

STUDIES ON THE POSTERIOR SILK GLAND OF THE SILKWORM, *BOMBYX MORI*

I. Growth of Posterior Silk Gland Cells and Biosynthesis of Fibroin During the Fifth Larval Instar

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ABSTRACT

Growth of the posterior silk gland and biosynthesis of fibroin during the fifth larval instar of the silkworm, *Bombyx mori*, have been studied. In accordance with the exponential increase in the wet weight of the gland, the amounts of DNA, RNA, protein, and lipids per animal increased rapidly in the early stage of the fifth instar (0–96 hr). Biosynthesis of fibroin, on the contrary, mainly proceeds in the later stage of the fifth instar (120–192 hr). Electron microscopical observations have shown that, in the very early stage (0–12 hr), a number of free ribosomes exist in the cytoplasm. Rough endoplasmic reticulum (ER) with closely spaced cisternae was also observed. Then rough ER starts to proliferate rapidly, and at the same time lamellar ER is rapidly or gradually transformed into vesicular or tubular forms. In the later stage of the fifth instar (120–192 hr), the cytoplasm is mostly filled with tubular or vesicular ER. Golgi vacuoles, free vacuoles (fibroin globules), and mitochondria are also observed. It is concluded that in the early stage of the fifth instar the cellular structures necessary for the biosynthesis of fibroin are rapidly formed, while in the later stage the biosynthesis of fibroin proceeds at a maximum rate and utilizes these structures.

INTRODUCTION

The posterior division of the silk gland of the silkworm, *Bombyx mori*, conventionally called posterior silk gland as used here, is known to synthesize exclusively a single exportable protein (1, 2), fibroin, which is the most important silk protein with a simple amino acid composition; it is rich in glycine, alanine, serine, and tyrosine (3). During the fifth instar, fibroin is synthesized very rapidly in the posterior silk gland, the maximum rate of synthesis having been reported to be much larger than that of the synthesis of albumin in liver (4). Probably because of these reasons, protein synthesis in the gland has been extensively studied,

especially in Japan. It is interesting, therefore, to study the ultrastructure of the gland in relation to the biosynthesis and secretion of fibroin.

Another interesting characteristic of the gland is that the extensive biosynthesis and secretion of fibroin are observed only in a very short period of the larval life, that is, only during the fifth instar. The posterior silk gland of the young larvae is quite rudimentary, and even in the fourth instar larvae it is very small. At the beginning of the fifth instar, however, the growth of the gland is triggered suddenly and the weight of it increases very rapidly, as will be shown later. This marked

growth of the gland is exclusively due to the enlargement of the individual cell; the total number of the posterior silk gland cells, which will be referred to as "(the) cells," is kept constant throughout postembryonic development (5, 6). It is expected, therefore, that the various structural elements of the cells, especially rough endoplasmic reticulum (ER) and ribosomes, are intensively produced during the fifth instar. If so, the posterior silk gland is an interesting material for investigation on the biogenesis of ER, ribosomes, and related structures.

Although the silk glands of the silkworm have been extensively studied by light microscopy (1, 5, 7, 8), no systematic study by electron microscopy appeared until Akai published recently a series of papers on the ultrastructure of the silk glands (9-11). In the present paper, biochemical as well as morphological studies on the growth and maturation of the cells of the posterior silk gland of the silkworm during the fifth larval instar will be described.

MATERIALS AND METHODS

Silkworm

Strains of the silkworm used and the seasons of rearing are the following: Shi 124 (autumn); Nichi 124 x Shi 124 (spring and autumn); and Shungyoku x Gunpo (spring). The larvae were fed exclusively mulberry leaves and cultivated at $25^{\circ} \pm 1^{\circ}\text{C}$. Events in the last (fifth) larval stage were precisely timed in hours from the moment of the fourth eclosion from exuvium, and this eclosion was also used for the synchronization by selection of the larvae within a fluctuation of ± 3 hr. Under these experimental conditions, the larvae stop eating and the bodies become yellowish and lustrous usually at 192 ± 9 hr (SD); these are the signs of full maturation. The fully mature larvae soon start spinning to make a cocoon, and the rectum empties a short time after the rest of the gut. This paper is concerned with studies on the larvae from the very beginning of the fifth instar until this fully mature state. The animals were sacrificed at intervals of 6, 12, or 24 hr, depending on the stage in this larval period.

Biochemistry

Shungyoku x Gunpo (spring) was exclusively used for biochemical analyses. Usually six larvae of average body weight (either sex) were selected and used for each time point. First, the body weight of the individual larvae was measured. The middle and posterior silk glands of both sides were dissected and washed briefly with 0.9% NaCl. The middle

silk gland was exclusively used for the extraction of fibroin. The wet weight of the right and left posterior silk glands was measured; one gland was then used for the extraction of intraglandular fibroin, and the other was used for determinations of RNA, DNA, and protein. For each determination usually two posterior silk glands were used at a time, and the average values of three independent determinations were calculated. These values are given in this report. Schneider's procedures (12) were used for the extraction of RNA, DNA, and protein. The amount of RNA was determined by the orcinol reaction (13) and use of purified liver RNA as a standard; DNA was determined by the Dische reaction (14) and use of purified calf DNA as a standard; and protein nitrogen was determined by nesslerization of Kjeldahl digests. Lipids were extracted directly from posterior silk glands by chloroform-methanol (1:1, v/v). After the solvent had been evaporated, the residue was dissolved in chloroform and the total lipids were estimated from the dry weight of the chloroform-soluble fraction. The phospholipid P of this fraction was determined by Allen's method (15). Chemical analyses of the lipids were carried out by Saito et al. and will be published in a separate paper.¹

Biosynthesis of Fibroin

Biosynthesis of fibroin from the beginning of the fifth instar until the fully mature state was estimated from the total amount of fibroin extracted daily from the lumens of the middle and posterior silk glands. The fibroin in the anterior silk gland is so small in amount that it was neglected in the calculation. The rate of increase in the amount of fibroin in the rest of the gland should be a good index of the biosynthesis, because no fibroin is secreted out of the larva during this stage of the fifth instar and almost all the synthesized fibroin is probably accumulated there. We have to assume, however, that there is hardly any reabsorption of the luminal fibroin during the fifth instar. Since it has been shown by Akai (16) that newly synthesized fibroin appears rapidly in the glandular lumen after administration of glycine-¹⁴C, increase in the biosynthesis of fibroin will result in the increase in the total amount of luminal fibroin, the time lag being small, probably of the order of several hours.

The following methods were used for the extraction of fibroin. Posterior silk glands were soaked overnight in cold 5% trichloroacetic acid (17), and the coagulated intraluminal fibroin was peeled out of the tissue and washed several times with 0.3%

¹ Saito, K., K. Sato, and M. Gamo. Manuscript in preparation. We thank these authors for allowing us to cite their unpublished data.

acetic acid. Middle silk glands were soaked overnight in 0.3% cold acetic acid (18), and the coagulated intraluminal silk proteins (sericin plus fibroin) were similarly peeled out of the tissue. This soaking of the middle silk gland in 0.3% acetic acid seems to be quite effective; the outer coagulated sericin layer became easily separable by forceps from the coagulated central fibroin column, except around the posterior end of the middle silk gland where the thin layer of sericin sticks to the central fibroin column. From these crude glandular fibroins, sericin was extracted twice with 0.2% Na_2CO_3 at 100°C for 1 hr (19), and the remaining fibroin was washed several times with hot and then with cold distilled water, and finally with alcohol. Contaminating lipids were extracted twice with alcohol-ether (3:1) at 37°C, and finally the fibroin was washed with ether and dried. The weight of the purified fibroin was measured after drying at 110°C for at least 5 hr.

Light and Electron Microscopy

For light microscopy, the posterior silk gland was fixed either with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, or with Bouin's fixative, then dehydrated with alcohol, embedded in paraffin, and observed after staining with Mallory's method. For electron microscopy, glutaraldehyde fixation (20) was essential, especially in the early stage of the fifth instar. The materials were fixed several hours with cold 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, washed several times with the same buffer, and then postfixed for 1-3 hr in 1% OsO_4 . Blocks were usually stained with uranyl acetate (21) after several washings with Veronal-acetate buffer, then dehydrated with alcohol, and embedded in Epon (22). Thin sections were cut either with an LKB Ultratome or with a JEM-5 microtome and were doubly stained with uranyl acetate (23) and lead citrate (24). The electron microscopes used were Hitachi HU-11, HS-7, JEM-6C, or JEM-7A.

RESULTS

Body Weight and Wet Weight of Posterior Silk Gland

During the fifth instar, the body weight of the larvae and the wet weight of the posterior silk gland increase logarithmically until they reach a stationary state at around 96 hr (Fig. 1). The two curves, however, are not identical but differ at least in the following two respects: (a) the wet weight of the gland increases more rapidly than the body weight; (b) at the very beginning of the

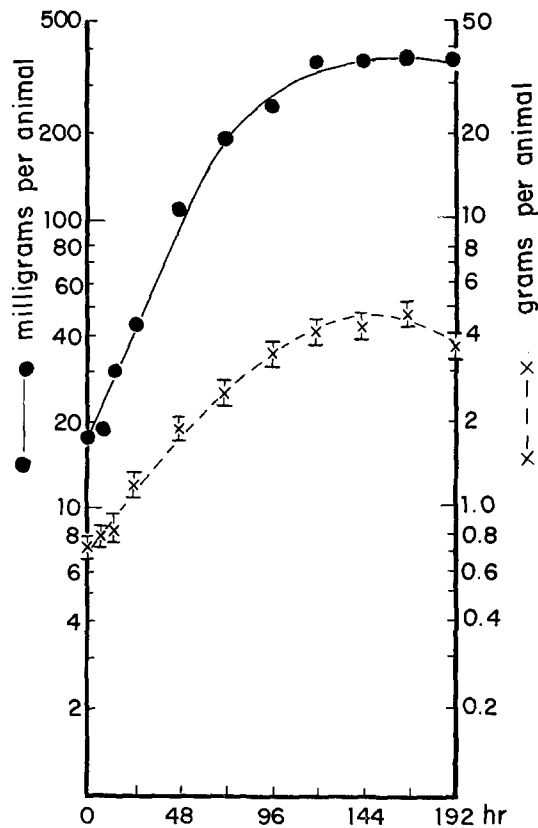


FIGURE 1 Increase in total body weight (crosses) and wet weight of the posterior silk gland (closed circles) in the fifth instar (from the end of the fourth ecdysis until the fully mature state).

fifth instar, the body weight does not increase and a lag phase is observed. This is to be expected because silkworms, just after fourth ecdysis, do not eat; moreover, they are usually fasted for several hours to synchronize the growth. In spite of this initial fasting, the wet weight of the posterior silk gland usually continues to increase (see, however, Fluctuation of the Data), probably at the expense of some other tissues.

Amount of DNA, RNA, Protein, and Lipids

As shown in Fig. 2, a remarkable increase in the amounts of DNA, RNA, protein, and lipids was observed from the very beginning of the fifth instar; no lag was observed comparable to that of the total body weight. The curve for DNA, however, is different from those for RNA, protein, and lipids. The increase in the amount of DNA slowed down

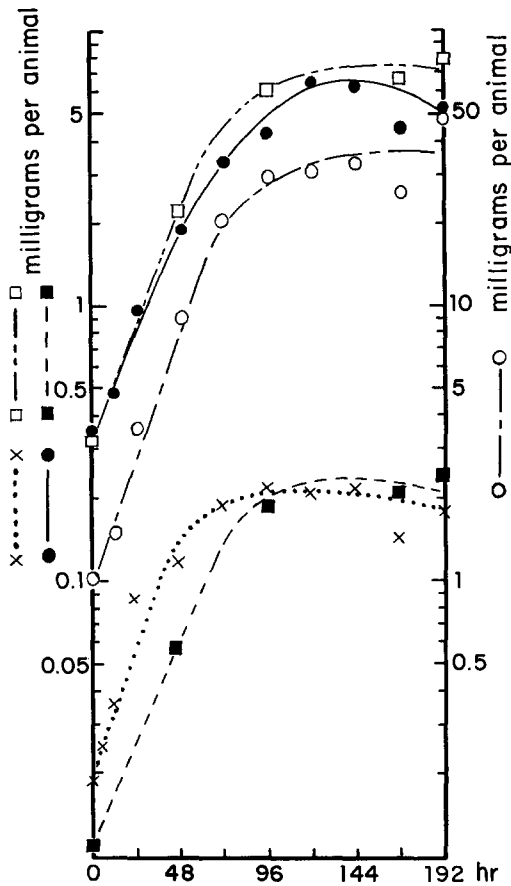


FIGURE 2 Increase in the amounts of DNA (crosses), RNA (closed circles), protein (open circles), total lipids (open squares), and phospholipids P (closed squares) during the fifth instar.

already at 48–72 hr and reaches a constant level of approximately 200 μg per animal. This result is in agreement with the qualitative radioautographic study by Akai and Kobayashi (25) who observed that thymidine- ^3H was incorporated only in the early stage of the fifth instar.

In contrast to DNA, the amounts of RNA, protein, and lipids continued to increase until about 96–120 hr, and then reached a constant average value of approximately 5.5, 34.7, and 7.3 mg per animal, respectively. A rapid increase in the amount of RNA and in the wet weight of the gland at the beginning of the fifth instar has also been reported by Hosoda et al. (26). Miura et al. (27) observed that orotate- ^{14}C is rapidly incorporated into the RNA fraction only in the early stage of the fifth instar. As shown in Fig. 3 the

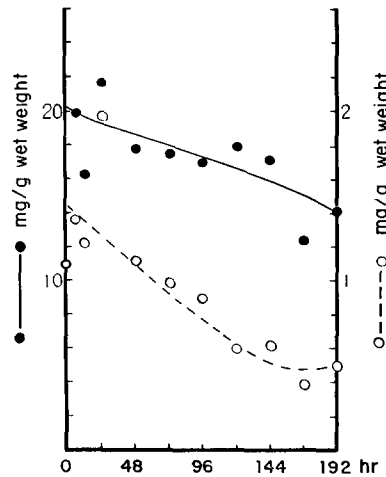


FIGURE 3 Change in RNA (closed circles) and DNA (open circles) contents (milligrams per gram fresh tissue) in the posterior silk gland during the fifth instar.

amount of RNA per gram wet weight of posterior silk gland calculated from our data was 19.9 mg/g at the beginning of the fifth instar but decreased gradually to the level of 14.2 mg/g at the end of the same instar, probably because of the accumulation of fibroin in the posterior silk gland towards the end of the fifth instar. It is to be noted here that these values are much higher than the corresponding values reported by Denuce (28), which are only 3–4 mg RNA per gram. This discrepancy is probably due to the difference in the strain of silkworm used; a similarly high RNA content in the posterior silk gland has recently been obtained in other laboratories in Japan.^{2, 3}

The protein curve may need some comments. One might expect a continuous increase in the amount of total protein in the posterior silk gland during this instar because fibroin may be continuously synthesized in the gland. Such an increase was not observed, as is explained in the next section.

Amount of Fibroin

The three curves in Fig. 4 show the total amount of fibroin (fibroin in the posterior plus the middle silk gland), the amount of fibroin in the posterior

² Tanaka, M., and K. Shimura. Manuscript in preparation.

³ Shigematsu, H. Personal communication. Calculated from the recent data of Shigematsu and Takeshita (29).

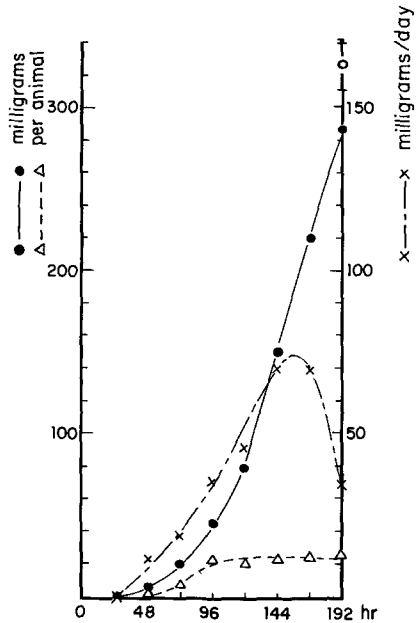


FIGURE 4 Biosynthesis of fibroin in the posterior silk gland of the silkworm during the fifth instar. Fibroin was extracted from the posterior and the middle silk gland and the dry weight of purified fibroin was measured. Total fibroin (closed circles) is the sum of the amounts of fibroin in the middle and posterior silk glands. Rate of synthesis of fibroin (crosses) was calculated from the curve for total fibroin. The amount of fibroin in the posterior silk gland (open triangles) increased at an earlier stage and soon reached a constant level of ~ 20 mg per animal. Open circle on the ordinate on the right side (open circles) is the average amount of fibroin extracted from the completed cocoons (average of eleven determinations). The fibroin was extracted from the cocoon after the larva within it had finished spinning, and the dry weight of the purified fibroin was measured.

silk gland, and the rate of biosynthesis of fibroin (derivative of the first curve), respectively. Although the amounts of RNA and lipids increase early as shown by the curves in Fig. 2, the synthesis of fibroin increases later. Even at 96 hr of the fifth instar, when the amounts of RNA and lipids have almost reached their maximum values, the rate of fibroin synthesis is still low, the maximum synthesis being observed only 2 or 3 days before the full maturation of the larvae (120–192 hr). This discrepancy in the biosyntheses of RNA and fibroin in the posterior silk gland is in agreement with the experiments of Takeyama et al. (30), who showed that glycine- ^{14}C is rapidly incorporated

into the gland protein only towards the end of the fifth instar. After full maturation, the larvae start spinning. The total amount of fibroin (fibroin in the silk glands and in cocoon) increases only slightly, as is shown by the open circle on the ordinate on the right side of Fig. 4. This problem will be discussed in detail in the following paper (38). The amount of fibroin in the posterior silk gland itself increased at an earlier stage (48–72 hr), but soon reached a constant level of approximately 20 mg per animal, this value being maintained until the end of the fifth instar. This means that most of the fibroin synthesized in the posterior silk gland is not accumulated there but is continuously sent to the middle silk gland to be stored until the larvae start spinning, as has been shown by Fukuda and his associates (31, 32).

The most important suggestion obtained from these biochemical analyses may be that the growth of the cell and the biosynthesis of fibroin do not occur simultaneously but sequentially; the former, of course, precedes the latter. It seems reasonable, therefore, to divide the fifth instar into the log stage (fifth instar 0–96 hr) and the stationary stage (96 hr to full maturation). It is during the latter stage that fibroin synthesis is carried out at a maximum rate.

Light and Electron Microscopy

Samples for morphological observation were prepared at 0, 6, 12, 24, 48, 72, 84, 96, 108, 120, 144, 168, and 192 hr after the fourth ecdysis (fifth instar, 0 hr). In agreement with the biochemical studies described above, the morphological studies also have shown that the development of the gland in this period could reasonably be subdivided into two stages, first or log stage (0–96 hr) and second or stationary stage (96–196 hr).

Since light microscopical observations on the posterior silk gland have been published repeatedly (1, 5, 7, 8), no detailed description will be necessary. It is interesting, however, to point out here that each branch of the cell nucleus is quite thick at the beginning of the fifth instar and that the ratio of nuclear to cytoplasmic volume is quite large (Fig. 5 a). As the cells mature, this ratio decreases gradually, and in the mature silk gland narrow branches of nucleus are observable buried within the cytoplasm (Fig. 5 d). The decrease in the ratio of nuclear volume to cytoplasmic volume is in good agreement with the sequential

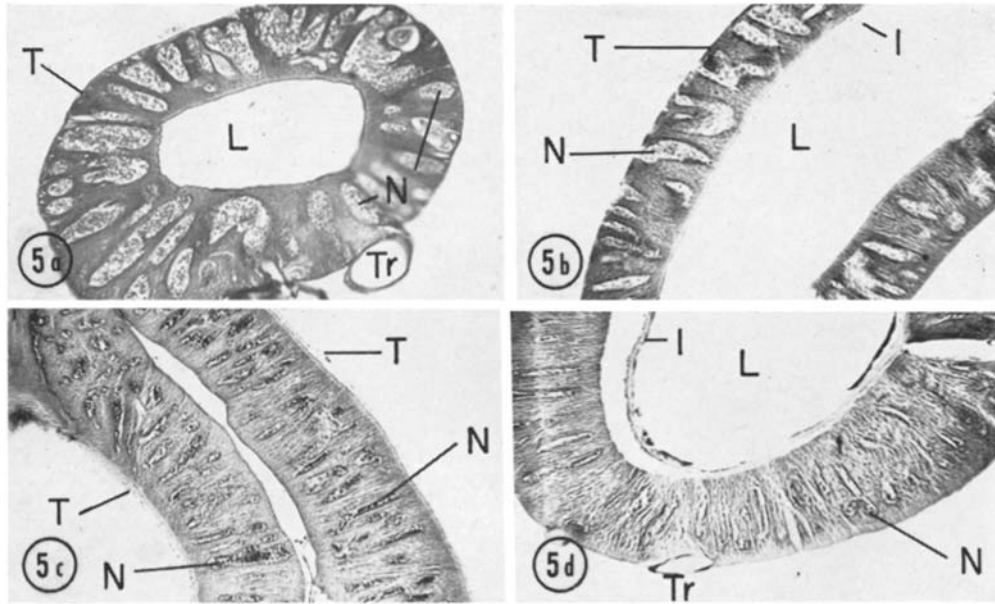


FIGURE 5 Light micrographs of the posterior silk gland in the fifth instar. *a*, fifth instar 0 hr, $\times 256$; *b*, fifth instar 48 hr, $\times 160$; *c*, fifth instar 96 hr, $\times 160$; *d*, fifth instar 144 hr, $\times 160$. *T*, tunica propria; *N*, nucleus (more exactly, branch of nucleus); *I*, intima; *L*, glandular lumen; *Tr*, trachea.

decreases in the amount of DNA per gram of gland in the fifth instar as shown in Fig. 3.

Electron microscopical observations in the log and the stationary stages will be explained.

LOG STAGE (FIFTH INSTAR LARVA 0-96 Hr Old)

FIFTH INSTAR LARVAE 0 AND 6 HR OLD: Fig. 6 shows the cell at 0 hr. The most striking finding is that the cytoplasm is filled with a tremendous number of free ribosomes, which frequently occupy the perinuclear region. It is not very clear whether the ribosomes exist as monosomes or as ribosomal aggregates, probably because they are too crowded. In some regions, however, ribosomes are clearly arranged in rows, clusters, or helices. The rough ER, which exists here and there in the cytoplasm, is exclusively lamellar in type, i.e. consists of flattened cisternae; no distension of the intracisternal space is observed in this very early stage of the fifth instar.

The Golgi apparatus at the beginning of this instar shows quite limited development, poorly developed flattened saccules and vesicles being found. Mitochondria are present throughout the cytoplasm; circular and oval profiles predominate, are usually small ($0.2-0.5 \mu$), and occasionally contain ribosome-like particles. Lysosomes and

multivesicular bodies are only rarely found. In the cytoplasm many microtubules run here and there. As pointed out by Ledbetter and Porter (33), microtubules are found only in specimens fixed with glutaraldehyde. The plasma membranes of adjacent cells are joined to one another by septate desmosomes, as described by Wood (34) and Locke (35). The luminal surface of the cell has microvilli, while the basal surface is surrounded by tunica propria and shows extensive invagination of the plasma membrane.

The cell at the beginning of the fifth instar has a large, extensively ramified nucleus with a number of nucleoli and chromatin blocks. Though the nucleoli are usually larger than the chromatin blocks, their similar appearance at this stage of development makes it sometimes difficult to distinguish between them, as pointed out by Akai (11). The nucleoli, however, are usually associated with ribosome-like particles and are slightly less dense than the chromatin blocks. The nucleus is surrounded by the usual nuclear envelope, and protrusion of the outer nuclear membrane into the cytoplasm has never been observed, at least during the fifth instar. When tangentially sectioned, many nuclear pores are observed on the nuclear envelope.

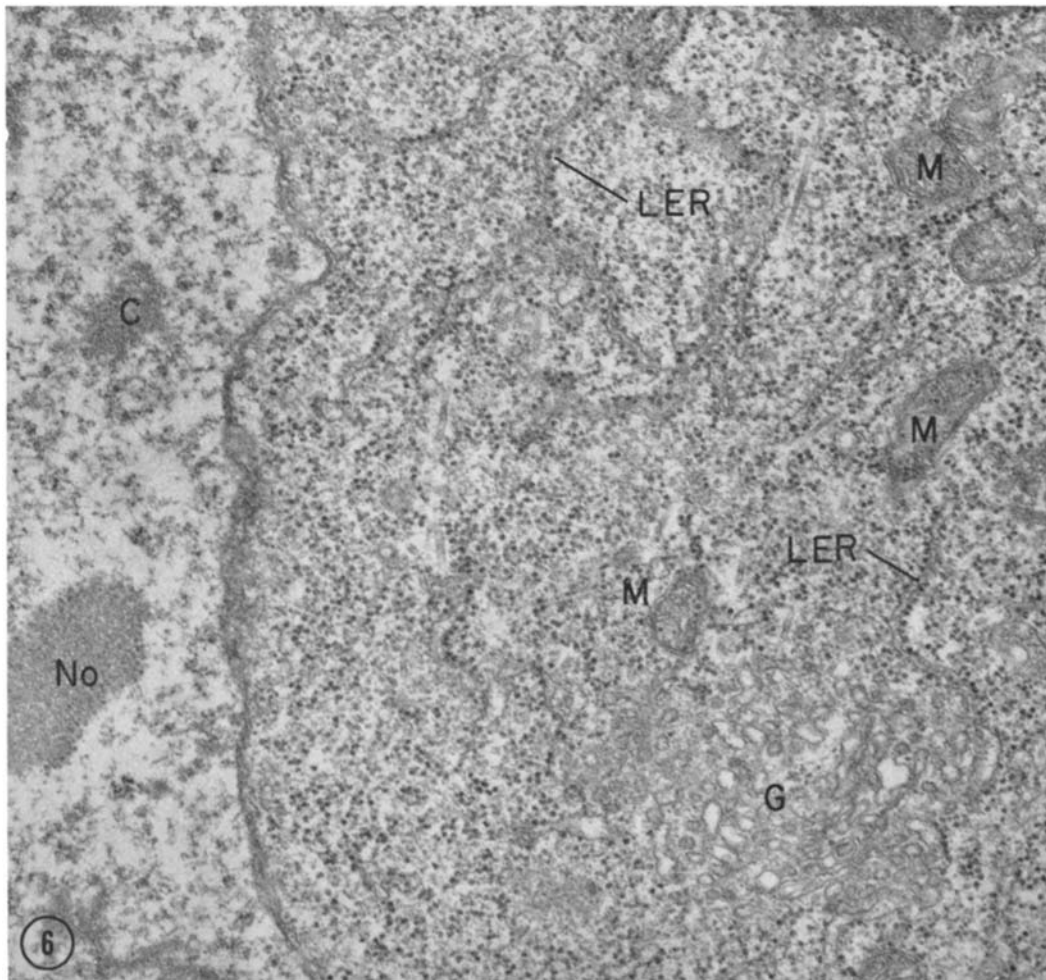


FIGURE 6 Electron micrograph of a posterior silk gland cell; fifth instar larva, 0 hr. Cytoplasm is filled with free ribosomes, and with lamellar ER (*LER*). Mitochondria (*M*) are usually small and oval in profile. The vacuoles in the Golgi complex (*G*) are not well developed. Nucleus contains a number of nucleoli (*No*) and chromatin blocks (*C*). $\times 32,000$.

Among the cells, tracheoles are also frequently observed. They are numerous at the basal part of the cell and are separated from it by their own cell membranes and frequently by an intercellular space.

FIFTH INSTAR LARVA 12 AND 24 HR OLD: At 12 or 24 hr, the rough ER starts to increase markedly. Figs. 7 and 8 show a part of the cell at 12 and 24 hr, respectively. This micrograph is characterized by elongated rough ER profiles which appear in the area previously occupied by free ribosomes; the ratio of ER to free ribosomes increases rapidly. In some parts of the

cytoplasm, distension of the intracisternal space of the ER already appears at 12 or 24 hr of the fifth instar, and at the same time the Golgi vacuoles start to enlarge.

FIFTH INSTAR LARVA 48-96 HR OLD: The rough ER further increases in amount until it fills almost the entire cytoplasm, while the free ribosomes continue to decrease in amount (Fig. 9). The intracisternal space of the rough ER is more distended, and the Golgi vacuoles increase in number. In some parts of the cytoplasm, however, the ER is still of the lamellar type, no distension of the intracisternal space being observed. Occa-

sionally, so-called concentric lamellar structures appear as shown in Figs. 10 and 11. These structures appear at around 96 hr, when the amount of RNA in the posterior silk gland almost reaches a maximum value, and disappear already at 120 hr. Weiss (36) and Herman and Fitzgerald (37) have reported the appearance of similar structures during the regenerative phase of the pancreatic exocrine cell.

In accordance with the development of the cytoplasm, the nucleus in this stage becomes extensively lobated and within the nucleus a number of chromatin blocks and nucleoli are observed. As in earlier stages, the nucleoli are associated with ribosome-like particles. At around 48 hr, some of the chromatin blocks start to attach to the inner nuclear membrane, as has been reported by Akai (11).

STATIONARY STAGE (FIFTH INSTAR LARVAE 96-192 HR OLD)

As shown in Fig. 12, the most striking change is observed in the rough ER and the Golgi apparatus. The lamellar ER, which predominated in the cytoplasm at least at the beginning of the fifth instar, completely disappears, and only vesicular or tubular ER is observed, the intracisternal spaces of which are now markedly distended. These spaces usually have a light content and show no accumulation of granules similar to intracisternal granules (39). The Golgi complexes also show prominent development, and a number of them are scattered throughout the cytoplasm. The Golgi complex is composed of three or four vacuoles and several, minute vesicles. In other parts of the cytoplasm, vacuoles very similar in appearance to the Golgi vacuoles are observed. The content of these two kinds of vacuoles is equally light except for the existence of a filamentous material. As shown in Fig. 13, a tremendous accumulation of these vacuoles is observed at the apical portion of the cells during this stationary stage, and some of these vacuoles are in direct contact with the cell membrane. They are probably ready to be secreted, as has been reported for similar vacuoles in the pancreatic exocrine cells (40). From these observations and from the fact that there are no other structures which could be a candidate for secretory granules, we may reasonably conclude that these vacuoles are the secretory granules of fibroin, or fibroin globules as named by Akai (41). Most of the

ribosomes seem to be attached to the membranes of the ER. Mitochondria are larger and longer and their cristae are better developed than in the log stage.

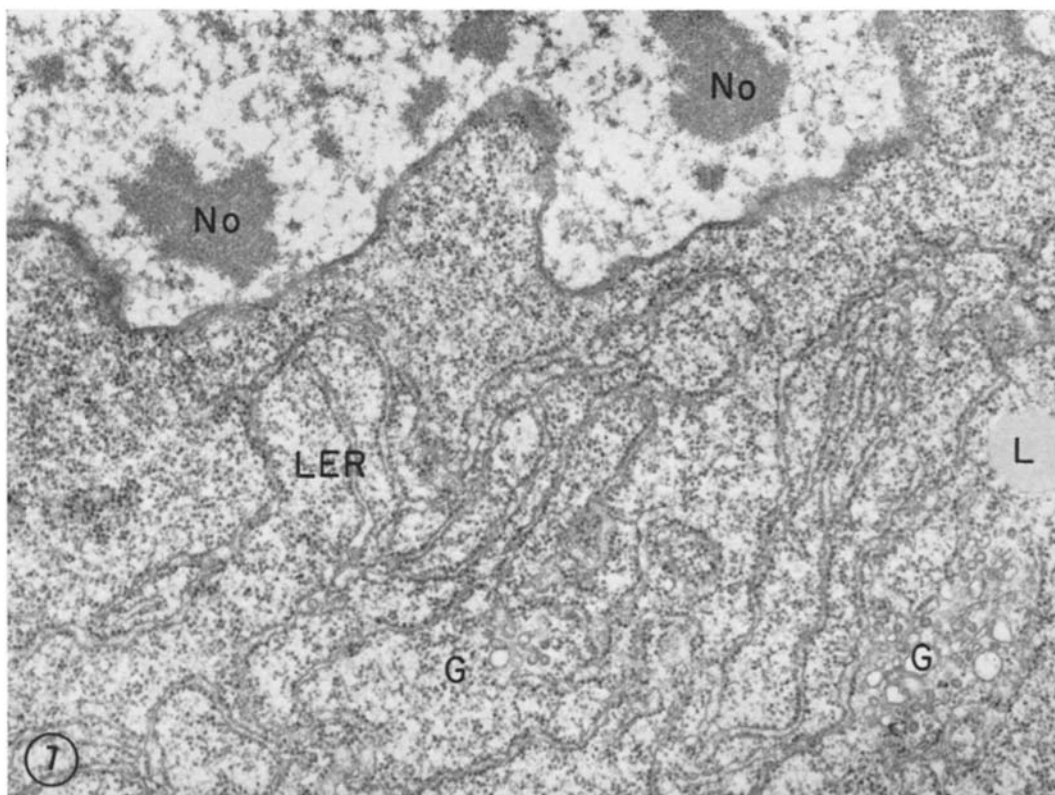
Fluctuation of Data

It has been known that the growth of silkworms is influenced by various factors, such as nutrition (quality of mulberry leaves or of artificial food when used), temperature, humidity, illumination, etc. Growth also depends on the strains of silkworms used and the seasons of rearing. Some of the factors, such as quality of mulberry leaves, are not always easy to control rigorously, and it is not surprising that some fluctuation of data appears in this kind of experiment. For example, the increase in the wet weight of the posterior silk gland is usually less in larvae reared in the fall than in larvae reared in the spring; and the increase in the wet weight of the gland has occasionally shown a lag at the very beginning of the fifth instar. Morphological observations also show some variations. For instance, in the Nichi 124 x Shi 124 strain (reared in the fall), the lamellar ER persists longer and concentric lamellar structures are found at 96 hr of the fifth instar, whereas in the Shung-yoku x Gunpo strain (reared in the spring), the lamellar ER is transformed earlier into tubular or vesicular ER and no concentric lamellar structures are observed. Under our experimental conditions (25°C; humidity was not controlled), variation in quality of mulberry leaves, especially a seasonal variation, is probably the main factor in these fluctuations. This problem has not been studied in detail.

DISCUSSION

Since the posterior silk gland is composed almost exclusively of glandular cells [(the) cells] and, moreover, since the growth of the silkworms has been synchronized within a fluctuation of several hours, it may be possible to correlate reliably the biochemical data with the morphological changes.

Biochemical analyses clearly showed that, in the early stage of the fifth instar (0-96 hr), the amounts of DNA, RNA, protein, lipids, etc. increase logarithmically. Since the number of the cells remains constant throughout postembryonic development (5, 6), these data strongly suggest that the various subcellular constituents, such as nucleus, ribosomes, ER, and other structures of the individual cell, proliferate rapidly. This possibility is sup-



FIGURES 7 and 8 Electron micrograph of a posterior silk gland and cell; fifth instar larvae, 12 and 24 hr, respectively. These micrographs suggest extensive proliferation of rough ER. In Fig. 8 some of the lamellar ER (*LER*) has already been transformed to vesicular or tubular ER (*VER*). Golgi apparatus (*G*) starts to develop. Microtubules (*Mt*) run here and there. Nucleoli (*No*) are associated with ribosome-like particles. *L*, lipid droplet; *Np*, nuclear pore. Fig. 7 \times 26,000; Fig. 8 \times 45,000.

ported by electron microscopical observations on the gland. At the very beginning of the fifth instar, the cytoplasm is filled with free ribosomes, and the nucleus contains a number of chromatin blocks and nucleoli associated with ribosome-like particles. Probably these ribosomes migrate from the nucleus to the cytoplasm. The biogenesis of ER seems to be triggered from the beginning of the fifth instar. As to the origin of the membranes of the ER, at least three possibilities have been proposed: the first, that the membrane is formed by the dilatation and detachment of the outer nuclear membrane (42, 43); the second, that the membrane is formed by the rough ER itself (44), and the third, that the membranes are synthesized on the free polysomes which are arranged in linear or helical array, as has been suggested in bleached grana (45). Since we have never observed the

protrusion of outer nuclear membrane during the fifth instar, we can safely neglect the first possibility. The other two possibilities are to be examined by cytochemical study in future.

As described in this report, the ER in the cells is exclusively of the lamellar type at the very beginning of the fifth instar; this suggests that the newly formed ER is of this type, though it is also possible that biogenesis also proceeds in the vesicular ER. At around 24 hr, the vesicular or tubular ER starts to appear, and it gradually or rapidly increases in proportion (see Fluctuation of Data) so that finally at 120 hr all of the ER is of this type. Since this transformation of ER apparently proceeds in parallel with the increase in the rate of biosynthesis of fibroin, it is suggested that the transformation is intimately correlated with the biosynthesis of fibroin. It is highly probable that

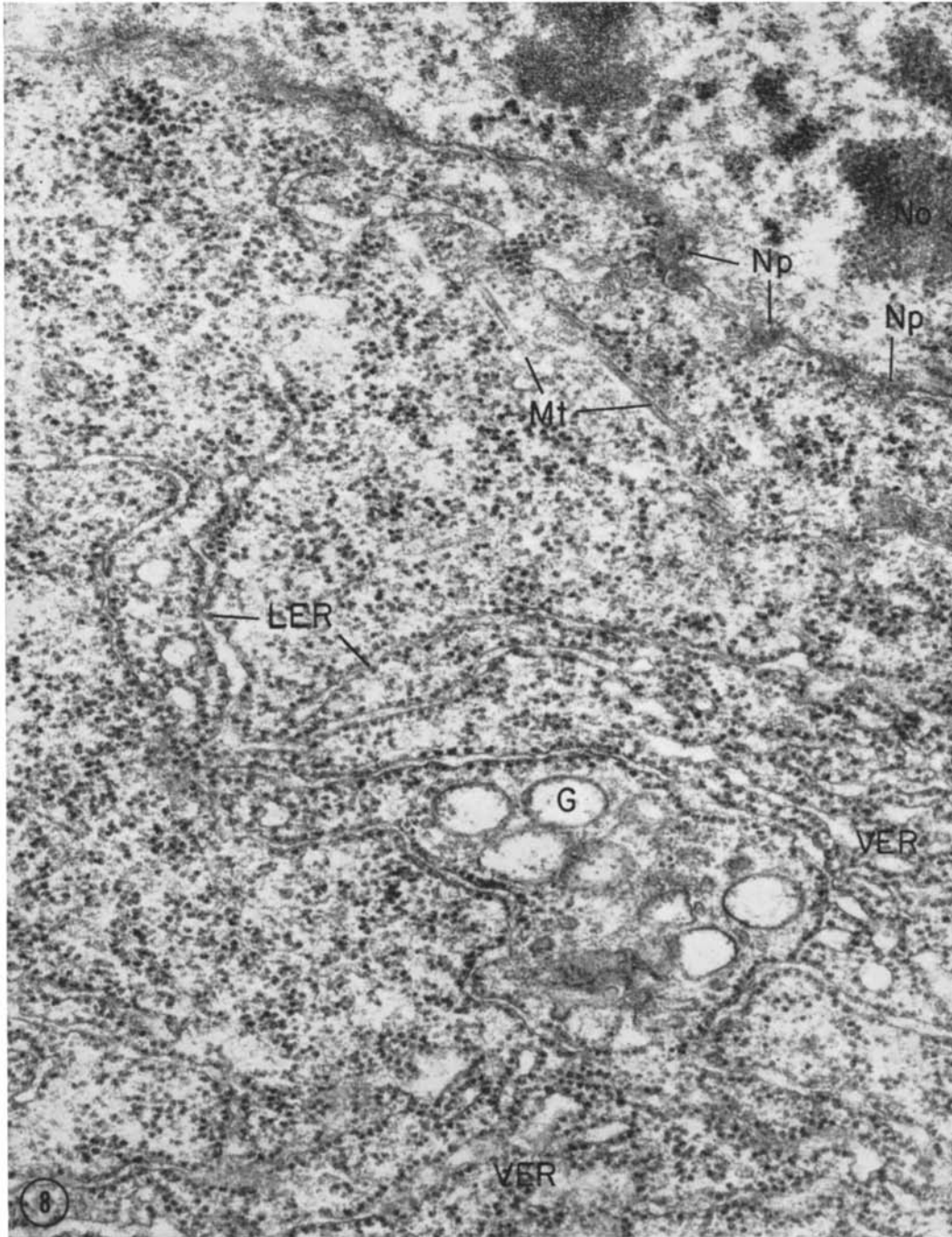


FIGURE 8 See legend under Fig. 7.

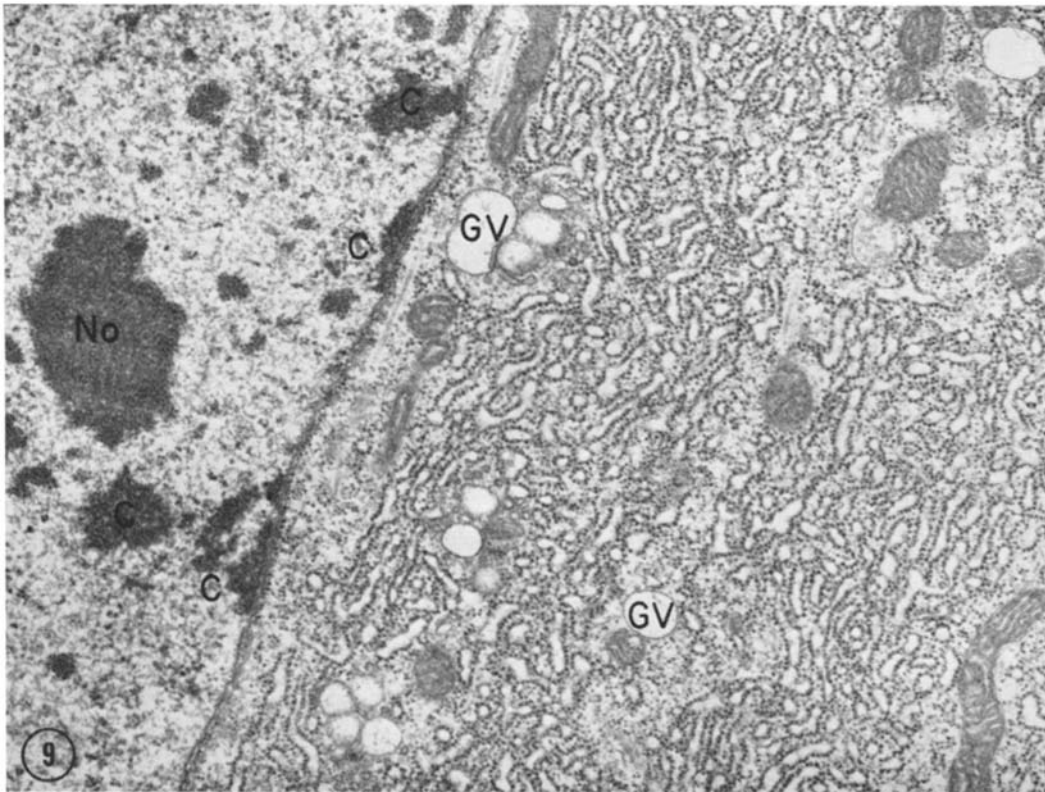


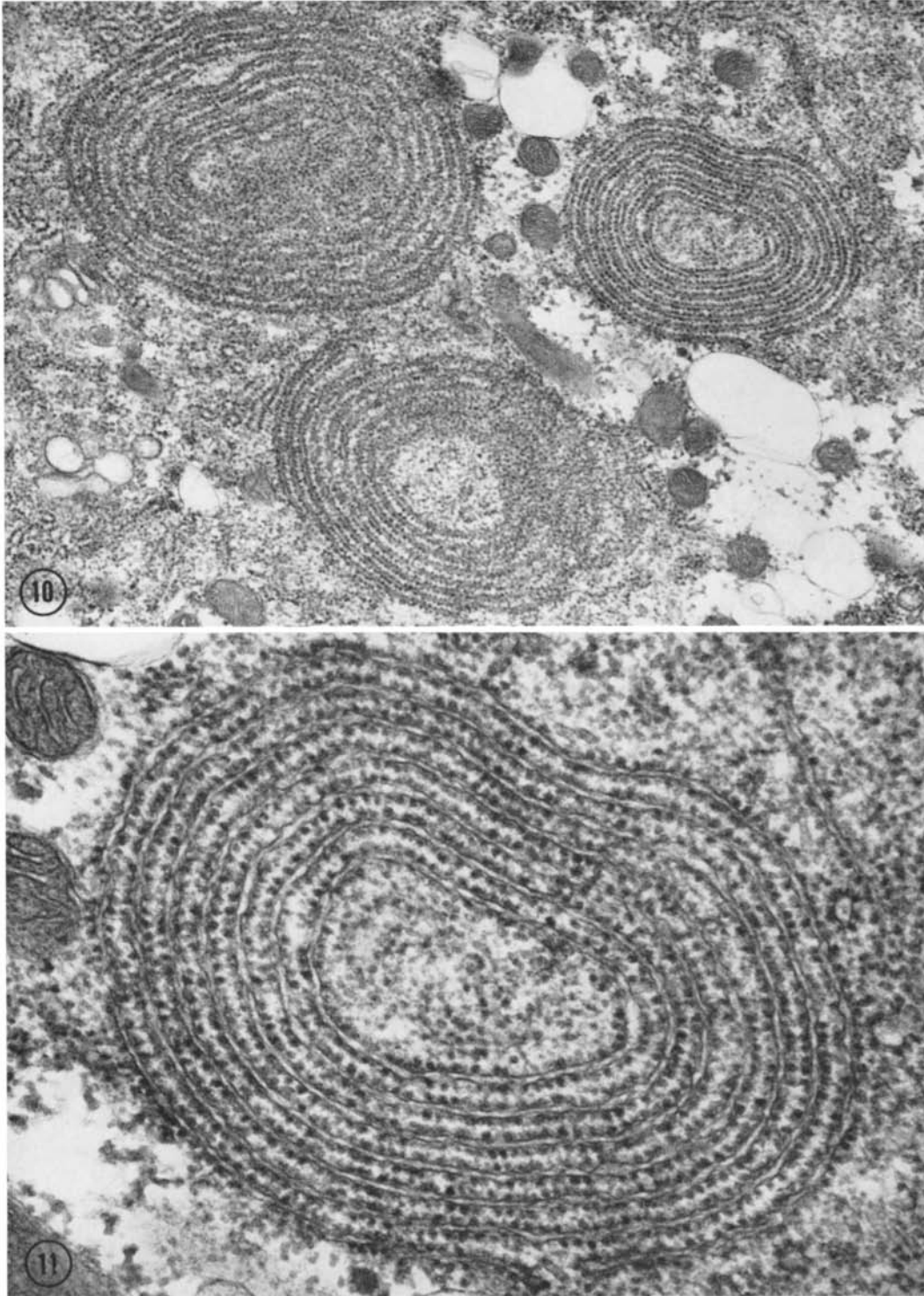
FIGURE 9 Electron micrograph of a posterior silk gland cell; fifth instar larva, 96 hr. The cytoplasm is filled with well developed rough ER of either the lamellar, vesicular, or intermediate type; free ribosomes are markedly decreased in number. Golgi vacuoles (GV) are also well developed. In the nucleus are seen nucleoli (No) and chromatin blocks (C); some of the latter are attached to the inner nuclear membrane. $\times 23,000$.

the fibroin which is synthesized on the ribosomes attached to the ER is transported directly into the intracisternal space of the ER and accumulates there for a while. This accumulation results in the distension of the intracisternal space and therefore in the transformation of ER into the vesicular or tubular type. Similar functional modulations in ER structure have been reported in pancreatic exocrine cells by Siekevitz and Palade (46) and Watanabe and Arakawa (47).

At the beginning of the fifth instar, the Golgi apparatus is quite poorly developed and no Golgi vacuoles are found. In parallel with the transformation of the ER, the Golgi complexes composed of several vacuoles surrounded by a number of minute vesicles start to develop. Probably the fibroin accumulated within the intracisternal space of the ER is transported to the Golgi vacuoles via

Golgi vesicles or transition elements, as suggested by Jamieson and Palade (48). Mature Golgi vacuoles leave the Golgi region as fibroin globules, move towards the glandular lumen, and then secrete their content, by a reversed pinocytotic process, into the luminal space. Akai and Kobayashi (49) have shown by radioautography that glycine- ^3H , injected into the fifth instar larvae, is accumulated within fibroin globules. From our observations, the synthesis and secretion of fibroin seem to be very similar to the biosynthesis and secretion of digestive enzymes in the pancreatic exocrine cells (50).

It may seem peculiar that the electron opacity of these fibroin globules is so low. It is suggested that either the fibroin is not stained by the heavy metals used here (Os, U, Pb) or the fibroin is not fixed and, therefore, is extracted from the speci-



FIGURES 10 and 11 Electron micrographs of the concentric lamellar ER structures which are occasionally observed in the cytoplasm of the posterior silk gland cell at 96 hr of the fifth instar. Fig. 11 is a higher magnification of a part of Fig. 10. Fig. 10, $\times 25,000$; Fig. 11, $\times 66,000$.

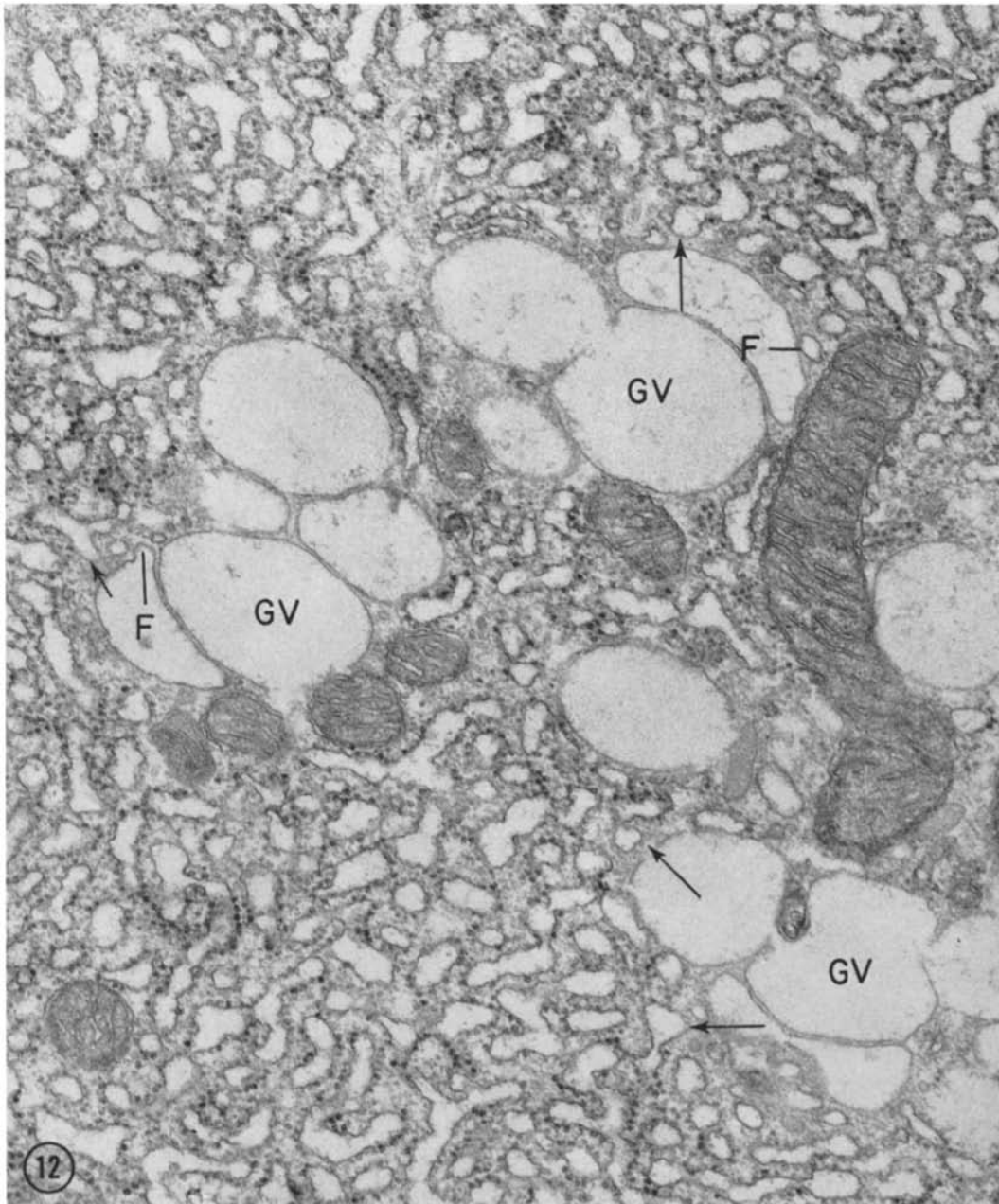


FIGURE 12 Electron micrograph of a posterior silk gland at 144 hr of the fifth instar. Vesicular or tubular ER predominates in the cytoplasm, the intracisternal space being extensively distended. Golgi vacuoles (GV) are large, and there are many Golgi vesicles, some of which are fusing with Golgi vacuoles (F). The Golgi vacuoles have a light amorphous content, except for some filamentous materials. A part of the rough ER elements facing the Golgi apparatus do not have ribosomes, suggesting that Golgi vesicles are formed by budding of ER membrane (arrows) after detachment of ribosomes, as usually supposed. $\times 44,000$.

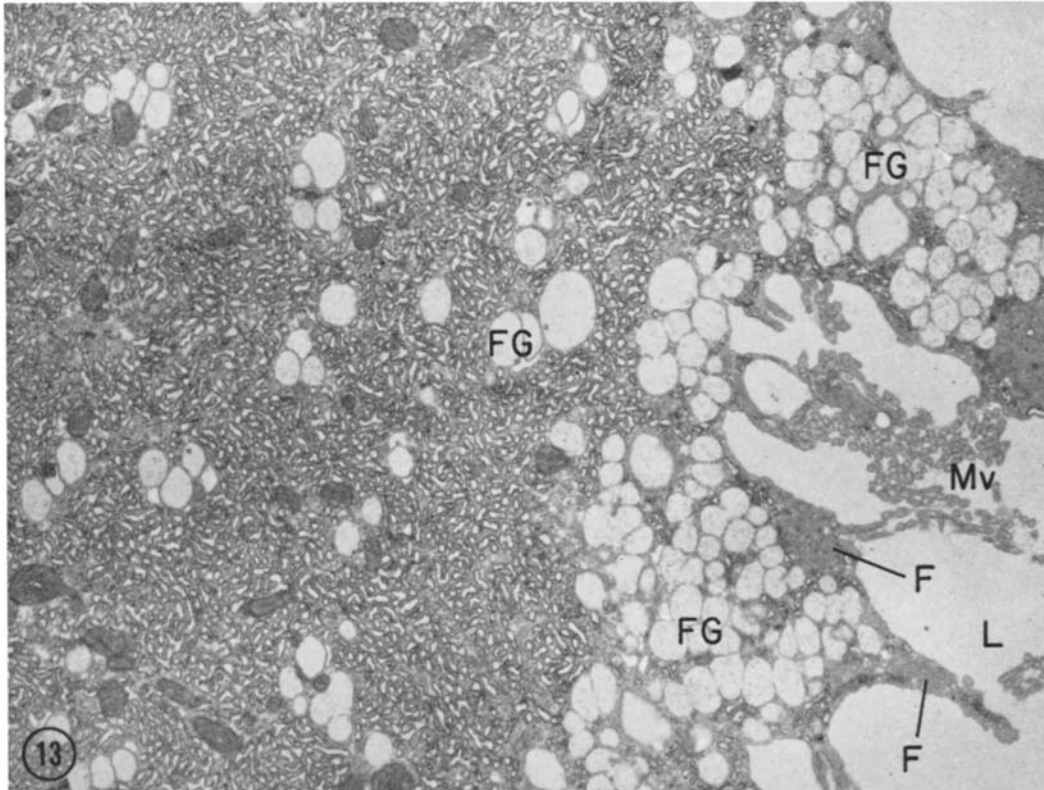


FIGURE 13 Accumulation of fibroin globules (FG) in the apical cytoplasm of a posterior silk gland cell. A number of microvilli (Mv) exist on the surface of the cell. Bundles of fine filaments (F) similar to those of terminal webs are not very clear in this picture. L, glandular lumen. $\times 8,000$.

mens during the fixation or dehydration procedures. The former possibility is supported by our experiments, because specimens for light microscopy, which were similarly fixed in glutaraldehyde and stained with Mallory's method, clearly show the existence of intraluminal fibroin.

At the transition from the logarithmic growth stage to the stationary stage, that is, around 96 hr of the fifth instar, the concentric lamellar type of ER occasionally appears. This type of ER probably occurs at this stage because the rate of biogenesis of ER exceeds the rate of transformation of lamellar ER into vesicular or tubular ER, so that lamellar ER is piled up in excess in cytoplasm. The problem of the formation of these concentric

lamellar structures will be discussed in detail in the third paper of this series (51).

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