STUDIES ON THE ANATOMY AND PHYSIOLOGY OF THE SILK-PRODUCING INSECTS.

I. ON THE STRUCTURE OF THE SILK GLANDS AND THE SILK FORMATION IN BOMBYX MORI.[1]

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With Pl. III–IX.

Introductory.

The Domestic Silkworm, *Bombyx mori,* evidently is one of the Insects which have been studied in several respects, owing no doubt to the economic importance of sericulture in combination with the material easily accessible. There are, however, met with in the literature numerous points on which the views of the previous authors are still widely divergent from one another; from the growing importance of the economic side and from the scientific interests, these questionable points are required to be elucidated, if possible, satisfactorily. Since 1909 I have been engaged in a work with my hope in throwing some light upon the minute structures of the silk glands, which remain at least in parts obscure in spite of their being in close relations to the silk-formation. In connection with this morphological study, I have observed the silk formation, the minute processes of which are, as it seems to me, not without interest. The present paper embodies the results obtained from the study in these two directions.

The material employed consists chiefly of the larvæ of the Japanese white univoltin race and Japanese white bivoltin race. The univoltins composing the material are those hatched out on June 3rd, 1907, and "mounted" on July 7th, that is, thirty five days after their day of hatching, being killed day after day during the course of the whole larval life. The bivoltin material was obtained from the larvæ reared in the summer season of 1910; this latter lot was collected at times in the course of culture.

[1] See TANAKA, Y., Preliminary Note on the Silk Glands of *Bombyx mori*. Trans. Sap. Nat. Hist. Soc., Vol. III, pp. 19–23. 1910.

The material was fixed in 0.6% watery solution of picric acid and was hardened in 70% alcohol in which it has been preserved until worked, the fluid having been renewed at times. The objects which were imbedded partly in paraffin and partly in celloidin paraffin were divided into series of transverse and longitudinal sections of 1/300 *mm.* in thickness. Staining was done on the slides chiefly with HANSEN's hæmatoxyline, but alum-carmine, borax-carmine, picro-carmine, RENAUT's hæmatoxyline, safranine, aniline red, etc. were also employed.

I wish here to express my sincerest thanks to Prof. S. HATTA for his constant advices and his kindness in looking through the manuscripts. My obligations are also due to Prof. K. MIYABE and Prof. K. SUDA for the warmest courtesy shown by them in the course of the present work.

Part I. On the Structure of the Silk Glands.

The silk glands consist, in the Domestic Silkworm, *Bombyx mori*, of a pair of long tubular bodies, the proximal ends of which are communicated with a common duct, while the distal ends terminate blindly; the whole body of the glands is shining whitish in both the races reared in our country.

During earlier stages of larval life, the glands are situated, in a symmetrical manner, on both sides of the central nervous system which lies flat in the body cavity in a close contact with the ventral body-wall. The silk glands undergo, however, a rapid growth in the course of larval life; especially when the fourth moult is over, they are suddenly added in dimensions *(Pl. III, Fig. 1)*, so that they fill up nearly the whole space of the body cavity, while this cavity was formerly to its greater part occupied by the alimentary canal which is, during the growth of the silk glands, translocated by stages towards the dorsal body-wall, being pushed by the growing silk glands, and is at present pressed on to the body-wall, so as to assume a band shape, being compressed dorso-ventrally *(Pl. III, Fig.2)*

Besides the common duct, the following three sections may be distinguished in each limb of the silk glands: *a*) the anterior division, *b*) the middle division and *c*) the posterior division. These three divisions are often erroneously regarded, from before backwards, as the part discharging, and the section reserving the silk secretes which are secreted in the glandular division representing the termienal division; hence they are most commonly called the *excretory tube*, the *reservoir* and the *secreting tube* respectively.

The gland tube on each side is thin in the proximal part of its anterior division and is imperceptibly added in its thickness towards the posterior part, until it is suddenly thickened at the commencement of the middle division. The middle division of the tube also grows thicker distally and attains its thickest bulk at about the middle part of its extent, to be again gradually decreased in bulk towards the posterior division, at the commencement of which the tube is rather abruptly decreased in its thickness. Thence backwards the tube becomes by degrees thinner and thinner; the hind tip of the gland tube is, however, markedly thicker as compared with the anterior section of the tube.

In the passage from one division to another the tube shows certain characteristics. The tube's lumen is suddenly diminished at the passage from the middle division to the anterior division which contains the narrow lumen; the epithelium forming the walls is likewise rapidly decreased in its thickness from this part forwards; and the very thick intima which lines the inner surface of the gland tube of the anterior division is almost lost from sight at the passage to the middle division *(Pl. III, Fig.3, A, C)*. In the passage from the middle division to the posterior the tube shows not, as a rule, the features so marked as in the one just referred to, passing rather gradually from one to the other.[1]

The anterior division extends from the distal end of the common duct to about the level of the fifth or sixth body-segment, which lies in the commencement of the middle division. The middle division forms two loops in its course, one curved posteriorly, and the other bent anteriorly. The first loop which is bent posteriorly, goes in younger larvæ, as far backwards as the sixth segment[2], and in full grown larvæ it reaches the ninth segment, whereas the anterior loop is, in younger larvæ, found in the fifth; in full grown larvæ, it grows farther anteriorly, so as to attain the second segment. The middle division passes over, in the fifth, sixth or often in the seventh segment, into the posterior division. The looping of the gland tube in question is already to be seen in embryos at least three days before hatching. It is a noteworthy fact that the looping remains always unchanged in its principle, being neither added nor simplified in turnings, although the middle division which is looped, as well as other parts, undergo striking changes in several respects in consequence of enormous growth and development.

The posterior division, the hind continuation of the division just referred to, is in younger stages very simple; it is almost straight, showing only a few undulations near its distal end *(Pl. III, Fig.1, A, B, C, D)*. It extends from the fifth, the sixth or the seventh segment to the ninth segment, about which it ends blindly. In full grown forms, on the other hand, the gland tube of the posterior division shows numerous convolutions and occupies almost the whole space between the fifth segment and the tenth. It is specially to be noted that, although the convolutions of the tube push their way into the tenth segment, yet its distal end is always found in the ninth segment where it lies from

[1] Very often is, however, seen a wide calibre which passes over suddenly into a narrow lumen of the posterior division, and we see the gland epithelium passing over into that of the posterior division which is increased abruptly in thickness. See *Pl. III, Fig.3, B.*

[2] The term "segment" always signifies in the present paper the *body-segment.*

the first, being fastened to the enteric wall in this segment by special muscles *(vide infra)* *(Pl. IV, Fig. 8)*. The convolutions of this division are numerous indeed: I have counted in some specimens no less than fifty of them *(Pl. III, Fig.1, F)*.

As to the histological structure, in the silk gland three layers are discernable: the tunica propria representing the external cover, the tunica intima or the innermost layer, and the layer of the epithelial cells, which builds up the fundament of the gland and is coated on both its surfaces by the above mentioned two layers. The tunica propria is thin transparent, structureless membrane which is nevertheless prettily resistant against the mechanical forces acting from outside. As a continuous membrane it stands in connection with the basal ends of the gland cells, which together form the external surface of the gland. Seen from the surface, the gland cells are polygonal in outline and are of very large size in full grown caterpillars, so as to be easily recognised by the naked eye; they are, however, not very thick and are curved to describe a semi-circle, and the two opposite components are so arranged to complete, on a cross section, a ring. It is, therefore, clear that the gland tube is composed of rings of the gland cells arranged in two rows.

According to the divisions of the tube, the gland cells differ more or less in their shape: the cells represent nearly hexagonal column in the anterior division; and those in the middle division are lower and broader, being elongated from side to side, while in the posterior division, the cells are not only irregular but variable in shape, being doubtless caused by the convolutions of the gland tube *(Pl. III, Fig. 4)*.

BLANC believes that the component cells of the silk glands are separated from each other by narrow spaces which are filled up with a substance remaining unaffected by staining matters. The spaces in question are not in reality a natural one, but due to mere artificial effects, as shown by the following facts. In living specimens, the gland cells are closely put together so as to cause the cells to be pressed one against another and to assume a polygonal shape as above mentioned; consequently there is left at any rate no space between the cells themselves as BLANC mentions. On the other hand, the spaces appear between the cells, when the gland is fixed and hardened, no doubt in consequence of contraction undergone by the cells. Then the inner and outer tunics, not affected by the reagents, remain unchanged.

The cytoplasm is granulated and vacuolated; this is especially the case during the period of active secretion of the cells. The numerous tracheal branches are divided into fine capillaries which pass through the substance of the cells. The cell nuclei also present a granular structure, except those of the anterior division in later ages, which are clear and homogeneous, being free from granulations.

Ramifications with which cell nuclei are provided, appear at first in the cells composing the anterior division. This fact disproves the view by HELM, according to which the middle division is earliest to show the dendric form of cell-nuclei; it is, however, to be remembered that the ramifications of the nuclei are, in full grown caterpillars, highly developed and much complicated in the middle and posterior divisions, although the changes of the nuclei commence their work in the latter two divisions later than in the anterior division.

The tunica intima lines the inner surface of the gland cells. This coat is thickest in the anterior division; in the middle division it is not only very thin, but is characterized by occurrence of numerous striations of filamentous appearance, which are probably brought about by local thickenings of the intima. In the middle division, the filamentous striations take a parallel course. Some adjacent filaments very often anastomose with each other, although this is, in the middle division, comparatively seldom in occurrence. The parallel course of the filaments is, however, gradually disturbed, and anastomoses are met with more frequently towards the hinder part of the gland, to be at last transformed into a complicated net-work, as in the case of the hinder half of the posterior division. According to HELM, the intima is, together with the body integuments and the tænidia of tracheæ, etc., cast off and renewed at every moulting. As the intima is nothing other than the cuticular coat secreted by the cells of ectodermal origin, this view by HELM seems not unreasonable; yet there is detected, in reality, no trace of changes suggestible as being due to such a renewal of this layer. On the contrary, in the certain structures which are actually renewed at every moulting, for instance, in the cuticular coat of the integument, in the tænidia of tracheæ, etc., a new layer is clearly recognised, being formed already before the old coat has been cast off, so that double layers, old and new, are seen at the same time during a moulting period *(Pl. V, Fig.16)*. Such an occurrence is never seen in the intima of the gland tube at all *(Pl. VII, Fig.24, C)*.[1)]

Muscles in Relation to the Silk Glands. The silk glands and the alimentary canal which are found in the perivisceral cavity, are suspended to the skin by means of nine pairs of long, slender muscles, each pair of which regularly occurs in each of the nine body-segments from the second to the tenth *(Pl. IV, Fig.5)*. For the convenience of reference, these muscles will be called the *dermo-visceral muscles*. A muscle of this series is represented by a band composed of the muscular fibres with large nuclei arranged in a row on the median line of the band. The dermo-visceral muscle bands arise, in each pair, from the body-wall represented by a cuticulated integument and are inserted on each side of the median line to the dorsal enteric wall. The muscles of the second pair, the strongest of all, show certain modifications as regards their origins and attachments:

1) A paper on the intima together with the post-embryonal development of the nuclei of the gland cells will appear as a separate article, and detailed accounts on these structures will be seen in that paper.

firstly, instead of being inserted to the enteric wall on the same side of the median line as their origins, the muscles of this pair are crossed on this median line to be attached to the enteric wall on the opposite side beyond this line; *secondly,* the second muscle band on either side is soon split, shortly from its origin, into several branches; the others are divided only near to their inserting points. The muscles of the first pair run from their origins on the skin almost transversely to the inserting points on the enteric wall. All the muscles of the remaining eight pairs take, on the other hand, an oblique course, owing to the backward shifting of the inserting points, in contrast to the case of the first pair of muscles, in which both the origin and inserting points lie on a straight transverse line.

Of the muscles above mentioned, the fourth and the eighth alone are those which stand in connection to the silk glands. Arising from the anterior boundary of the fifth segment, the fourth muscle on each side passes into the ventral side of the silk gland, where it is divided into two branches, one of which is attached to the anterior part, and the other is inserted to the middle part of the middle division. Not unfrequently this muscle sends off a third branch to be connected by it with the hind part of the middle division. Towards their insertion, the branches given by the muscles are further subdivided repeatedly, and at length these branchlets are fastened by means of connective tissue tendons which are interwoven in the distal part of the muscular branchlets, to the external surface of the gland, *i. e.* the tunica propria *(Pl. IV, Fig.6).*

Turning to the muscles of the eighth pair, two branches which are given by each counterpart of them are attached to the distal part of the gland and to the tip of it. The main trunks of the eighth muscles have, on the other hand, nothing to do with the gland, but go directly into the enteric wall, in order to suspend the distal end of the gland to the wall of the alimentary canal *(Pl. IV, Fig.8, 9).*

A. LENTTICCHIA believes that the silk glands have a muscular wall, in virtue of which the secretes in the gland lumen are pressed and moulded to take a cylindrical form. But this view is evidently erroneous. The silk glands contain not a single fibre of muscle in their bodies.

Distribution of Air-tubes in the Silk Glands. The silk glands are provided with the tracheal tubes which vary in their strength and occurrence according to the division of the gland as well as by stages. The middle division is supplied with the tracheal branches given off by the trunks from the fourth stigma and by a part of those from the third stigma; after the fourth moult the ventral surface of this division is almost wholly covered by the tracheal branches *(Pl. V, Fig.11).* Several branches of the tracheæ from the fifth, sixth and seventh stigmata are distributed to the posterior division. The anterior division is always free from the tracheal tubes.

The tracheæ are already to be detected a great deal in connection with the silk glands in larvæ just hatched, although the branches are greatly added thereafter. The tracheæ attain, however, their full development only when the fourth moult is over; They acquire then their strength of the branches sent off fully.

The tracheal branches on the gland walls give off their branchlets which enter the bodies of the gland cells penetrating the tunica propria *(Pl. V, Fig.15)* On series of cross sections through the middle and posterior divisions are found several stages of the tracheal tubules in making their way into the cell-bodies. There are tubules which come in contact with the tunica propria of the gland by their tips. The tip of the tubule shows a characteristic shape; it is provided with a basal knob, looking like an injection cannula. In other cases, the tubules, and often those of large size, are piercing the cell body; then the basal knob is lost from sight; and lastly, the cell bodies contain a system of the tracheal capillaries into which the branchlets are divided in the cell-bodies. It is worthy of a special notice that the finest parts of the tracheal capillaries, which are destitute of the tænidian rings, are communicated neither with one another, nor with any other part, but end freely, showing every indication in opening by fine terminal pores in the cytoplasm. Though peculiar as it seems, yet the cytoplasm of the silk gland cells is in fact richly supplied with the tracheal capillaries[1] *(Pl. V, Fig.17)*

Every ecdysis is preceded by the formation of a new series of the tænidia; the tracheal branches often show on their cross-sections at the moulting periods double tænidian rings, the inner old and the outer new. Not unfrequently are further seen two small rings of the old tænidia in the inside of the new just formed *(Pl. V, Fig.16)*. These peculiar occurrences are the case in the tracheal tubes contained within the cell-bodies as well as in those outside of them.

Having given in the preceding pages the chief facts concerning the structures of the silk glands made out in the present work, I will now turn to the results obtained by the previous authors.

Concerning the observations made as early as the seventeenth and eighteenth centuries, the following are to be referred to. ALDROVANDUS (1602) believes that the silk fibre is drawn out from the mouth of the silkworm. We owe the discovery of the blind ending of the gland tube to the authentic observation by MALPIGHI (1669). And by his splendid discovery of a special opening for the silk spinning, the author corrected the erroneous

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1) The peculiar features which are seen in the distribution of the air-tubes in the silk glands draw attention to two points: *firstly*, the tracheæ are supplied in the middle and posterior divisions, but not in the anterior division; this is due to the active secretion performed by the former two divisions; *secondly*, the vacuoles or babbles loaded in the silken column, and often in the cytoplasm of the gland cells are doubtless those emitted from terminal openings of the tracheal capillaries within the cytoplasm.

assumption by ALDROVANDUS who claims that the silk fibre is drawn out from the mouth. His descriptions of the silk glands are, however, confined chiefly to their external features. The "double structure" of the cocoon fibre was pointed out for the first time by LEEUWENHOEK (1719). REAUMUR (1734) gives detailed statements on the spinning apparatus which is called by him *filière*.[1] It is by LYONET (1726) that the anterior, middle and posterior divisions have been distinguished, and the wall of the gland is, accordiug to him, composed of several layers. We learn also from him more detailed structure and more accurate physiological function of the filière[2], than its discoverer, REAUMUR, shows; the author pointed out the "corpus bulbeux," as he calls a small mass of glandular tissue, in the caterpillars of *Cossus ligniperda*. The glandular body in question is nothing else than one of the paired glands found by DE FILIPPI in the silkworm and at present known as FILIPPI's glands which open to the common duct of the silk glands and give some slimy secrete into it.

H. MECKEL (1846), one of the distinguished observers, undertook a study of the silk glands, the results of which made, for the first time, clearly known the tunica propria, the glandular cells and the tunica intima, as well as the branched nuclei of the gland cells, and these splendid results are confirmed by later investigators, especially by LEYDIG (1857) who extended his work over several silk-producing insects. As one of the discoveries by MECKEL to be mentioned are the so-called "Porencanäle" which bore, according to the author, the intima in directions of radi of the gland tube.

Our knowledge concerning the structure of the silk glands has been greatly pushed forwards by the extensive work of E. H. HELM (1876), which comprises various kinds of spinning insects and is illustrated with nearly sixty figures given in main exactly. The author pointed out in this work the tracheal branches within the cell-bodies of the silk glands and gives post-embryonal development of the nuclei of the gland cells, together with the structure and fuuctions of the "Spinnapparat" and those of the FILIPPI's glands. According to HELM the intima of the anterior division is, as MECKEL maintains, provided with the "Porencanäle," and the intima itself is renewed at every moulting. The metamorphoses of the silk glands of *Bombyx mori*, in which he distinguishes the progressive and regressive, have been observed by him with great caution.

Whilst LIDTH DE JEUDE (1878) positively denies the nerve supply to the glands, JOSEPH (1880) gives accounts on the innervation, together with the embryological development of the silk glands, assuming that the glands are supplied with certain branches from the infra-œsophageal ganglion and "Verdauungsnervensystem" (sympathetic nerve system) which form the nerve plexi spreaded between the peritoneal membrane and the

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[1] REAUMUR means by the term "filière" the spinneret itself.

[2] By the term "filière" LIVONET designates not only spinneret, but the entire body of the labium.

glandular cells.

So far as I am aware, no writer, subsequent to JEUDE and JOSEPH, gives an account on the innervation of the silk glands. When the fact that the salivary glands of Insects have a special nerve supply is drawn into consideration, the silk glands, the homodymous organ of the salivary glands, ought, as I assume, to be supplied by the homologous nerves as supplied to the salivary glands, although the glands have undergone a "Funktionswechsel". Standing on this assumption, I dissected numerous individuals of various stages, with great caution in seeking for nerve fibres standing in connection with the glands, but all my efforts were at length fruitless, proving nothing else than the occurrence of the connective tissues connecting the dermo-visceral muscles and fatty tissues with the glands, together with the tracheal branches distributed thereon. These structures, the connective tissue and trachea, show, shining whitish, a great resemblance to the nerve fibres; this resemblance is still further intensified in younger individuals which formed the chief material of JOSEPH's work. After all, I am obliged to conclude that JOSEPH might have mistaken these white structures or some of them for the nerve. It is of course a striking fact that a body conspicuous in form and volume, and active in function, such as the silk gland, is entirely destitute of nerves specially supplied to it; the fact positively denies, however, the presence of such nerves.

Next we come to the work by WISTINGHAUSEN (1890); the weight of the results by him falls upon his affirmation of the so-called "Tracheenkapillarendnetz". According to the author, the finest twigs of the tracheal branches terminate not in the gland cells, but are united with one another and give rise to a system of finest network, the "Tracheenkapillarendnetz", as he calls it. The Tracheenkapillarendnetz is, according to WISTINGHAUSEN, a system of fine capillary tubes constiuting the continuation of, and homologous to, the "Tracheenkapillaren", and the network in question is formed of only the intima which is probably chitinized and is coated with the "Peritonealhaut". This system of network intervenes between the tunica propria and the layer of the gland cells, without entering within the latter. This view is, however, not correct. The end-twigs of the tracheal tubes are not so united as to give rise to a network, but end freely in the cytoplasm of the gland-cells, as stated in the descriptive part. The free terminations of the tracheal capillaries are, however, by no means easy to make out; I used a very high power (Zeiss apochromatic objective 2 *mm.* × compensating ocular 18) for this purpose. Under such a magnifying power, the capillaries are clearly revealed to their termini in the cell-bodies. In addition to this, I have in my possession some preparations in which very obvious are several interesting stages of the tracheal twigs making their way into the cell-bodies of the gland: in some the tips of the twigs are coming in contact with the propria, and in others these are penetrating to some extent this structureless membrane, while in still others they completely have bored into the cytoplasm *(Pl. V, Fig.13, 14, 15)*.

It should not be uninteresting to infer the mode, in which the tracheal twigs thrust the propria. It is of course doubtful that the tapering fine tracheal twigs are efficient enough to tilt the tunica propria by the pressure exerted by the growing tracheal twigs themselves. Actually there is not perceived a depression which ought to result at the point of the membrane coming in contact with the tip of the tracheal twig, if the assumption is vaild. I come at length to assume that the tracheal twigs secrete from their tips some enzyme substance, by the action of which the tumica propria is first of all dissolved at the points lying in contact with the twigs to allow the latter to make their way into the cell-bodies. The basal knobs with which the tracheal capillaries are provided near their tips represent, it is highly probable, the reservoirs of such enzyme, as is suggestible from the disappearance of the knobs when the capillaries penetrate, by their tips, the cell-bodies which might be dissolved at the point of the penetration by the supposed enzyme discharged from the reservoir.

In spite of my efforts there is detected at length no trace of network of the tracheal capillaries in the place WISTINGHAUSEN made out, *i. e.* between the tunica propria and the layer of the gland-cells. WISTINGHAUSEN made use of comparatively low magnifying power, × 200 to 700, a power under which the terminal portion of the twigs in question can not at any rate distinctly be revealed; the author seems to have been misled in recognising some other bodies as the network of the tracheal capillaries. WISTINGHAUSEN does not touch at all the results by HELM who gives his discovery of the tracheæ within the cell-bodies in his paper published in the same journal as WISTINGHAUSEN's appeared, Zeits. f. wiss. Zool., XXVI, 1876.

1889 L. BLANC published a work entitled "Étude sur la sécrétion de la soie", which is, perhaps, of the most important after the publication of HELM's work above cited. It comprises the whole extent of the anatomy and histology of the silk glands, the secretion of the silk substances, the formation and structure of "brin" and "bave" and makes known a complete fragmentation of the cell-nuclei, and the parallel filaments on the intima of the reservoir. In a work published by the author two years later, he deals with the anatomy and physiology of the head of *Bombyx mori*, in which he elucidates the structure of the "appareil fileur", giving the descriptions and numerous figures on the excretory tube and common duct, filière[1] and its muscles, spinneret, FILIPPI's glands, etc.

The works by BLANC just cited are followed by several notable publications of investigation, for instance, the works by GILSON (1890), in which is confirmed the view of BLANC concerning the structure of the intima, a study of the silk glands by A. LENTICCHIA (1906) affirming the wall of the glands as muscular in nature, and a joint work by

[1] He applies the term "firière" to a part of the common duct.

R. MATHESON and RUGGLES (1907), according to which the nuclei of the cells composing the conducting division are "oval to rounded in shape...never branched" and the FILIPPI's glands are totally absent in *Apanteles glomeratus*.

Hithertofore the *dermo-visceral* muscles have drawn little attention of the investigators. We find in the literature only a brief note on them in a manual of "Practical Anatomy of the Silkworm"[1] by K. TOYAMA and S. ISHIWATA; the authors, however, do not go further than a brief mentioning of the presence of the muscles. As stated above, the muscles in question stand in important relation to the mode of looping and convolution of the silk glands; accordingly they are further utilized as the landmarks in researching the changes undergone by the looping and convolution of the gland tubes during larval development. First of all, the fourth muscles of the series are subserved in retaining the three parallel limbs of the middle division in their primary relative position which has been assumed in the embryonic stages, throughout whole larval life. The similer fact is recognized in the posterior division, the distal end of which is fixed by the muscles to the body-wall; this end retains always its previous position, *i. e.* on the level of the ninth segment, in spite of subsequent enormous prolongation of the tube, which has been carried on in its extent between the points fixed by the muscles.

Part II. The Silk Formation.

The *fibroin*, the silk proper, and the *sericin*, or gum, as it is often called, of which the *brin* and *bave* are chiefly composed, differ in amount according as the divisions of the glands, although both the substances are usually found at the same time in the greater parts of the gland tubes. In the middle division where the sericin is as a rule most abundantly present, this gummy substance coats the fibroin column and forms a tube with its walls of uniform thickness; the silk substances are to be traced in this condition to the anterior division *(Pl. VI, Fig.19, A, B, C; Pl. IX, Fig.35, D)*.

In some cases, however, the sericin layer is thickened on a side or two, while in the remaining parts of it the gummy layer is much thinner so as to expose the fibroin column almost bare *(Pl. VI, Fig.20, A–J; Pl. VIII, Fig.30, A)*. In other cases the sericin forms the axial cord, and the fibroin layer is laid over the sericin column *(Pl. VII, Fig.25, B)*. Not only this, but the sericin column is not unfrequently represented merely by the sericin alone *(Pl. VII, Fig.25, A ; Pl. VIII. Fig.27, A)*. On the contrary, the gland tubes of the anterior, middle and posterior divisions contain the fibroin alone, but this condition is restricted to the embryonic stages.

The posterior division contains, as a rule, only the fibroin throughout the larval

[1] This manual is written in the Japanease, published in 1900.

life. In certain stages of age, it is often found that the sericin layer encloses the central fibroin axis as in the two other divisions; this is not all, for the entire silken column is often represented by the sericin alone.

Three cases are thus discernable: *1*) the silken column composed merely of the sericin substance which sometimes occurs in all the divisions; *2*) the sericin cover of the fibroin cord in the posterior division; and *3*) the fibroin cortex of the sericin column in the middle division. All of these striking facts are of course quite new to science, and these facts are further very interesting so far as they make intelligible the precise mode of the silk formation, on which I will return later on.

The secretes differ in their consistency according to the regions of the gland in which they are contained. The secretes are denser in the region near the anterior end than in the hinder sections *(Pl.IX, Fig.34)*. The secretes cause certain modifications in their consistency in the substance of both their components, the fibroin and sericin, the latter component being in most cases more vacuolated than the former

In the fixed material of full grown larvæ the fibroin and the sericin are easy to distinguish from each other by the naked eye; the sericin looks to be a semi-transparent layer, while the fibroin is an opaque white mass. Both the substances are further distinguishable by their reactions against certain reagents, such as dilute alkali, acids, hot-water, etc., the sericin being more easily soluble in the reagents than the fibroin. In regards the special affinity for staining matters, the sericin is proved to be intensively affected by hæmatoxyline, carmine, etc., which I used in differentiating the sericin from the fibroin always with satisfactory results.[1]

The silken column is, in the anterior division, cylindrical in outline and compact in consistence, containing no vacuole in it *(Pl. VI, Fig.19, A)*; this is however not always the case, but often the silken cord assumes an irregular shape or contains vacuoles within it *(Pl. VII, Fig.24, C; Fig.25, A)*. The silken column is furthermore comparatively slender in the division spoken of, and is set off from the gland-wall, being intervened by a large space between the latter and it.

The silken column is in the middle division not always cylindrical in shape, assuming often an irregular outline, and moreover it shows not a sharp demarkation against the gland-walls, but is connected with the inner surface of the glandular cells by numerous threads of the fibroin. The connection which is established by the fibroin threads between the silken colunm and the gland-cells, is not without significance. It is a striking fact that the fibroin threads in question are most numerous during the active secretion of the substance, while they entirely disappear, when the secretion is inactive, as is obvious during the period of moulting. Occurrence of the connecting threads stands in close relation to

[1] The fibroin is less intensely affected by these staining matters.

the secreting activity; it grows more or less according as the secretion is going on actively or inactively, as seen on several series of sections either in different stages of development or in each section of the glands. We are thus forced to conclude that the fibroin threads in question are nothing else than the fibroin fluid which has been fixed by the reagents of fixation in just the moment of its being poured out.

The sericin layer defined by an irregularly frayed contour is also connected with the glandular cells by means of the sericin threads, in just the same manner, as the fibroin columns and the gland-cells are brought in relation by a series of the fibroin threads. The sericin thread connection is therefore to be looked upon as being caused by the same agencies as we affirm in the case of the fibroin thread connection; in short, the connection in the present case is brought about by the sericin fixed at the moment of its secretion. It is then obvious that *the same gland-cells, which give the fibroin, secrete the silk substance in the form of sericin at certain periods.* It must be noted that the secretion of the sericin contrasts in regards its activity to that of the fibroin: the fibroin is, as above stated, confined almost to active secretion, while the sericin thread connection is seen only in the period of inactive secretion, for instance, as the beginning and the last stages of an age *(Pl. VI, Fig.19, D; Pl. VII, Fig.23, 25).*[1]

The silken column in the posterior division usually differs from that in the middle division, wanting the sericin which is represented in the middle division as a component of it *(Pl. VIII, Fig.30, B).* Here is observed, as in the middle division, the characteristic connection of the fibroin column with the gland-cells by means of the fibroin threads *(Pl. VI, Fig.19),* making obvious the secretion actively going on in this division.

The posterior division occasionally contains the sericin which occurs either singularly or accompanied by the fibroin. A series of the sections through the gland from a larva immediately after the fourth moult shows the silken column in the posterior division which consists merely of the sericin *(Pl. VIII, Fig.29, C).* In other series of sections is also revealed a series of the sericin threads connecting, as in the middle division, the sericin mass with the gland-cells, and suggests the sericin secretion of the cells. In still other series of sections through the posterior division of the gland from a larva, in which the third moult is just over, the sericin surrouuds as the distinct cortical layer the fibroin mass which forms the axial cylinder *(Pl. VIII, Fig.33).*

Besides the cylindrical forms, the fibroin is seen in drops occuring in the substance of the gland-cells in the posterior division as well as in the middle division; the fibroin is in this way ready to be discharged into the gland tube. When the secretion is active, the fibroin drops occur very abundantly and are very minute in their individual size. On the contrary, at the stages of less active secretion occur a few of the large fibroin

[1] See future p. 15.

drops, into which the minute drops first formed became, as it is highly probable, confluent, owing to the delay of the discharge of them into the gland lumen.

From these facts we learn the very precise mode of the fibroin formation; the fibroin is not a product first formed when it is given off by the gland-cells into the gland lumen, but is produced already in the substance of the gland cells and becomes visible only when it is accumulated to give rise to the fine fibroin drops which are united further into comparatively large drops, as this is the case when the process of secretion is less active, viz. when the fibroin has to stay longer in the substance of the cells. The *secretion* in its usual meaning, as it has been denoted hithertofore in the silk glands, is then in reality not secretion but *discharge* of the substance already formed, *i. e.* of the substance secreted in *the interior of the gland cells.*

The similar occurrence refers to the sericin which frequently appears in drops in the substance of the gland cells, provided that the secretion, *i. e.* discharge of it into the gland lumen is very inactive.

Next we have to give explanations of the occurrence of the *vacuolation* which is seen in both the sericin and fibroin mass. The vacuoles grow more or less in occurrence according to stages of the ages: usually abundant in the stages of active secretion, and scarce at other times. In their every feature, the vacuoles are nothing else than the air foams or bubbles enclosed in the silk substance. The source wherefrom the bubbles come *into the places found, is most probably the air contained in the space separating the silken* column from the gland cells; and the air in this space is no doubt supplied by the gland cells themselves, in the substance of which it is given off by the tracheal capillaries opening freely by their termini. The fibroin discharged on the inner surface of the gland is drawn into the fibroin threads extended across the space around the column, to be connected with the latter in the similar way as stated in the foregoing pages (*p.* 12); the column is, in this way, added in its bulk. From this fact, it is inferable that the column itself is at first brought about, in the centre of the gland tube, by fusion of threads produced from the gland cells; subsequent growth of the column in its thickness is made, as just stated, by addition of the threads subsequently produced. As the column incessantly shifts forwards the fibroin threads are likewise drawn forwards and fused to the column. It is obvious that, under such a circumstance, the air contained in the space around the column is likely to be enclosed within the substance of the column produced and added from time to time by the fibroin threads, being divided into pieces of various sizes, *i. e.* into foams, although a certain amount of the air may be absorbed in the silk substance, or chemically combined with it. These foams or bubbles in enclosure are what we called above the vacuoles, on account of their looking like the vacuoles in the cytoplasm of certain cells.

The vacuoles found in the sericin are, as I believe, to be looked upon as being brought about by the similar processes and under the same circumstances as those in the fibroin.

In the foregoing pages I have given various states of the silk substance, as it shows in various sections of the glands and in various stages of development; and so far as concerns the silk components and their relative positions in the silken column formed of them, I have made intelligible four varieties of cases: *1*) the silken column consists of the fibroin representing the axis of it, and of the sericin which coats the fibroin axis forming cortex of it; *2*) the silken column is represented by the bare fibroin, the sericin cortex wanting; *3*) the silken column is formed merely of the sericin; *4*) lastly, the sericin column constitutes the silken column which is coated with a thin fibroin cortex. In addition, I have pointed out the fibroin drops which are secreted in the substance of the gland cells themselves and are destined to be incorporated into the fibroin column, having been discharged in the gland lumen. And further I have found the sericin drops which have the fates to incorporte into the sericin mass and contribute to the formation of it in the same way as in the case of fibroin drops.

We have now to deal with the genetic relation between the two components of the silken column, the sericin and fibroin. In the most usual cases in which the process of secretion is actively going on, the silk gland content of the posterior division is represented by the fibroin, and in the middle division is contained the silken column formed of the fibroin axis with the sericin cortex, which latter grows thicker towards the anterior part of the gland tube, passing over into the silk substance in the anterior division, which is represented by the more slender silken column with the sericin cortex of nearly homogeneous thickness. There is little room to doubt that the sericin cortex is transformed from the superficial layers of the fibroin column by some *chemical changes* undergone by it during its transportation from behind forwards; the sericin cortex is thicker in the anterior part than in the posterior simply because the chemical action displays longer and accordingly goes into layers deeper than in the posterior part.

In some cases, the fibroin column is entirely transformed into the sericin, owing to nothing else than longer exposition of it in the chemical action; such cases are often observed in the glands of larvæ at or just after a moult, *i. e.* in stages when the secretion stops. In the cases of secretion quite inactive, the sericin is discharged actually as such, as referred to in the foregoing pages; the fibroin is in the cases under consideration to be looked upon as having been converted, in the form of drops, into the sericin before it is discharged into the gland lumen owing to a greatly delayed discharge of it.

According to the view advocated by Blanc and others, the chemical changes in question consists in convertion of the sericin from the fibroin when the latter is oxidised and is combined witn the molecules of water; the circumstances under which the sericin is formed are in harmony with this view. The chemical changes refer, in the gland lumen, to the air contained in the space around the silken column and in the cell substance, into which it is given off from tracheal openings. In both the cases the air which is saturated with the vapour is, as it need not further explanations, in a condition very favourto able in oxidising the fibroin, as in the space of the gland tube, or the drops of it, as in the cytoplasm, and in giving off the water molecules from the vapour to be combined with this silken substance.

Then it is clear that the fibroin is *the first substance which is secreted by the gland cells, and that the sericin is secondary in formation.* The sericin formation is quite obvious: first, it is converted from the fibroin through the chemical changes undergone by the latter; secondly, the chemical changes takes place *either within the cell substance or in the gland lumen,* into which the fibroin is discharged by the gland cells.

This view does not crash with the cases in which the sericin axis is coated with the fibroin cortex; the cortex is added by its secretion reappearing after a cessation of secretion in the moulting period.

So far as I am aware, all the previous observers overlook the secreting power of the anterior division, or else this power is positively denied. According to the results of my present observation, this division of the gland is also able to produce certain secretes. In embryos more than three days before hatching, the fibroin secrtion is distinctly recognized, as shown by means of staining reaction *(Pl. VI, Fig.18, A).* Then it is obvious that the silken fluid is, in the embryonal stages, given by the whole extent of the silk gland, without distinction of the divisions so far as the secreting power concerns. It is further to be noted that the silk secretion of the anterior division is soon given up after the hatching, its secreting power is however not deprived: it secretes the chitinous substance which gives rise to the intima which attains its considerable thickness in that division. The intima never undergoes ecdysis throughout life; on the contrary it is added by ages in thickness,—a fact which goes a long way in proving the secretion of this chitinous substance through the whole larval life.

The so-called "mucoidine", which was pointed out by BLANC, could not be made out in the present work, notwithstanding I took all the methods possible. According to BLANC the "mucoidine" shows a strong affinity to staining fluids; but various staining matters I employed in this respect give no differentiation in demonstrating the substance in question at all.

There is a fact which perhaps wants a special notice. A peculiar feature is often seen in the sericin contained in the anteriormost part of the middle division; the sericin layer often sends out the processes of dendric form *(Pl. III, Fig.3, C)* and gives off in other cases simple protuberances into the fibron mass *(Pl. IX, Fig.36, C).* There are also the cases in which a part of the layer is simply thickened, striking against the surface of the fibroin mass *(Pl. III, Fig.3, A)*. From the affinity to the staining matters as well as from the place found, this peculiarly outlined outgrowth of the sericin layer might be suggestible to answer to the "mucoïdine" of BLANC.

Lastly, with reference to the secreting activity of the glands the following sketches will be given, although this power has incidentally been touched upon in the foregoing statements. During the ecdysis, the silk secretion is temporally given up; consequently, at the later stages of a moulting period or immediately after it, the posterior division is completely empty, as not unfrequently happens *(Pl. VII, Fig.24, A)*. As soon as the moulting is over, however, the secretion recurs and recovers its activity day after day. The secreting activity attains its maximum in the period of greatest voracity; and then the secretion again declines gradually, until it is totally given up when the following moulting period sets in.

On the forces by which the silk fibre is set forth, GILSON remarks that the "press" acts as the organ forcing exterior the silken thread. BLANC assumes the mechanism as consisting in the effect of two forces combined, a negative pressure and a positive pressure; the former is produced, according to the author, in the "press" when the lumen of it is brought into full distension by the contraction of the muscles inserted to the gland walls of the filière, while the latter arises from the added blood pressure brought about by the general contraction of the body. The combined action of these forces is, as BLANC believes, efficient enough to pass forwards, as the negative pressure is so arranged that the positive pressure exerted upon the gland contents is almost wholy utilized in pushing forwards the silk thread. I have no fact, which accounts for or opposes the negative pressure suggested by BLANC; the general contraction of the body may make higher the blood pressure, but this selfevidently does not afford the positive pressure desired by BLANC as being indispensable in the function under consideration: it grows necessarily higher and lower at times—it is intermittant in action. It is beyond doubt that the air contained in the gland tube preserves a considerable amount of pressure which grows higher in the stages of active life from time to time by the air, increasing in amount, being put off from the tracheal twigs. This pressure—the positive pressure—exerts necessarily its action upon the gland contents; when this pressure is regulated by the negative pressure, the combined action is, I believe, sufficient enough to press forwards the gland contents, the silk substances.

This action is evident to be absolutely functional so far as concerns the free emit of the silk either in the first beginning of the spinning or in accidental cases when the silk thread is broken in the filière while spinning. In the case when the spinning is smoothly carried on, the spinnig function is, however, assisted a great deal by the tenacity of the produced thread which is fixed on a rigid object such as the cocoon, or the branches of trees.

Observations of the stages of inactive life show further that the distal part of the gland tube, is often empty, as this is the case in the posterior division. From this fact we learn that the secretion is occasionally given up entirely. This condition of the gland is seen often in the later period of moulting. From the circumstances under which this condition is realised, it is suggestible that the silken column still shifts forwards with some activity, after the secretion is given up.

The silk formation was comparatively late in drawing the attention of special investigators. As early as 1839 there was only a view put forwards by STRAUSS-DÜRKHEIM; according to this view the silk fibres are contained as such already in the reservoir of the gland. This assumption was afterwards proved by ROBINET (1844) to be false. BOLLEY (1864) worked on the chemical composition of the silk fibre and distinguishes for the first time the two components of the cocoon fibre, the fibroin and the sericin, and the author further affirms that the sericin which forms the cortex on the fibroin axis in a silk fibre is brought about by oxidation undergone by the cortical part of the fibroin column *when it is spinned out* and is exposed in the atmospheric air. HABERLANDT (1871) agrees with the view prevailing in his day; according to this view the essential silk material (fibroin) is secreted in the posterior division, while the sericin is produced in the middle division; on the process of production the author gives no account at all.

LIDTH DE JEUDE (1878) believes that the fibroin is probably the product of the gland cells constructing the posterior division, while the sericin is exclusively secreted by those of the middle division. H. SICARD and RAULIN (1887) regard the "grés" as the secrete of the middle division of the gland.

As regards the fibroin and sericin, "La fibroïne", L. BLANC says, "est fabriquée par la paroi du tube sécrèteur seul****. Le grès se montre dès l'origine du resérvoir". The author failed to make out the "mecanisme" of the sericin formation: he gives, "S'il est aisé de constater que c'est dans le réservoir, et en particulier dans les deux tiers postérieurs de celui-ci que ce produit le grès, le mécanisme de cette production nous a échappé"; and comes to the assumption as the follows: "la fibroïne, en arrivant dans le réservoir, se trouve au contact d'une membrane étendue, très mince et particulièrement riche en trachées qui lui apportent une quantité d'air considérable. Sous l'influence de cet air, avec lequel elle se trouve en contact presque immédiat, et aussi par suite de l'action directe des cellules de la paroi, la fibroïne est modifiée dans ses couches superficielles. Ces modifications, dons la principale est une oxydation, la transforment partiellement en grés". On the "mucoïdine" which has been known by BLANC, he says: "en examinant le réservoir dans sa partie antérieure, on remarque, sur les coupes convenablement colorées au vert de méthyle, qu'il *existe a la périphérie du grès, entre cette couche et l'enveloppe, une troisième substance, qui fixe les matières colorantes bien plus énergiquement que les deux premières"* (the fibroin and sericin). The mucoidine is formed, as the author believes, in the anterior portion of the reservoir, and it completely covers, in the excretory tube, the silken column, shifting forwards accompanied by the brin and bave to the filière; but its ultimate fate was unknown to him.

The results arrived at by G. GILSON (1890) seem to be peculiar. The fibroin and sericin are, according to him, not separated from the first each other, but are represented by a mixture which is secreted as such from the whole extent of the gland, and is separated *into the two substances, the fibroin and sericin, by means of a kind of selection going* on in the secreted mixture when it is still not apart from the cells secreting it. In their joint work, VERSON and QUAJAT (1896) come to the assumption that the fibroin secretion is confined to the posterior division, while the sericin is secreted in the whole extent of the reservoir. The authors confirm BLANC's view about the "mucoïdine".

MAILLOT and LAMBERT (1906) are in accordance with JEUDE in assuming that the fibroin is the secrete of the posterior division, while the sericin is the product of the middle division. MATHESON and RUGGLES (1907), who observed the silk glands of *Apanteles glomeratus*, incline to assume the secretes to be somewhat different in nature according as the parts producing them.

The views advanced by previous authors, as above given, may be classified into four principal groups, namely: *1*) the fibroin is secreted from the gland, and the sericin is produced by oxidation of the fibroin *after the spinning* (BOLLEY); 2) the fibroin and sericin is represented by a mixture when secreted; they are separated from each other by a peculiar process called "selection" which is performed in the peripheral parts of the secreted matter (GILSON); *3*) both the fibroin and the sericin are secreted from the glandular cells, the former from those of the posterior division, and the latter from those of the middle division (LIDTH DE JEUDE, RAULIN, SICARD, MAILLOT, LAMBERT, etc.); *4*) the fibroin is secreted exclusively from the posterior division, and the sericin is transformed in the middle division from the cortical part of the fibroin column, which undergoes oxidation therein (BLANC, SILBERMANN, VERSON, QUAJAT, etc.). The first view represented by BOLLEY had lost its basis long ago, since the discovery of the sericin in the gland's interior. The second view advocated by GILSON is unintelligible, as much as unreasonable is the spontaneous separation of the secreted matter by means of the "selection". The third and fourth views can not be also confirmed; yet these two views are those which are, at present, in general acceptance. The third view is incorrect, first of all, so far as it assumes the physiological division of labour of the gland cells forming the middle and posterior divisions, the fibroin secretion confining to the gland cells of the posterior division, and the sericin secretion to those of the middle division. The fourth view can no longer be supported in the present form, which expresses only a part of the fact, as the following accounts will show. This view does not make intelligible, first of all, all the cases of the sericin formation, because it fails to notice this process displayed by the gland cells of the posterior division; in the second place, the secreting power of the middle division is not recognized, and this section of the gland is regarded merely as a reservoir for the secretes shifting from the posterior division in which they are produced. The factors which mislead the view under consideration consist in abundant occurrence of the sericin in the middle division and in copious accumulation of the fibroin in the posterior division, as these conditions are seen in a case usually observable. Such a case is in fact to be regarded as *usual* because very easy to observe, being displayed in the silk gland of active secretion; it represents, however, only a side of the fact, other sides of which are manifested in the silk gland of inactive secretion—the cases by no means easy to work out, referring to the stages of moulting, or to those just after it. In the cases of inactive secretion we can distinguish, as stated in the foregoing pages, two ways of the sericin formation, namely the sericin formation within the cytoplasm of the gland cells and that in the interior of the gland tube on account of its long stay therein. The latter case is, so far as the sericin formation itself concerns, fundamentally not different from that advocated by the fourth view; however, the sericin secretion of the cells of the middle division is erroneously negated, being mislead by the observations which overlooked this peculiar case of inactive secretion. The former case, the case of sericin formation within the cytoplasm of the gland cells, is so important to make known a peculiar form of the sericin formation under influence of the air emitted from the tracheal capillaries, and by which is intelligible the sericin secretion of the gland cells. There is, then, no controversy to conclude that *the fibroin is produced by virtue of the physiological function of the gland cells, without reference to any part of the gland tube; the sericin is, in its formation from the fibroin, quite indifferent from the physiological function of the cells; on the contrary this transformation goes on merely physico-chemically.* From this fundamental conception the divisions of the gland are not to be discerned as regards the kinds of secretes they give.

Summary.

1) The silk glands are in earlier stages inconspicuous bodies found on either side of the central nervous system, but after the fourth moult, they grow to fill up the body-cavity occupied formerly in greater part by the alimentary system which is then set aside to a part of the cavity.

2) In the silk gland a common duct and two limbs are to be discerned; the limb

represents the gland proper in which three divisions are to be distinguished: the anterior, the middle and the posterior; the middle division is twice bent, whereas the posterior division shows, in a full grown larva, numerous convolutions.

3) The gland walls are composed of three layers; the structureless tunica propria, the layer of the gland cells and the tunica intima; the gland cells constitute the gland tube composed of two rows of the cells which together form a ring on a cross section and are closely put together being not separated by spaces as BLANC believes.

4) Ramifications of the nuclei appear at first in the cells of the anterior division, although in full grown larvæ they are most developed in the middle and posterior divisions.

5) The *tunica intima* is very thick in the anterior division and is suddenly thinned in passing over into the middle division with the very thin intima which is also the case in the posterior division. This layer in the middle division shows further characteristic filamentous appearance in consequence of its probable local thickenings; the filaments are transformed in the posterior half of the posterior division into a complicated network. In contrast to the cuticular structures of other parts, the tunica intima is not renewed at moultings.

6) A series of muscles which I called above the *dermo-visceral muscles*, is nine pairs in number, of which those of the fourth pair and certain branches from the eighth pair muscles are inserted to the middle and posterior divisions respectively, being subserved in suspending the gland between the integument and the enteric wall. The muscle elements have no share in the construction of the gland tube; the view by LENTICCHIA is therefore incorrect.

7) The branches of tracheæ which are supplied to the gland walls form not a network spreading between the tunica propria and the layer of the gland cells, but enter the cytoplasm of the cells to give within it the branches splitted again and again; the tracheal capillaries thus brought about end freely in the cytoplasm. The old tænidia is cast off at every moulting to be replaced by a new; the inner old and the outer new are often present at the same time. The anterior division is quite free from the tracheæ.

8) There is detected no trace of the nerves specially supplied to the silk glands.

9) One of the components of the silk fibre, the fibroin, is produced in virtue of the physiological functions of the gland, whereas the other, the sericin, is transformed from the fibroin by a certain chemical action. The transformation is carried on either in the substance of the gland cells or only in the interior of the gland tube into which the fibroin is discharged beforehand. In the latter case, *i.e.* in active secretion, the silken column in the posterior division is formed of the fibroin alone, and in the middle and anterior divisions, the fibroin represents the central axis coated by the sericin cortex which

is converted from the superficial layer of the fibroin column by the chemical action on the way of the silken column shifting forwards. In the former case, *i.e.*, in inactive secretion, the silken column is often represented by the pure sericin mass, being chemically transformed in the cytoplasm of the gland cells. Accordingly it is not peculiar that the silken column with the sericin axis and the fibroin cortex occurs when an inactive secretion is followed by an active secretion.

10) In embryonal stages, the cells forming all the *three* divisions secrete the silk substance; the silk secretion is given up in the anterior division when the larva hatched, but this division loses not at all its secreting power: it secretes, thenceforth the chitinous substance, the intima.

11) There is not found any substance which represents the "mucoïdine" made out by BLANC.

12) The vacuoles of the silken column which vary in size and occurrence according to stages, and from which the silken column in the anterior division is usually free, are produced by air contained in the gland lumen.

13) The air given off from the open termini of the tracheal capillaries acts in oxidizing the fibroin which is transformed into the sericin; that discharged in the gland lumen is at the same time utilized as the motive power in forward shifting of the silken column, in such a way that increasing pressure of the air in the gland lumen carries forwards the silken column in connection with a negative pressure produced in the filière by distension of its walls.

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Explanation of Plates.

The most figures represent the preparations of univoltin race, and a few show those of bivoltin race; the latter are distinguished by putting the name of the race.

List of Abbreviations.

a...........anterior end of the anterior division.
a. c.........alimentary canal.
am.........boundary of the anterior and middle division.
c. f.........tendon of fatty tissues.
c. m.........tendon of muscles.
f...........fibroin mass.
f. t.........fibroin thread.
g. l.........gland lumen.
m^1-m^9.......dermo-visceral muscles from first to ninth.
mp.........boundary of the middle and posterior divisions.
m. s........muscles of alimentary canal.
n...........nucleus.
p...........posterior end of the silk gland.
s...........sericin mass.
s'...........peculiar structure of the sericin layer.
s. c.........silken column.
s. g.........silk gland.
s. t.........sericin thread.
t...........trachea.
t. i.........tunica intima.
v...........vacuole.

Plate III.

Fig. 1. Silk glands in various stages of growth. Magnification: *A-E,* 5/1; *F,* natural size. From *a* to *am,* anterior division; from *am* to *mp,* middle dlvision; from *mp* to *p,* posterior division.

A, first day of first age (the day in which the larva hatched out).

B, first day of second age (9 days after hatching).

C, first day of third age (13 days).

D, first day of fourth age (20 days).

E, first day of fifth age (27 days).

F, eighth day of fifth age (34 days, full grown).

Fig. 2. Cross-sections through the body showing the relative sizes of the alimentary canal and silk glands.

A, cross-section through the fifith segment of a larva in the day of hatching; the section passing through somewhat obliquely. Zeiss 1 × E.

B, cross-section through the fourth segment of a full grown larva, slightly magnified.

Fig. 3. Optical longitudinal sections through the passages from one division of the silk gland to another. Zeiss 1 × E.

A, passage from the middle division to the anterior, second day of third age, showing merely thickened layer of the sericin;

B, passage from the middle division to the posterior in the same individual as that shown in *Fig. A.*

C, passage from the middle division to the anterior, in which the peculiar structure of the sericin is to be seen, fourth day of third age,

Fig. 4. Diagramatic view of various shapes of gland cells.

A, from the anterior division;

B, C, D, from the middle and posterior divisions.

Plate IV.

Fig. 5. Semi-diagramatic view of the body of silkworm, cut open the skin along the median ventral line, and showing the alimentary canal and the arrangement of the dermo-visceral muscles, slightly magnified.

Fig. 6. Ventral view of the middle division and the muscles inserted to it, magnified.

Fig. 7. Dorsal view of a part of the alimentary canal with the distal section of the silk gland *in situ,* showing the manner in which the gland is fastened to the canal by

the eighth muscle; first day of fifth age, magnified. *VIII, IX, X* indicate the eighth, ninth and tenth segment respectively.

Fig. 8. Distal end of the silk gland in a full grown larva. Leitz 3×3.

Fig. 9. Distal end of the silk gland and the muscles connected with it, first day of fifth age. Leitz 3×3.

Fig. 10. Diagramatic view of the posterior part of the silk gland, showing structures attached to the gland and resembling nerve fibres in appearance.

Plate V.

Fig. 11. Ventral view of the middle division with tracheal tubes distributed on it, first day of fifth age, magnified.

Fig. 12. Tracheal tubes with their termini, fifth day of first age. Leitz 3×7.

Fig. 13. Tips of trachea in the moment coming in contact with the silk gland, seventh day of first age. Leitz 3×7.

Fig. 14. A part of the cross-section through the middle division, first day of fourth age. Leitz 3×7.

Fig. 15. Cross-section through the middle division, in first day of fifth age, bivoltin, showing glandular cells which are richly distributed with tracheal tubes. Zeiss $1 \times$ E

Fig. 16. A part of the cross-section through the middle division, during the fourth moult, showing the double rings of tænidia; bivoltin. Zeiss $4 \times$ E.

Fig. 17. Extremities of trachea ending freely in the cell bodies. Zeiss compensat. ocul. $18 \times$ apochr. object. 2m.m.

Plate VI.

Fig. 18. Cross-sections of the silk gland in a larva three days before hatching; *A, B, C* represent the cross-sections through the anterior, the middle and the posterior division respectively. Zeiss $2 \times$ homog. immers. 1/12.

Fig. 19. Cross-sections through various parts of the silk gland in a larva just hatched. Zeiss $\times 4$ homog. immers. 1/12. *A–C,* from the anterior division; *D–J,* from the middle division; *K,* from the posterior division.

Fig. 20. *A–J.* cross-sections through various parts of the silk gland in a full grown larva, showing the distribution of the sericin and fibroin. The sections from *A* to *J* pass respectively through the places indicated by the dotted lines with the same letters as in the diagram *K,* magnified. *K,* a diagram of a part of the silk gland to indicate the planes of the cross-sections represented in *A–J.*

Plate VII.

Fig. 21. A part of the longitudinal section through the posterior division of a full grown larva, showing elongated vacuoles in the silken column. Zeiss 1 × E.

Fig. 22. A part of a section from the same series as that given in *Fig. 21,* showing numerous round vacuoles. Zeiss 1 × E.

Fig. 23. Cross-section of the silken column in the middle division, first day of fifth age, showing vacuoles contained in the silk substance. Zeiss 1 × E.

Fig, 24. Cross-sections through the posterior *(A),* the middle *(B),* the anterior *(C)* division, during the fourth moult; bivoltin. In *A,* no secretes are to be seen; in *B* is contained the fibroin alone secreted by the glandular cells, and in *C,* no content else than the sericin ring is to be seen. Zeiss 1 × E.

Fig. 25. Cross-sections through the anterior *(A),* and the middle *(B)* division immediately after the fourth moult. In both the divisions the silken column consists almost absolutely of the sericin. In the middle division, however, the newly secreted fibroin is laid upon the sericin column. Zeiss 1 × E.

Fig. 26. Cross-section through the middle division, seventh day of first age. Zeiss 2 × homog. immers. 1/12.

Plate VIII.

Fig. 27. Cross-sections through the anterior *(A)* and the posterior *(B)* division, first day of fifth age; bivoltin. In the posterior division no secrete is found, while in the anterior division, the silken column consists merely of the sericin. Zeiss 1 × E.

Fig. 28. Cross-section through the middle division, in second day of fifth age, bivoltin, showing the sericin block imbedded in the fibroin mass. Zeiss 4 × A.

Fig. 29. Cross-sections through the posterior division of the same gland, as that from which the preceding figure was taken, showing gradual increase of the silk secretion towards the anterior portion, the secrete being represented by the sericin. Cross-sections *A, B* and *C* pass through respectively the middle and the fore-part of the division. Zeiss 5 × A.

Fig. 30. Cross-sections through the middle *(A)* and the posterior *(B)* division in a full grown larva. The silken column contained in the posterior division consists of the fibroin only, and that in the middle division is made of both the fibroin and sericin. Zeiss 1 × A.

Fig. 31. A part of the section given in *Fig.30, A.* Highly magnified. Zeiss 1 × E.

Fig. 32. Cross-sections of an irregularly shaped silken column in the posterior division,

fourth day of fourth age. *A*, foremost, *B*, middle and *C*, hindmost section. Zeiss 5 × A.

Fig. 33. Cross-section through the posterior division, first day of fourth age, showing the sericin rings enclosing the fibroin column and fibroin drops in the gland lumen. Leitz 3 × 7.

Plate IX.

Fig. 34. Cross-sections through various planes of the middle division, seventh day of third age. *A*, the foremost and *E*, the hindmost, *B*, *C*, and *D*, the intermediate part between *A* and *E*. The figures show, according to successive portions of the gland, the successive change of the relative positions of the fibroin and sericin, and that of the consistency of the silken column. Zeiss 2 × E.

Fig. 35. *A*, a diagram of the middle division, the dotted line *xy* indicates the plane of the cross-section given in *B–D*. *B–D*, cross-section through the dotted line *xy* in the diagram *A*, showing the gradual change of substances in the external layer, in correspondence to the sections of the gland following one after another. Zeiss 1 × E.

Fig. 36. Optic longitudinal sections through the passage from the middle division to the anterior, showing peculiar structure of the sericin layer therein, in several stages of larval life.

A, seventh day of first age. Zeiss 4 × E;
B, fourth day of third age. Zeiss 1 × E;
C, first day of fourth age. Zeiss 1 × E.

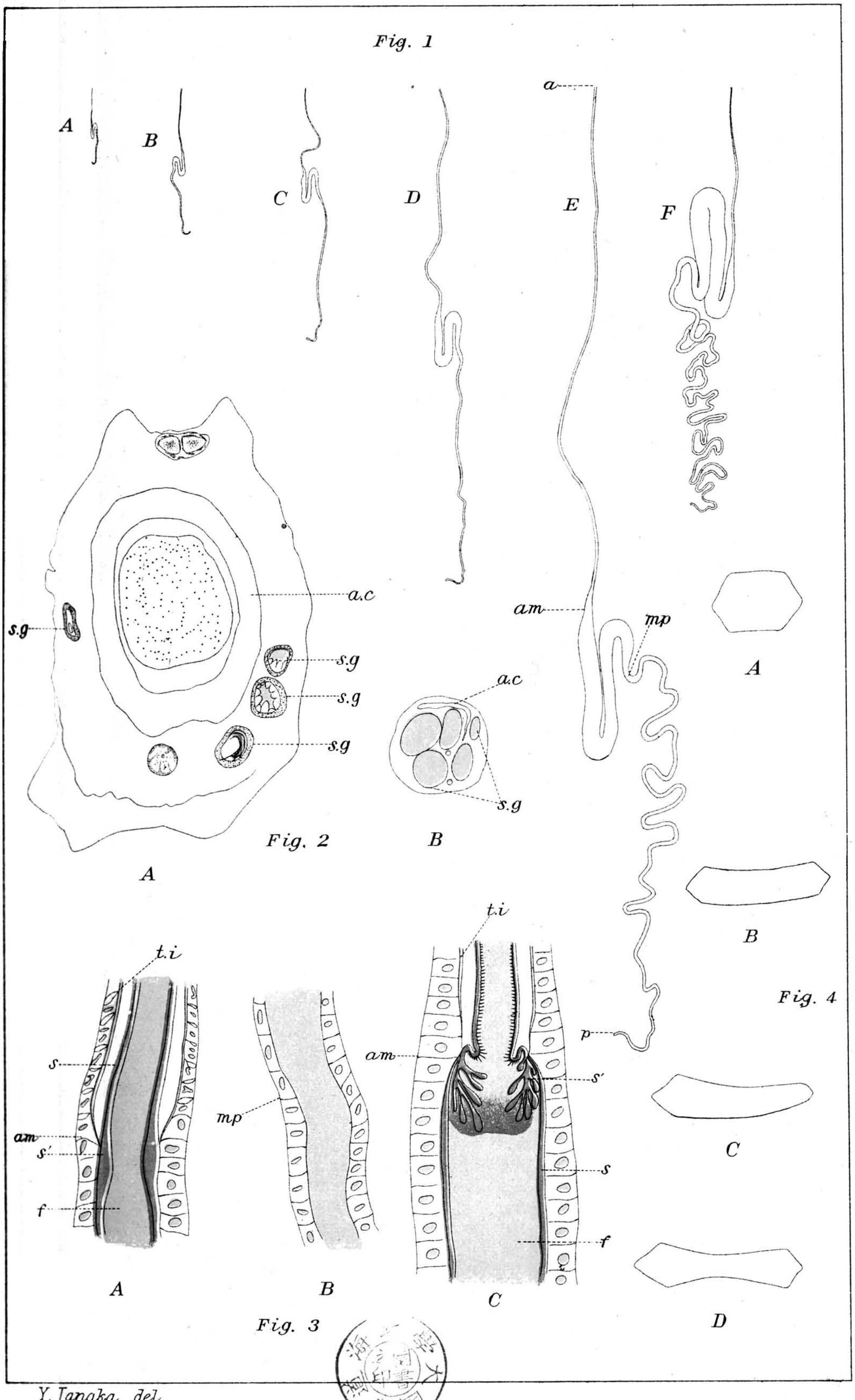
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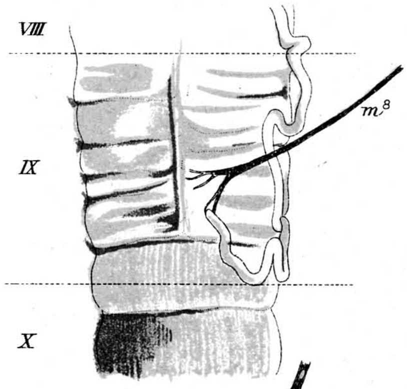
Fig. 7



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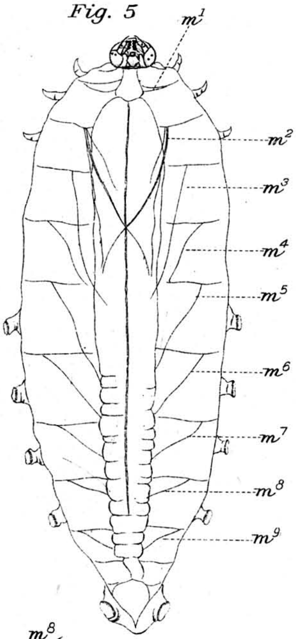

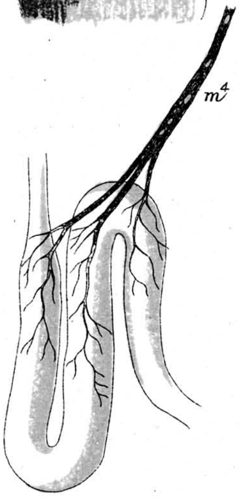

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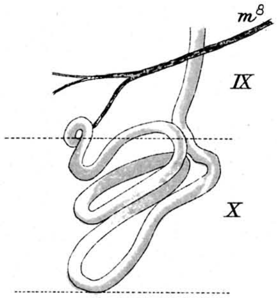

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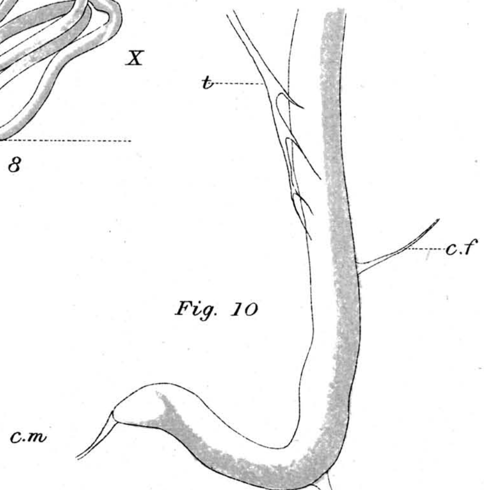

Fig. 10

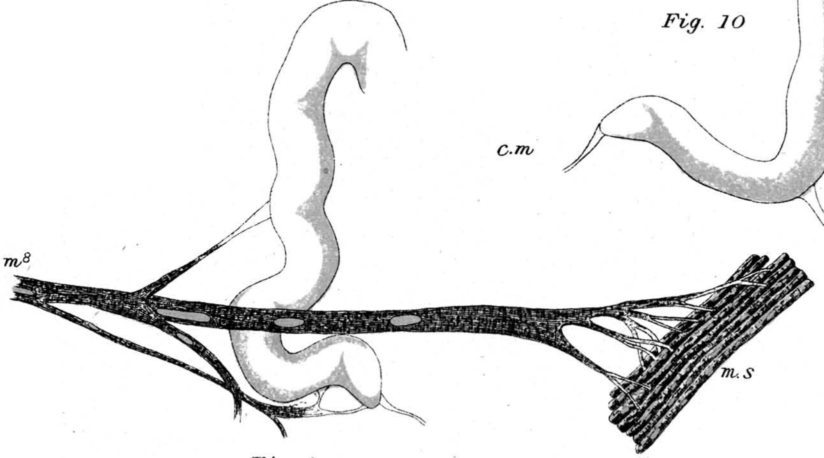

Fig. 9

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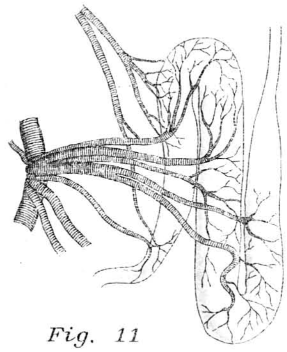


Fig. 11

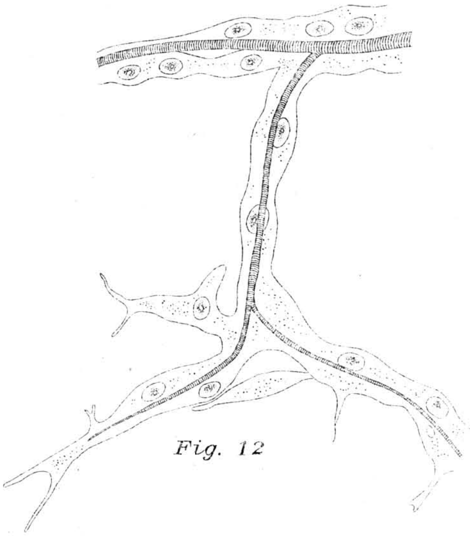
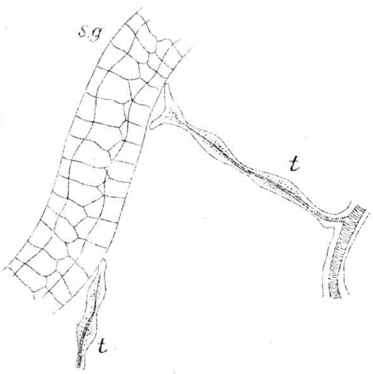


Fig. 12

Fig. 13



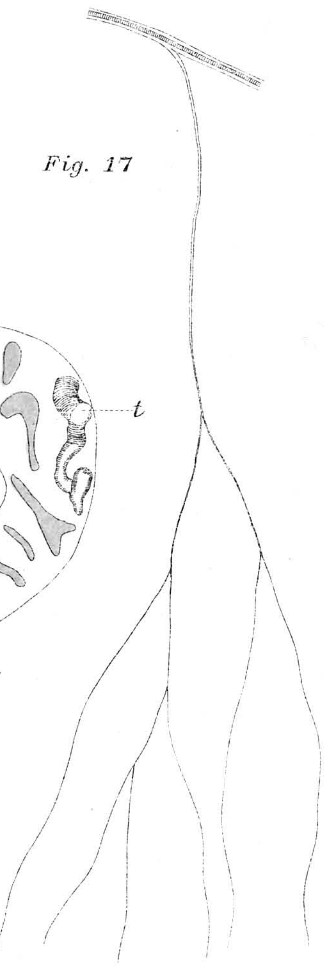


Fig. 17

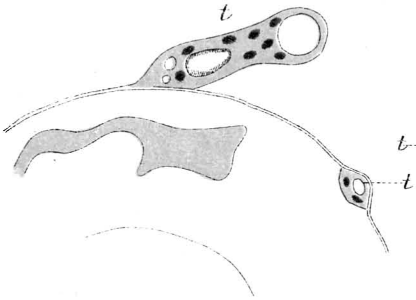
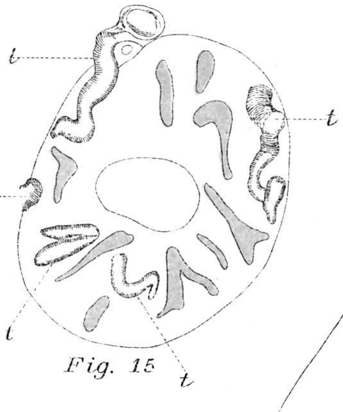


Fig. 14



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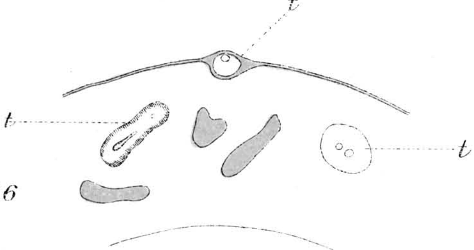

Fig. 16

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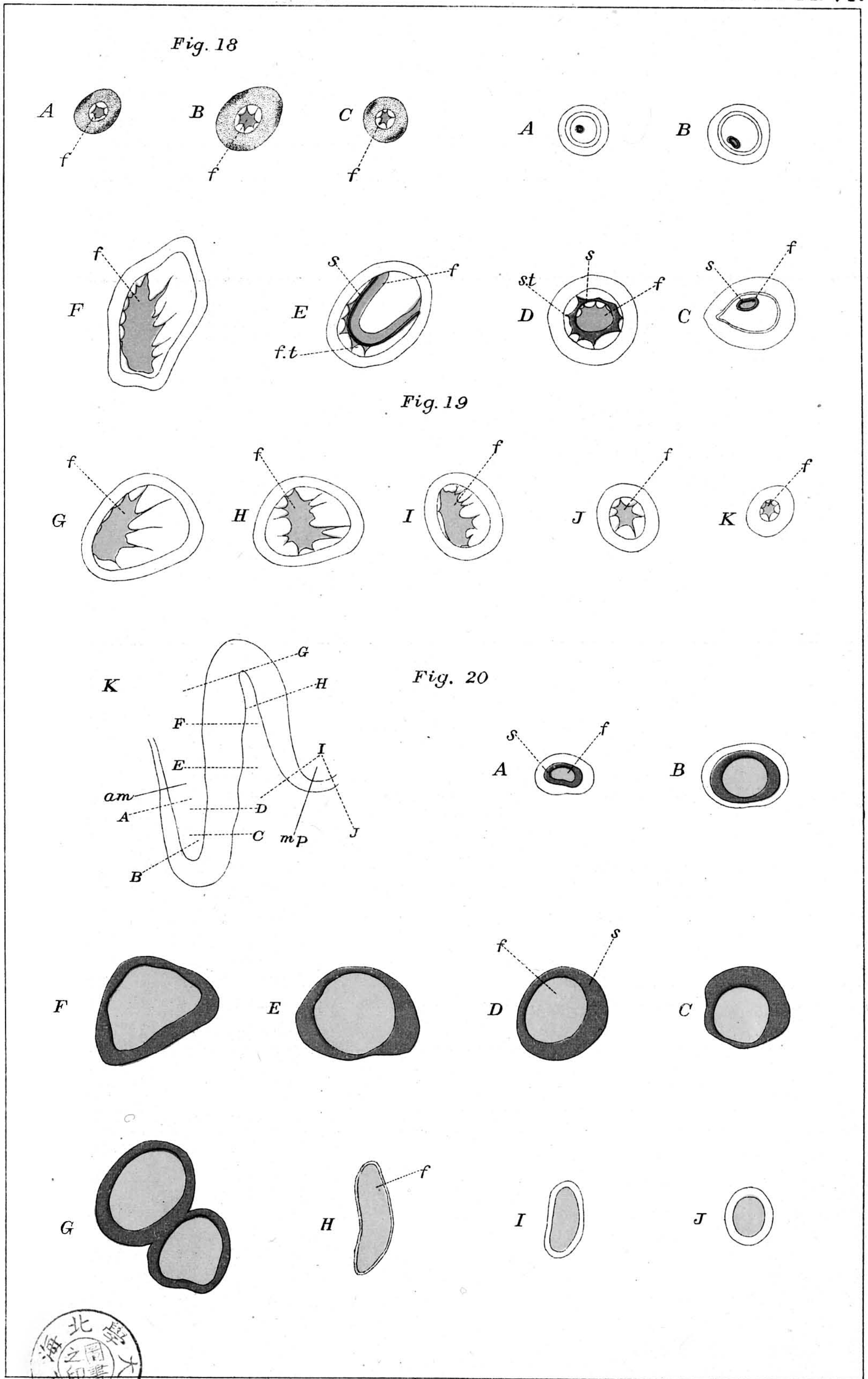

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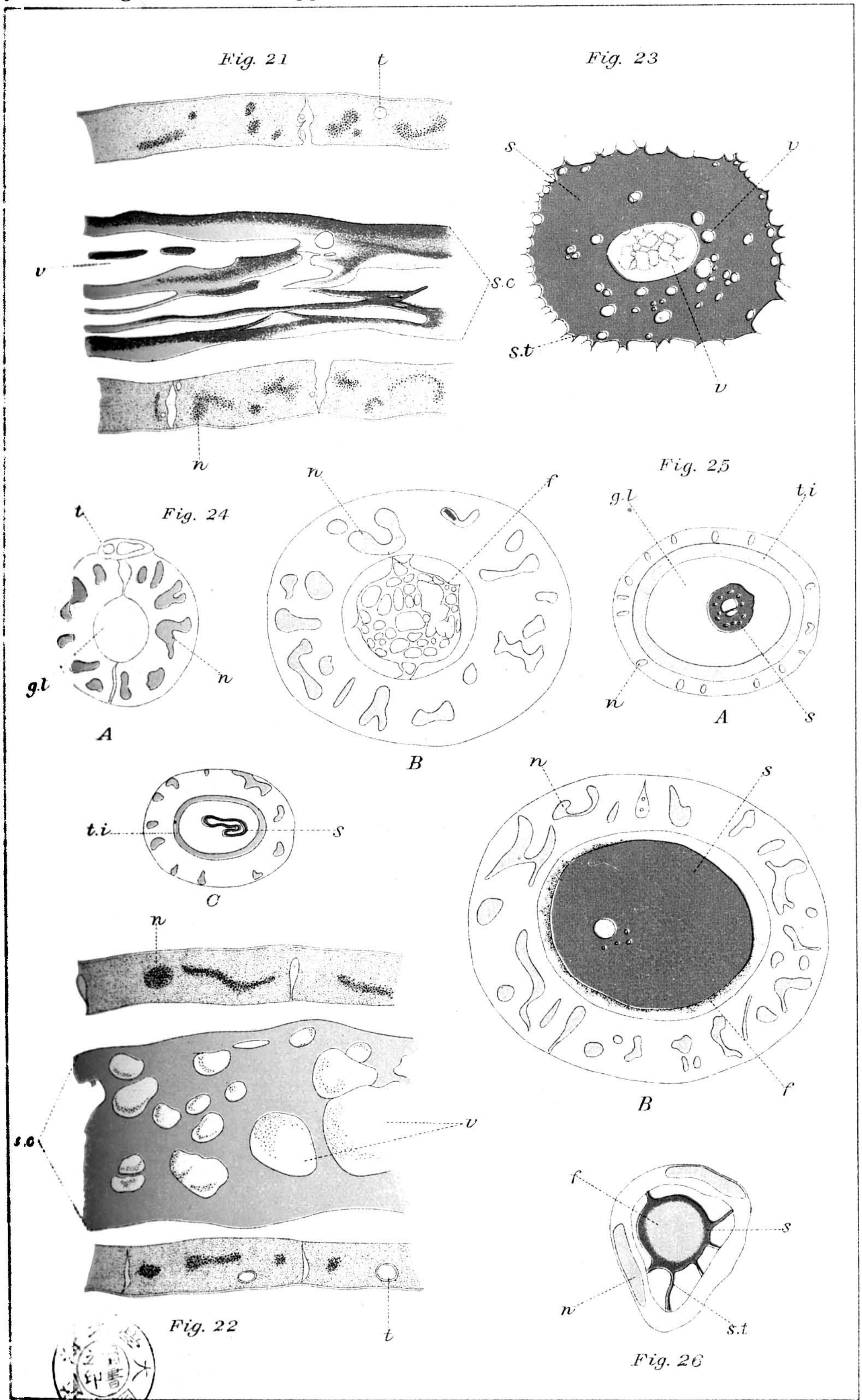
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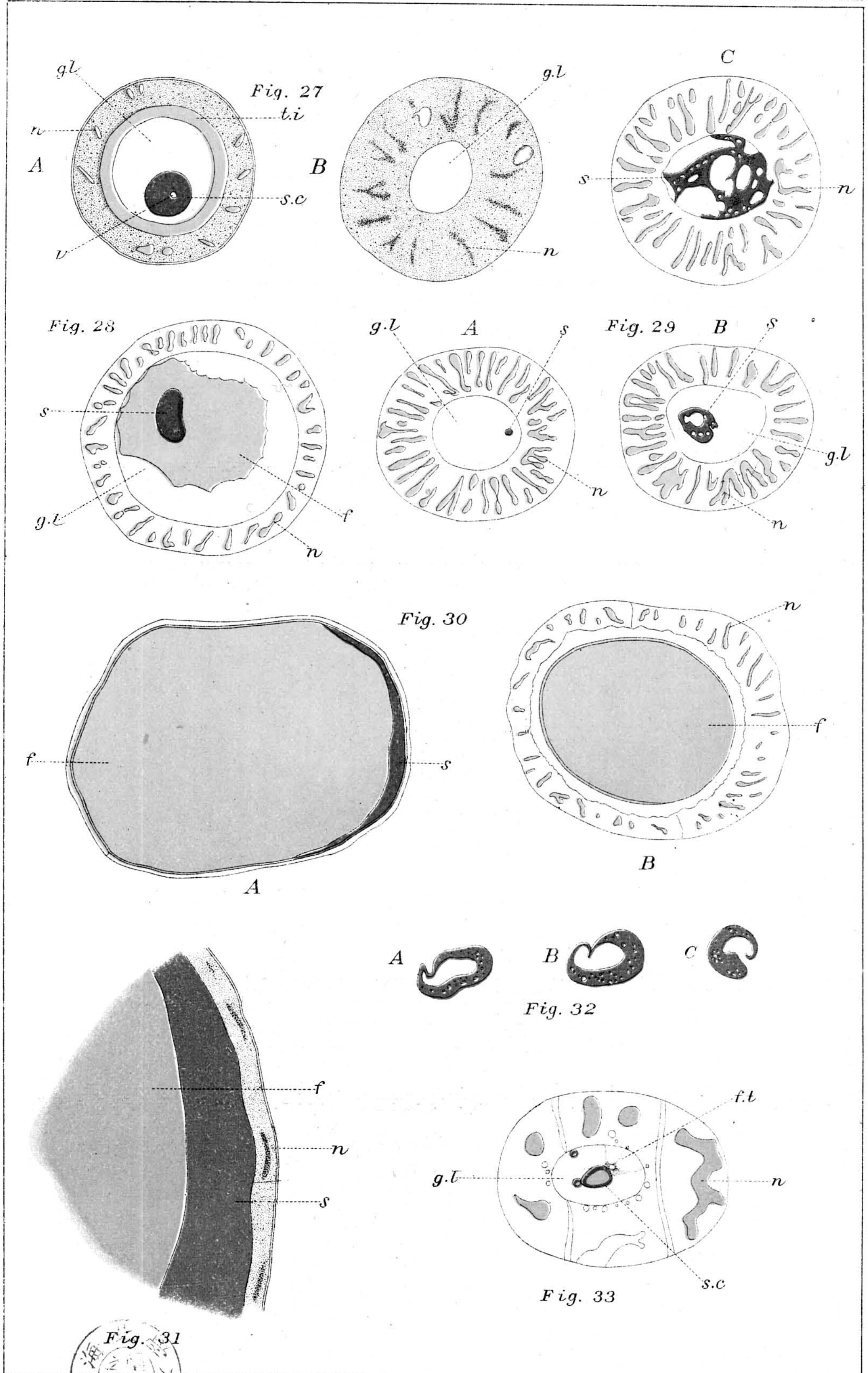
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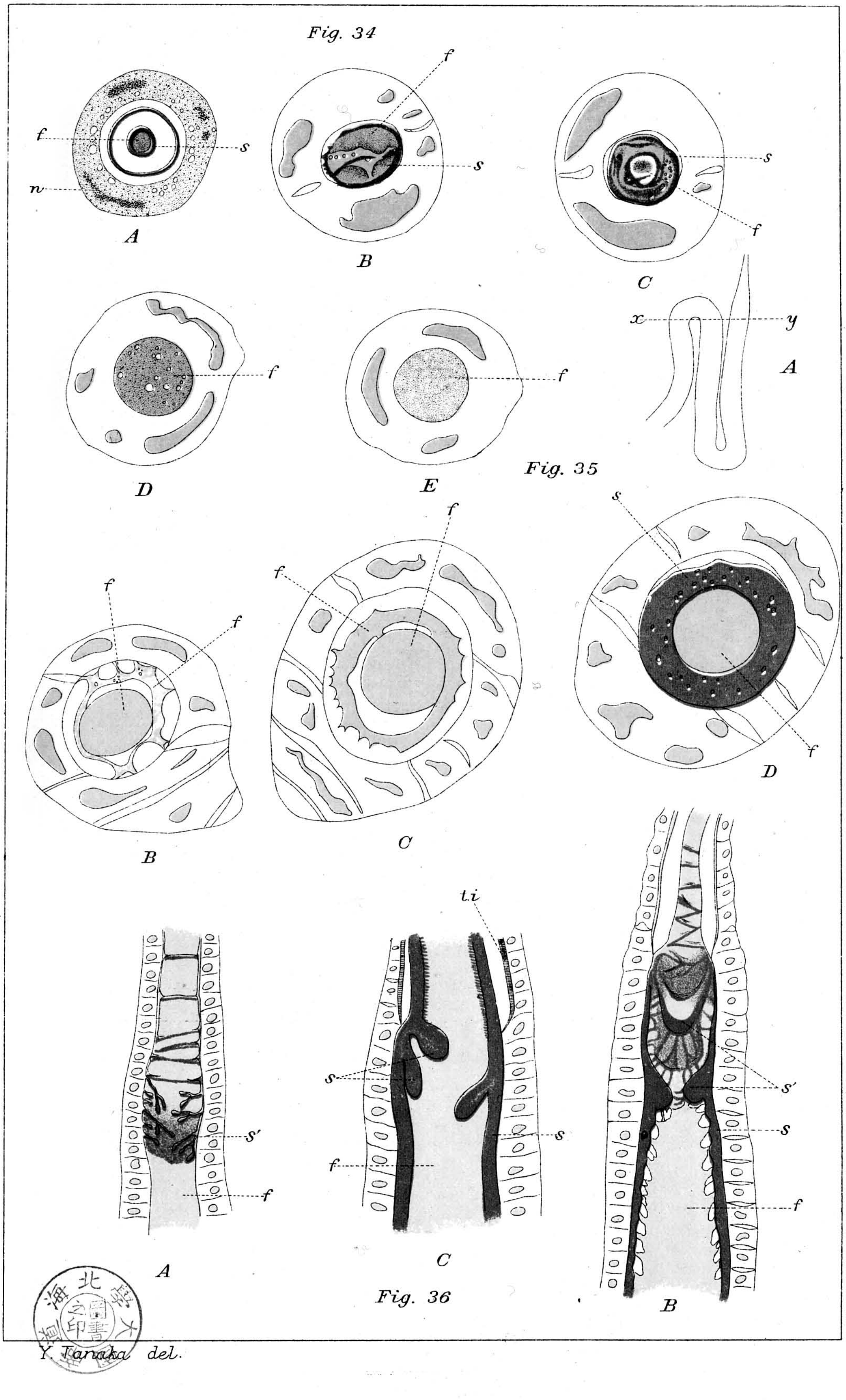
Fig. 34
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Fig. 36

Y. Tanaka del.