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VII. Concluding remarks

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In this review spinning of silk proteins in the silkworm, *Bombyx mori,* has been discussed from morphological, biophysical, biochemical and endocrinological points of view. As the DNA in the silk gland cells is replicated in the absence of cell division after the later period of the embryonic development, they grow up to become cells of an extremely large size at the 5th instar. The nucleus of the fully grown cell shows a highly ramified shape (fig. 2 in chapter II). A single posterior gland cell nucleus on the 6th day of the 5th instar has 200,000 times more DNA than a diploid cell. This value suggests that DNA replication has occurred about 17–18 times without cell division during the growth of the silk gland. The ramification of the nucleus may be helpful to enlarge its surface area, and to increase the transfer of materials required for the silk protein synthesis between nucleus and cytoplasm.

In the early stage of the 5th instar, a number of nucleoli are detected in the ramified nucleus, accompanying many ribonucleoprotein-granules. The stage coincides well with that of the maximal synthesis of ribosomal RNA (fig. IV-1). Fibrous materials were detected in the Golgi vacuoles of the posterior silk gland and termed 'elementary fibroin fiber' (fig. 4 in chapter II). The elementary fibroin fibers are also observed in fibroin globules in the gland cells and in secreted fibroin in the lumen of the silk gland. These observations may support the occurrence of microstructure in which fibroin polypeptide chains are bundled partly into a micro-fiber.

The process of fiber spinning is performed by joint forces of drawing and ejection of aqueous silk through the spinneret of the silkworm. The spinning speed ranges from 0.4 to 1.5 cm/sec. By these mechanical stresses, there occurs an 'α- to β-transition' of silk fibroin. In this transition process the shearing stress seems to play an important role in extending the chains of fibroin molecules.

The biosynthesis of silk fibroin in *Bombyx mori* has been extensively investigated as a model system of protein synthesis in eukaryotes. It is noteworthy that at the last instar the metabolic activities concerned in protein synthesis in the silk gland are highly organized both qualitatively and quantitatively to produce a large amount of specific proteins, that is, fibroin in the posterior silk gland and sericin in the middle silk gland, respectively.

Much effort has been made to elucidate the mechanism of the accumulation of specific tRNA species in the silk glands in concert with the amino acid compositions of the proteins being synthesized. At present, the selective transcription of tRNA genes at the last instar seems to be the most possible explanation for the functionally adaptive population of tRNA species in the tissue. Its detailed mechanism, however, remains unexplained.

Fibroin mRNA was isolated in a highly pure form from the posterior silk gland using its unique features of high G+C content and high molecular weight. This also made it possible to separate the fibroin genes from *Bombyx mori* DNA. The sequence analysis of the fibroin gene, especially around the 5′ end and the intervening region, has revealed some interesting features of the sequence that might be involved in the gene expression in eukaryotic cells.

Recently, another species of RNA containing a poly A tail has been isolated from the posterior silk gland. This mRNA exhibits a high template activity for the synthesis of fibroin small subunit in a wheat germ cell-free system. This finding of the small size mRNA strongly supports the opinion that 2 polypeptide chains of large and small subunits in a fibroin molecule are synthesized individually in the posterior silk gland. The site at which the 2 polypeptides are jointed to a whole fibroin molecule presumably by disulfide bond(s) remains to be determined.

The program of the synthesis of silk proteins in the silk glands and the spinning of the proteins synthesized is apparently under direct or indirect control of 2 major hormones, ecdysteroids and juvenile hormones. Throughout most of the larval instar period, the ecdysone level is very low or not detectable in the hemolymphs of *B. mori* and *Galleria mellonella.* However, before ecdysis - at the 4th day of the 4th molt and day 8 of the 5th molt - the ecdysone level sharply rises, the peak at the last larval instar being twice as high as that at the 4th instar. This peak is preceded by secondary peaks: spinning starts during that period.

On the other hand, juvenile hormone levels in *G. mellonella* hemolymph show 2 peaks during the 4th instar and decrease during the 4th ecdysis. They rise immediately after re-feeding at the last instar and then decrease throughout the period of the last instar. The ratio of juvenile hormones to ecdysteroids in the hemolymph of the silkworm at the last instar is an important factor for larval developmental processes. Treatment of the larvae with juvenile hormones during the early stage of the last instar induces a prolongation of the instar (that is, a delay in cocoon spinning), and a 30–50% increase in silk secretion. Thus, it is most likely that the silk gland is a target tissue for juvenile hormones which act as a potent inhibitor of protein synthesis, presumably, at the transcriptional level.

Although many steroid compounds play important roles in insect metabolism, insects lack the capacity for de novo sterol synthesis, and therefore, they require a dietary or exogenous source of sterol for their normal growth, development and reproduction. This sterol requirement of insects is, in most cases, satisfied by plant sterols such as sitosterol. The conversion of sitosterol to cholesterol has been shown to occur in a number of species of insects including the silkworm, *B. mori.* Recent biochemical works by Ikekawa demonstrated that fucosterol epoxide is a key intermediate in the conversion of sitosterol to cholesterol and that the site of ecdysone synthesis is the prothoracic gland, which is under the control of brain hormones. Further detailed studies in this area provide a great deal of valuable information not only on insect but also general sterol metabolisms.

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Biochemistry of liver development in the perinatal period

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Summary. Just before birth, changes occur in the metabolic capacities of rat liver so that the animal can adapt to changes in the substrate supply. In utero, glucose is the main energy-generating fuel and the liver metabolism is directed towards glucose degradation. The activities of the rate-limiting enzymes of glycolysis, hexokinase and phosphofructokinase, are high. In preparation for post-natal life, when the continuous glucose supply from the mother is interrupted, very large amounts of glycogen are stored in the late fetal liver. With the intake of the fat-rich and carbohydrate-poor milk diet, the animal develops the ability to synthesize glucose de novo from non-carbohydrate precursors. During suckling, metabolic energy is derived mainly from the β-oxidation of fatty acids, which in turn is an essential prerequisite for the high rate of gluconeogenesis, by yielding acetyl-CoA for the activation of pyruvate carboxylase and by generating a high NADH/NAD ratio for the shift of the glyceraldehyde 3-phosphate dehydrogenase reaction in the direction of glucose formation. - The developmental adaptation of metabolism and the process of enzymatic differentiation are closely connected with the maturation of the endocrine system and the changes in the concentration of circulating hormones. The neonatal regulation of phosphoenolpyruvate carboxykinase and of tyrosine aminotransferase by variations in the hormonal milieu around birth, and also the interaction of hormonal and nutritional factors in the induction of serine dehydratase and glucokinase at the end of the suckling period, will be discussed in detail.