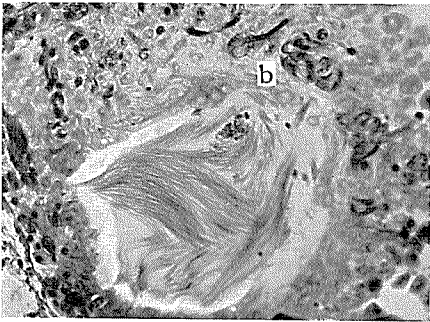
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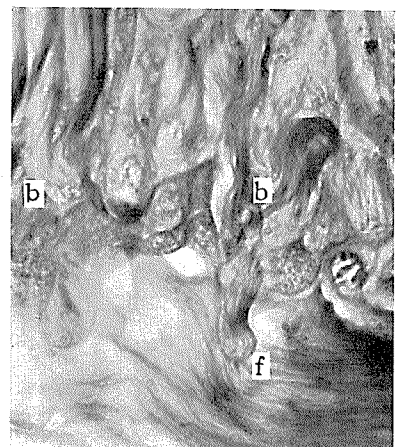
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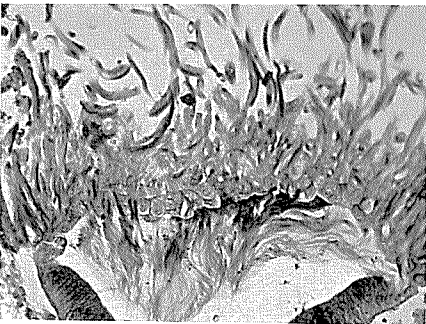
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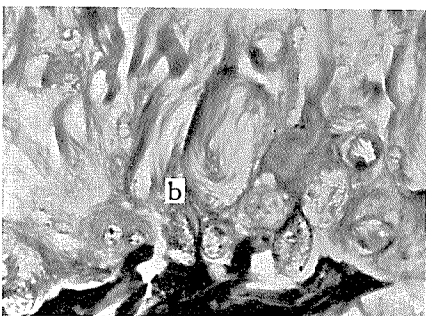
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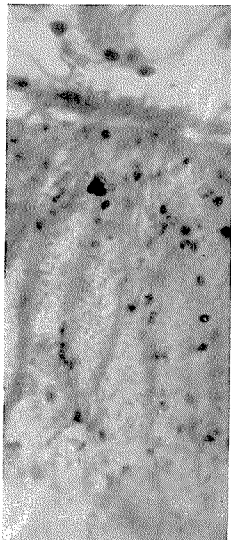
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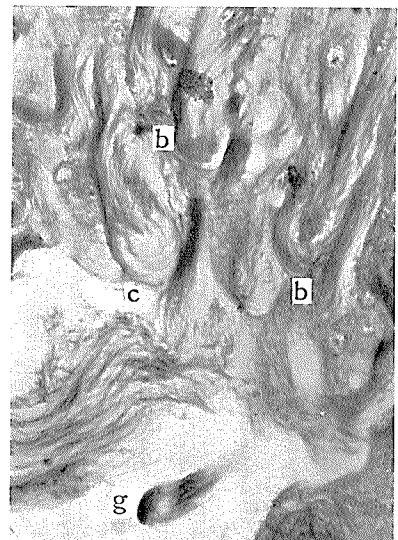
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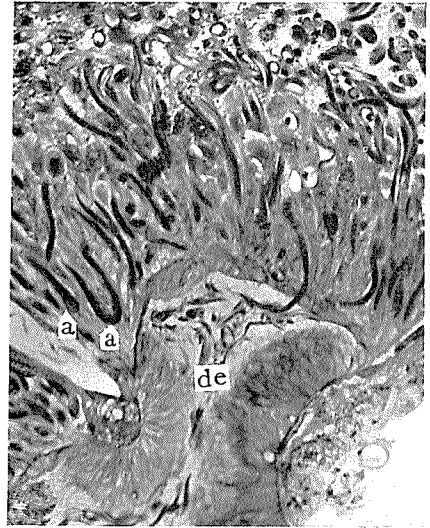
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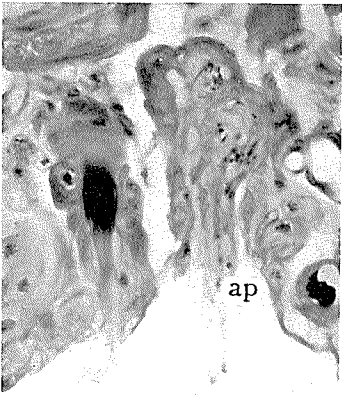
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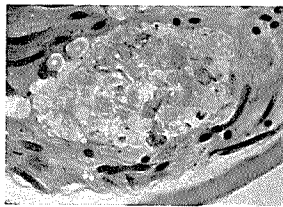
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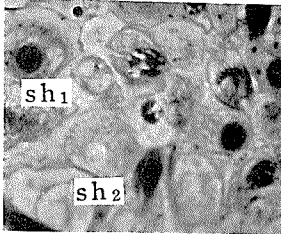
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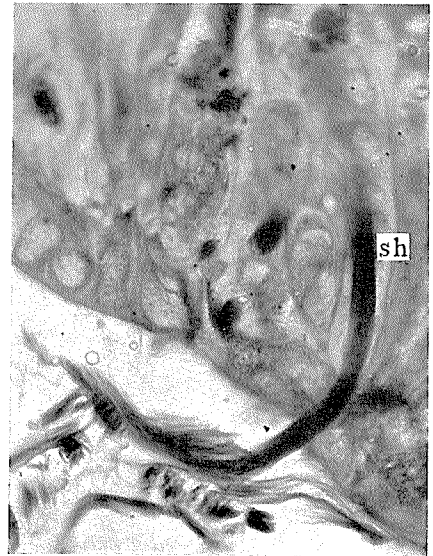
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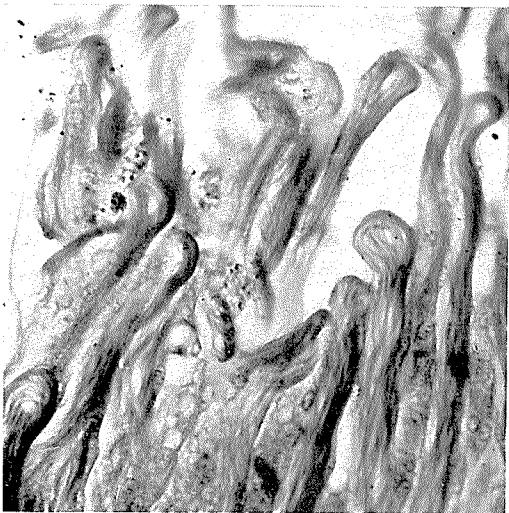
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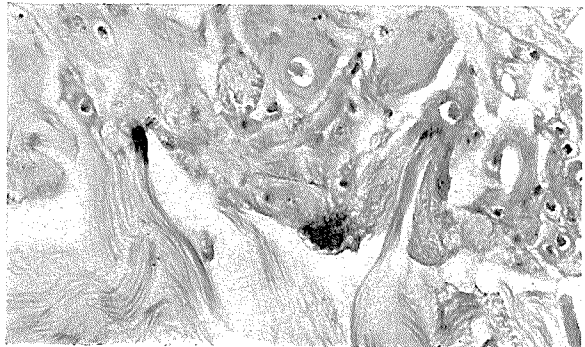
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