obscure - this is particularly true of their hypothetical specificity for silk genes and related genes.

In my opinion, there is no experimental evidence at present to suggest a direct relationship between JH activity and silk protein synthesis. Accurate quantitative assay of JH within the silk glands of *B. mori* should be available in order to confirm or refute the possible high level of JH I at the early stage of the 5th instar, which would not prevent the irreversible initiation of silk mRNA transcription. In addition, the protein synthesis inhibition directed by JH application - including inhibition of silk production - should be found at the transcription level, since this has been blocked, whereas the ribosomal apparatus is degraded at its normal turnover rate. Lastly, when JH dissociates from chromatin as a result of esterase activity and the larval developmental program resumes, silk protein synthesis is also resumed. Its accumulation is always proportional to the corresponding amount of mRNA available within the cell, which in turn depends upon the instar duration associated with a lower RNA turnover[28].

Finally, JH improves silk production because of the prolonged larval programming of the larvae. It is not necessary to postulate any affinity for the silk subprogram. This silk sub-program of larval character is controlled by other specific effector(s), possibly of a neurohumoral nature, independent of the major ecdysteroids and JH. This 'silk secretion factor', assuming it exists, has to be detected within the few hours which follow the 1st meal.

We hope that active and suitable research on ecdysteroids and JH action in the silkworm, as well as research on the silk program, will continue to progress. We are convinced that both are largely independent, but both are required for a better understanding of gene expression and developmental regulation.

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1 Maekawa, H., and Suzuki, Y., Devl Biol. *78* (1980) 394-406.
2 Couble, P., Moine, A., Garel, A., and Prudhomme, J.C., Devl Biol. (1983) in press.
3 De Reggi, M., Hirn, M.H., and Delaage, M.A., Biochem. biophys. Res. Commun. *66* (1975) 1307-1315.
4 Ohnishi, E., Ohtaki, T., and Fukuda, S., Proc. Japan Acad. *47* (1971) 413-415.
5 Mizuno, T., and Ohnishi, E., Devl Growth Differ. *17* (1975) 219-225.
6 Legay, J.M., Calvez, B., Hirn, M., and De Reggi, M., Nature *262* (1976) 489-490.
7 Coulon, M., Calvez, B., De Reggi, M., Legay, J.M., and Hirn, M., Experientia *35* (1979) 1120-1121.
8 Ohnishi, E., Sericologia *21* (1981) 14-22.
9 Calvez, B., Hirn, M., and De Reggi, M., FEBS Lett. *71* (1976) 57-61.
10 Bollenbacher, W.E., Vedeckis, W.V., Gilbert, L.I., and O'Connor, J.D., Devl Biol. *44* (1975) 46-56.
11 Wielgus, J.J., Bollenbacher, W.E., and Gilbert, L.I., J. Insect Physiol. *25* (1979) 9-16.
12 Plantevin, G., De Reggi, M., and Nardon, C., Gen. comp. Endocr., submitted 1983.
13 Calvez, B., J. Insect Physiol. *27* (1981) 233-239.
14 Coulon, M., Biochimie *61* (1979) 147-152.
15 Akai, H., J.A.R.Q. *13* (1979) 116-122.
16 Chinzei, Y., Appl. Ent. Zool. *10* (1975) 136-138.
17 Pratt, G.E., and Brooks, G.T., eds, Juvenile Hormone Biochemistry. Elsevier, Amsterdam 1981.
18 Akai, H., and Kobayashi, M., Appl. Ent. Zool. *6* (1971) 138-139.
19 Strambi, C., Strambi, A., De Reggi, M., Hirn, M.H., and Delaage, M.A., Eur. J. Biochem. *118* (1981) 401-406.
20 Shigematsu, H., Silkworm Satellite Meeting, Special FEBS Meeting, Athens, April 1982.
21 Akai, H., and Kiguchi, K., Bull. seric. Exp. Stn Japan *28* (1980) 1-14.
22 Aomori, S., Ozawa, Y., and Nihmura, M., J. seric. Sci., Tokyo *46* (1977) 69-76.
23 Kamada, K., and Shimada, S., J. seric. Sci., Tokyo *46* (1977) 77-78.
24 Kurata, K., and Daillie, J., Bull. seric. Exp. Stn Japan *27* (1978) 507-530.
25 Daillie, J., Biochimie *61* (1979) 275-281.
26 Kurata, K., Sericologia *21* (1981) 323-332.
27 Shigematsu, H., Kurata, K., and Takeshita, H., Comp. Biochem. Physiol. *61* (1978) 237-242.
28 Prudhomme, J.C., and Couble, P., Biochimie *61* (1979) 215-227.
29 Pave, A., Biochimie *61* (1979) 263-273.
30 Murakoshi, S., Chang, C.F., and Tamura, S., Agric. Biol. Chem. *35* (1972) 695-696.
31 Muroga, A., Nakajima, M., Aomori, S., Ozawa, Y., and Nihuma, M., J. seric. Sci., Tokyo *44* (1975) 267-273.
32 Plantevin, G., French-Japan Seminar, Lyons, April 1982.

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VI. Sterol metabolism in insects and biosynthesis of ecdysone in the silkworm

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Insects lack the capacity for de novo sterol synthesis and require a dietary or exogenous source of sterol for their normal growth, development and reproduction. Thus they differ from vertebrates and most plants, which are capable of endogenous biosynthesis of sterols from acetate. This sterol requirement of the insect is in most cases satisfied by cholesterol (**1**); this is one of the principal sterols in insects, and serves as

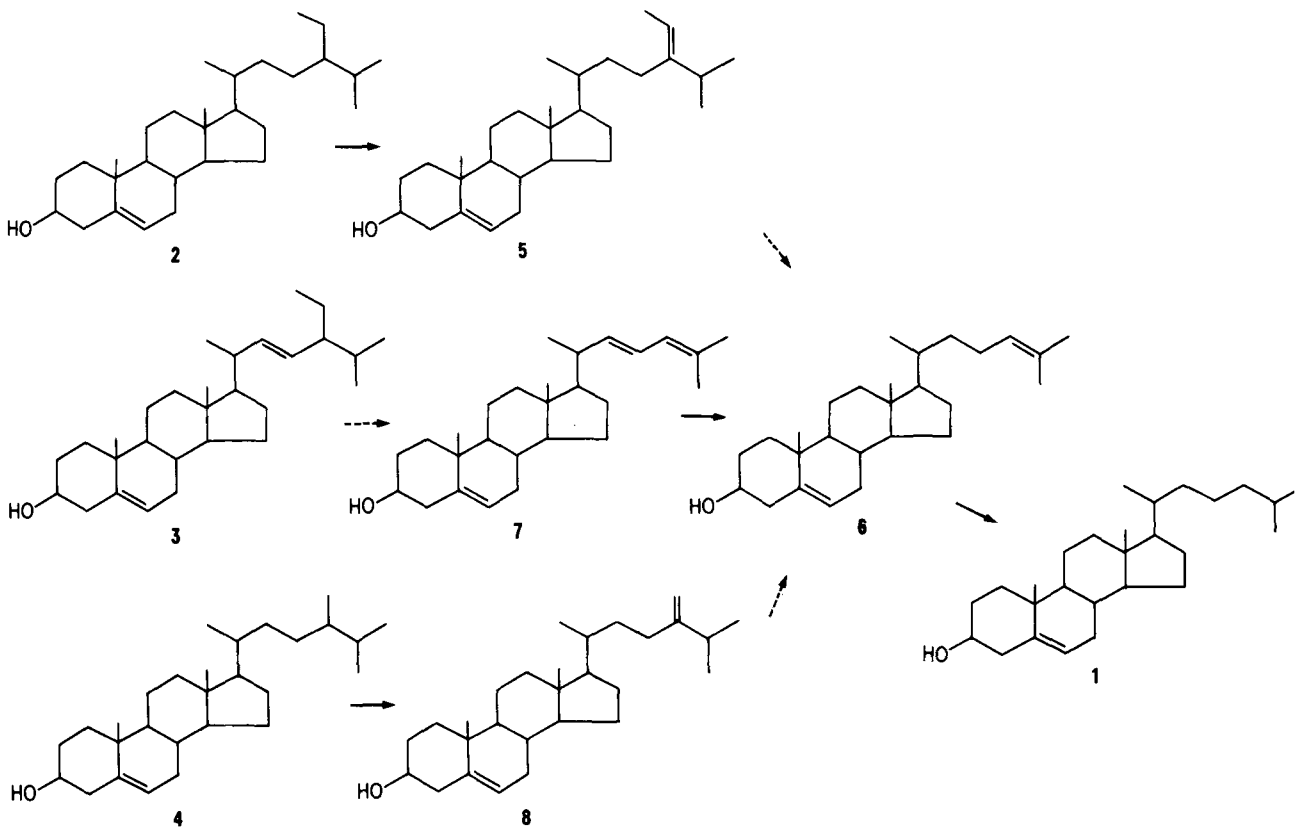
an important component of the cell membranes and as a biogenetic precursor of the moulting hormone ecdysone. In phytophagous and omnivorous insects, however, the requirement can be fulfilled by plant sterols such as sitosterol (**2**), stigmasterol (**3**) and campesterol (**4**), since these C-24 alkylated sterols are metabolically converted to cholesterol (**1**). It appears that dealkylation of phytosterol is an important reaction in sterol metabolism in insects. But not all insects are capable of converting phytosterol to cholesterol. The zoophagous species, such as the house fly *Mucosa domestica*, are unable to convert phytosterol to cholesterol. For this reason cholesterol is an essential nutrient for zoophagous insects.

Clark and Bloch[1] first demonstrated in 1959 that the German cockroach *Blattella germanica* converts ^{14}C-ergosterol to ^{14}C-22-dehydrocholesterol. Since then, the conversion of sitosterol to cholesterol has been shown to occur in a number of other insect species, including the silkworm *Bombyx mori*, the pine sawfly *Neodiprion pratti*, the boll weevil *Anthonomus grandis*, the tobacco hornworm *Manduca sexta*, and the cockroach *Eurycotis floridana*[2]. The biochemical mechanism of the conversion has been investigated in detail by the Beltisville group in the United States Department of Agriculture, using the tobacco hornworm. They identified several intermediates, and proposed the pathway summarized in scheme 1[3]. However, the precise carbon-24,28 bond cleavage mechanism for plant sterols has not yet been clarified, as indicated by the dotted lines in scheme 1.

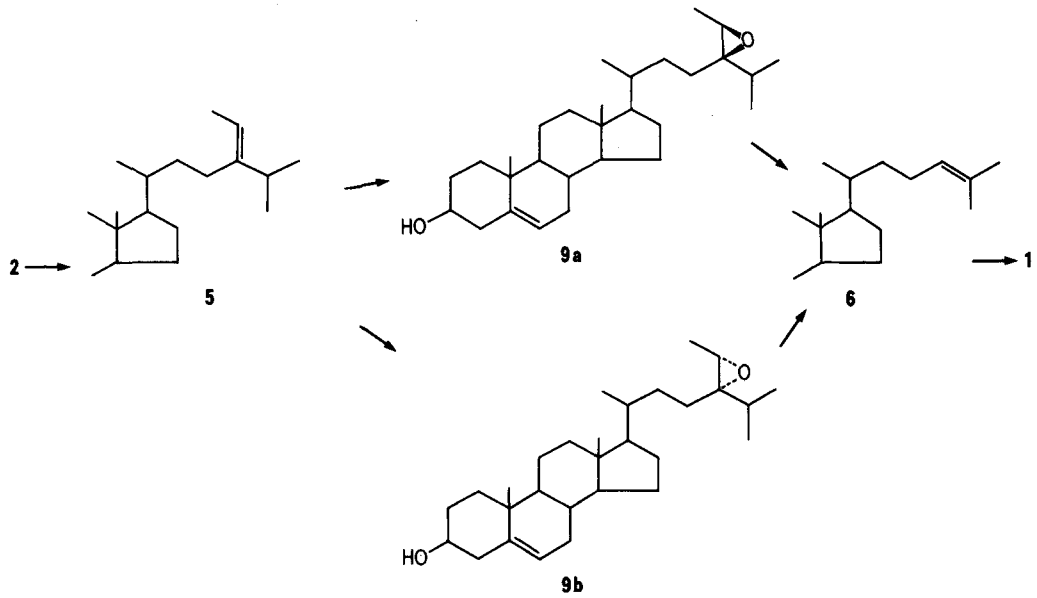
The silkworm has been demonstrated to be an ideal organism for such research work in our laboratory because a satisfactory agar-based artificial diet is available, the conversion of ^{3}H-sitosterol to cholesterol has already been shown, and a large number of the larvae can be cultured. The observation that fucosterol epoxide (**9**) yields desmosterol (**6**) when treated with boron trifluoride etherate led us to propose that fucosterol epoxide is a key intermediate in the conversion of sitosterol to cholesterol[4]. This was subsequently verified, with several lines of evidence. When ^{3}H-fucosterol epoxide was ingested by silkworm larvae, it was converted to cholesterol with a high yield, and tritium from ^{3}H-fucosterol was trapped in fucosterol epoxide[5]. Tritium from 25-^{3}H-sitosterol migrated to the C-24 position during its conversion to desmosterol. Fucosterol epoxide completely satisfied the silkworm sterol requirement[6]. The data subsequently reported support the biogenetic pathway shown in scheme 2 in other insects, including *Tenebrio molitor*[7] and *Locusta migratoria*[8].

For a detailed investigation of the mechanism of this dealkylation, it became necessary to determine the

Scheme 1



Scheme 2



configuration of the epoxide. In order to know which of the 2 stereoisomers of fucosterol epoxide, the 24R, 28R (**9a**) or the 24S, 28S (**9b**), is the true intermediate, each was chemically synthesized[9]. We developed a cell-free enzyme system prepared from the midguts of the silkworm. When samples of the supernatant obtained from a homogenate of silkworm gut were incubated separately with the ^{3}H-24R,28R-epoxide and ^{3}H-24S,28S-epoxide, only the former was effectively converted to desmosterol (**6**). However, subsequent in vivo experiments and more precise in vitro studies demonstrated no absolute stereospecificity, either in the formation of the epoxide from fucosterol or in its conversion to desmosterol, although it was observed that the 24R,28R-epoxide was a little more effectively converted to desmosterol than the 24S,28S-epoxide[10]. These data suggested that both epoxides could be intermediates. This supposition was substantiated when the two epoxides were found to satisfy equally the silkworm sterol requirement and the sterol profiles of the insects reared on them showed no significant difference. Moreover, both isomers of the epoxides were isolated from 5th instar larvae reared on mulberry leaves[11]. The low stereospecificity observed with fucosterol epoxide appears to be in line with the facts that both fucosterol and isofucosterol were identified in the silkworm, and there is no difference in the nutritional effects of sitosterol and clionasterol[12]. Although the intermediary role of isofucosterol epoxide is probably of less importance in *B. mori,* judging by its poor nutitional effect and its absence from the larvae, this may not be case in *Tenebrio molitor,* in which the 24R,28S-isofucosterol epoxide was converted into cholesterol, but not the 24S,28R-isomer, and both of the fucosterol epoxides were transformed into cholesterol to about the same extent[13].

24-Methylenecholesterol (**8**) has been identified as an intermediate of the dealkylation of compesterol (**4**). Recently, the transformation of 24-methylenecholesterol into the corresponding 24,28-epoxide, and of the latter into desmosterol and cholesterol, have been demonstrated in the silkworm and other insects[14].

It is worthy of note that low stereospecificity in utilizing 24-methylcholesterol was demonstrated with *Manduca sexta.* However, it was reported that in *Dermestes maculatus* there was a preference for 24R-alkylsterols over 24S isomers, and *Drosophila melanogaster* utilizes both 24R- and 24S-methylcholest-5,7-dien-3β-ol, while *D. pachea* preferentially utilizes the 24S isomers and *D. mojavensis* prefers the 24R isomers as reported by Kircher[15]. Recently, some phytophagous insects, such as the large milkweed bug, *Oncopeltus fasciatus,* and the khapra beetle *Trogoderma granarium* Everts, were found to lack capacity for dealkylating plant sterols to produce cholesterol. The Mexican bean beetle *Epilachna varivestis* Mulsant, is unique, because Δ^5-phytosterols are reduced to stanols and then dealkylated to produce cholestanol. The honey bee *Apis mellifera,* may be incapable of converting phytosterol to cholesterol[16]. It is interesting to note that makisterone A (24-methyl-20-hydroxyecdysone) is a major ecdysteroid in the egg of the large milkweed bug, which is incapable of dealkylating C-28 and C-29 phytosterols[16].

Inhibition of sterol metabolism has proved valuable for studying the metabolism of plant sterols in insects. Thompson[17] reported that certain azasteroids, 22,25-diazacholesterol and 25-azasteroids, and nonsteroidal amines block the conversion of 24-alkylsterols to

cholesterol and/or disrupt molting and development in insects. We postulated that a structural analog of the sitosterol dealkylation intermediate would be a potent and specific inhibitor of sterol metabolism in *B. mori*; to test this, 24,28-iminofucosterol (**10**), stigmasta-5,24(28),28-trien-3β-ol(allene I) (**11**) and cholesta-5,23,24-trien-3β-ol(allene II) (**12**) were prepared and investigated as inhibitors[18].

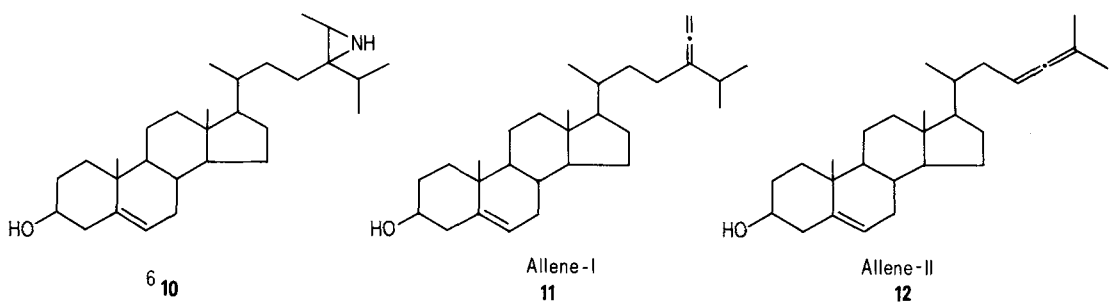
When the imine (**10**) or the allene II (**12**) were administerd in the silkworm diet in combination with sitosterol or cholesterol, the growth and development of *B. mori* were markedly retarded. The one likely target of the imine inhibition was expected to be the step of conversion of fucosterol epoxide to desmosterol, and this was verified by in vitro experiments where the imine, at the same level as the substrate ^{3}H-fucosterol exposide, completely blocked the transformation into desmosterol. However, the imine may not exert its effect solely by limitation of desmosterol or cholesterol formation, because cholesterol used as the sole dietary sterol was unable to prevent the imine inhibitory effect. In contrast, the allene II seemed to exert little effect on sitosterol dealkylation because the sterol component in silkworms fed on the allene II in combination with sitosterol was essentially not different from that of controls.

The other allene I (**11**) has more interesting properties. When *B. mori* was reared on fucosterol epoxide, desmosterol or cholesterol, the addition of the allene I to the diets at the same concentration as those of nutritional sterol caused no significant inhibition of insect growth and development. In contrast, when the nutritional sterol was replaced with sitosterol or fucosterol, marked growth retardation was observed. In agreement with these results, allene I induced the accumulation of sitosterol or fucosterol when these sterols were the dietary sterol. These results strongly suggest that the allene I would be a highly specific inhibitor for the steps involving fucosterol; that is, for the conversion of sitosterol to fucosterol and/or fucosterol to the epoxide.

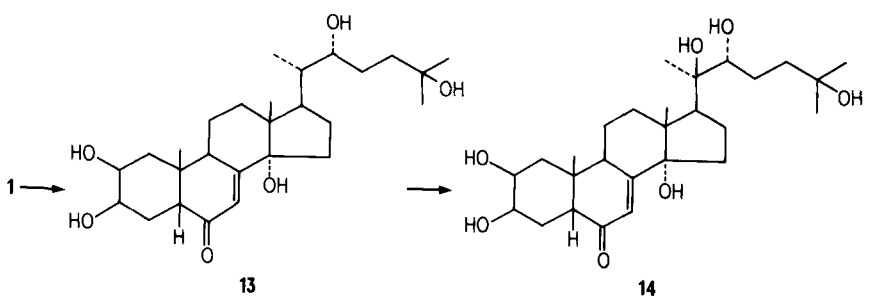
B. mori can grow and develop on a semi-synthetic diet containing cholesterol or phytosterols at 0.1–0.5%. Several cholesterol analogs with the side chain modified in carbon chain length and branching pattern were tested for their nutritional effect in order to have an insight into the relationship between biological function and side chain structure of cholesterol, which is ubiquitous and may be a functional sterol in insects. Most of the larvae fed on the analogs with the longest(C-32 sterol) or the shortest(C-24 sterol) side chain died during the 1st instar. The other cholesterol analogs tested(C-25–C-29 sterols) were 'partially effective' sterols, inducing a slower growth rate as the structure deviated more from that of cholesterol[19]. Insect sterol analysis revealed that the particular sterol given in the diet was almost intact in *B. mori* and the contents of other sterols were less than 5% of the total sterol. Accordingly, the major membrane sterol would be the dietary sterol itself. It may be considered that insect sterols with modified structures are probably only partially effective for exerting normal biological functions.

The insect molting hormone, ecdysone(α-ecdysone) (**13**), was first isolated in crystalline form from the

Scheme 3



Scheme 4



silkworm by Butenant and Karlson in 1954, and the structure was determined in 1965. Soon after that, the 2nd molting hormone was isolated and the structure was elucidated as 20-hydroxyecdysone(β-ecdysone, ecdysterone) (**14**). The same hormone was also isolated from some crustaceans. Pioneer work demonstrating the role of cholesterol as a precursor of ecdysone was done by Karlson using *Calliphora erythrocephala,* though the incorporation was low (0.0001%). Recently, higher incorporations of labeled cholesterol into ecdysone(0.015%) and 20-hydroxyecdysone(0.018% or 0.035%) have been reported in in vivo experiments.

In 1940 Fukuda proposed that the prothoracic gland (PG) secreted molting hormone, although there was no direct evidence to establish this. Endocrinological and chemical studies during 1966–1970 on the insect molting hormone led to considerable controversy regarding the site of ecdysone biosynthesis. For instance, it was reported that cholesterol is converted into ecdysone (0.0008%) and 20-hydroxyecdysone (0.00083%) in the isolated abdomens of larvae of the silkworm, and that the isolated ring glands do not convert cholesterol into ecdysone[20].

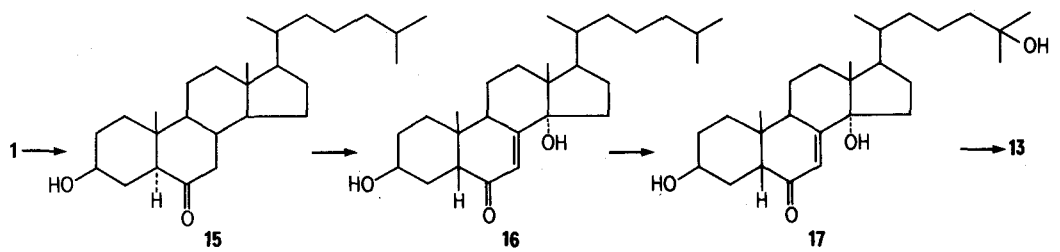
In order to obtain definitive proof that the PG is the organ which produces ecdysone, it was necessary to carry out organ culture of isolated PG, extraction of hormone from the culture system, and analysis by both bioassay and chemical studies. This was achieved by Chino et al.[21] using isolated PG of the silkworm and a medium containing hemolymph. A hormonal substance isolated from the culture medium was shown to be ecdysone, indicating that the PG is the site of ecdysone synthesis[21]. It was demonstrated that the conversion of ecdysone to 20-hydroxyecdysone occurred in fat body, Malpighian tubule, gut and body wall tissues[22]. Although ecdysone may have direct hormonal effects, it is generally held that ecdysone serves as a pre-hormone that is converted to 20-hydroxyecdysone which functions as the active hormone. This is because in many bioassay systems 20-hydroxyecdysone appears to be much more active than ecdysone.

Since the elucidation of the structure of ecdysone, a number of synthetic cholesterol derivatives have been demonstrated as possible intermediate in the conversion of cholesterol to ecdysone. However, no attempts have been made to identify the intermediates of ecdysone synthesis in the PG. Only 3β-hydroxy-5α-cholesta-6-one was isolated from the organ culture medium of silkworm PG as a possible intermediate[23]. The dehydrogenation of cholesterol to 7-dehydrocholesterol in some insects has been well documented in the PG is a site of synthesis or storage of this compound. Thus, this compound may be one of the intermediates[24]. Recently Goodwin's group reported that the formation of the A/B cis ring junction of ecdysteroids in plants and in insects apparently involves different mechanisms, and the 3-oxo-Δ^4 compound may be a intermediate in the formation of the A/B ring of ecdysteroids at least in the locust *Schistocerca gregaria,* ovarian system[25]. A tentative scheme for ecdysone biosynthesis, resulting from in vivo and in vitro studies with possible precursors and intermediates, by several groups working on a number of insect species, is presented in scheme 5.

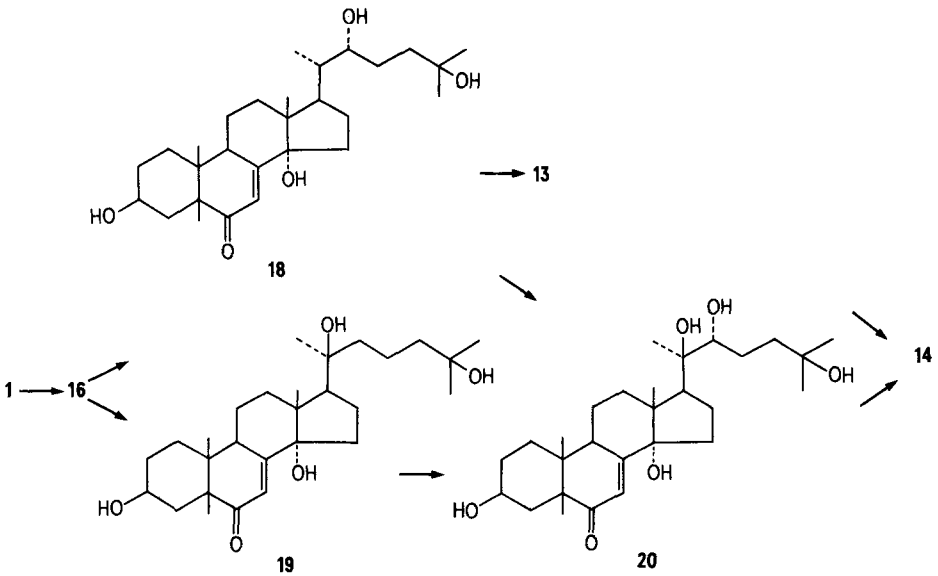
In 1971 high molting hormone activity was found in the ovaries of *B. mori* by Ohnishi[26]. Kaplanis also found a high level of active ecdysteroid, 26-hydroxyecdysone, in tobacco hornworm eggs[16]. Since then accumulation of ecdysteroids in ovaries and eggs has been reported in other insects, and now evidence that the ovary is also the organ of biosynthesis of ecdysteroids has been obtained. In contrast to the rather simple pattern of ecdysteroids found at the postembryonic stage, those found in ovaries or eggs are characterized by the presence of a variety of molecular species and a high proportion of conjugated forms. We coined the term 'oo-ecdysteroids' to distinguish them from 'prothoraco-ecdysteroids' which are found at post-embryonic stages[27].

The ovary of *B. mori* occupies about 60% of the body weight at later stages of pupal development and ecdysteroids amount to several µg per g wet weight of this organ. Thus the ovaries of *B. mori* are an excellent source of materials for ecdysteroid isolation. We have isolated and identified the following ecdysteroids: 2-deoxyecdysone (**18**)[28], ecdysone (**13**), 2-deoxy-20-hydroxyecdysone (**20**)[29], 20-hydroxyecdysone (**14**) and 2,22-dideoxy-20-hydroxyecdysone (**19**)[30]. Our studies on the mode of accumulation of these ecdysteroids during the ovarian development showed that the ecdysteroids initially accumulated in the ovary

Scheme 5



Scheme 6



were 2-deoxyecdysone, 2-deoxy-20-hydroxyecdysone, and 2,22-dideoxy-20-hydroxyecdysone, and that ecdysone and 20-hydroxyecdysone were synthesized de novo in the ovary[29]. The most plausible biosynthetic pathway interrelating the ecdysteroids found in the ovary is shown in scheme 6.

Prothoracic glands seem to have no capacity to hydroxylate at the C-20 position, whereas other peripheral tissues lack the capacity to produce ecdysone. This differs from the *B. mori* ovarian system, where successive hydroxylations at C-20 and C-22 occur in either order. The ovary of *B. mori* is a remarkable organ. It is induced to mature by the action of prothoraco-ecdysteroids; it takes up the ecdysteroids from hemolymph; it metabolizes the ecdysteroids, makes conjugates and accumulates them. The ovary synthesizes a variety of oo-ecdysteroids and accumulates them in free and conjugated forms.

Accumulation mainly of 2-deoxyecdysteroids in the *B. mori* ovary differs from the ovarian systems of *Galleria mellonella, Schistocerca gregaria* and *Macrotermes* species, in which ecdysone and 20-hydroxyecdysone are predominant. The fact might be related to the rather rapid maturation of the ovaries at the late pharate adult stage in *B. mori*. Kaplanis reported the metabolic route of ecdysteroids in *Manduca sexta* as follows. Ecdysone serves as a precursor for 26-hydroxyecdysone, an ecdysteroid that so far has only been found during embryonic development in *M. sexta*. Ecdysone is also converted to 20-hydroxyecdysone and 3-epi-ecdysone. Likewise 20-hydroxyecdysone is either further hydroxylated at the C-26 position to 20,26-dihydroxyecdysone or epimerized to 3-epi-20-hydroxyecdysone. 26-Hydroxylation and 3-epimerization of ecdysone in *M. sexta* could be considered as inactivation steps[31]. However the precise physiological role or function of these free and conjugated 'oo-ecdysteroids' in embryonic development remains to be clarified.

1 Clark, A.J., and Block, K., J. biol. Chem. *235* (1959) 2589.
2 Robbins, W.E., Kaplanis, J.N., Svoboda, J.A., and Thompson, M.J., A. Rev. Ent. *16* (1971) 53.
3 Svoboda, J.A., Kaplanis, J.N., Robbins, W.E., and Thompson, M.J., A. Rev. Ent. *20* (1975) 205.
4 Ikekawa, N., Morisaki, M., Ohtaka, H., and Chiyoda, Y., J. chem. Soc., D. *1971*, 1498.
5 Morisaki, M., Ohtaka, H., Okubayashi, M., Ikekawa, N., Horie, Y., and Nakasone, S., J. chem. Soc. chem. Commun. *1972*, 1272.
6 Fujimoto, Y., Morisaki, M., and Ikekawa, N., Steroids *24* (1974) 367; Fujimoto, Y., Awata, N., Morisaki, M., and Ikekawa, N., Tetrahedron Lett. *1974*, 4335.
7 Randall, P.J., Lloyd-Jones, J.G., Cook, I.F., Rees, H.H., and Goodwin, T.W., J. chem. Soc. chem. Commun. *1972*, 1296.
8 Allais, J.P., Alcaide, A., and Barbier, M., Experientia *29* (1973) 944, FEBS Lett. *82* (1977) 333.
9 Fujimoto, Y., Murakami, K., and Ikekawa, N., J. org. Chem. *45* (1980) 566.
10 Fujimoto, Y., Morisaki, M., and Ikekawa, N., Biochemistry *19* (1980) 1065.
11 Ikekawa, N., Fujimoto, Y., Takasu, A., and Morisaki, M., J. chem. Soc. chem. Commun. *1980*, 709.
12 Morisaki, M., Ying, B., and Ikekawa, N., Experientia *37* (1981) 336.
13 Nicotra, F., Pizzi, P., Ronchetti, F., Russo, G., and Toma, L., J. chem. Soc. Perkin I *1981*, 480; J. chem. Soc. chem. Commun. *1980*, 479.
14 Maruyama, S., Fujimoto, Y., Morisaki, M., and Ikekawa, N., Tetrahedron Lett. *1982*, 1701; Nicotra, F., Ronchetti, F., and Russo, G., Lipids *17* (1982) 184.
15 References cited in Fujimoto et al.[10].
16 Svoboda, J.A., Thompson, M.J., Robbins, W.E., and Kaplanis, J.N., Lipids *13* (1978) 742.

17 Marks, E.P., Robbins, W.E., and Thompson, M.J., Lipids *13* (1978) 259.
18 Awata, N., Morisaki, M., Fujimoto, Y., and Ikekawa, N., J. Insect Physiol. *22* (1976) 403.
19 Isaka, Y., Morisaki, M., and Ikekawa, N., Steroids *38* (1981) 417.
20 References cited in Chino et al.[21].
21 Chino, H., Sakurai, S., Ohtaki, T., Ikekawa, N., Miyazaki, H., Ishibashi, M., and Abuki, H., Science *183* (1974) 529.
22 King, D.S., Gen. comp. Endocr., suppl. *3* (1972) 221.
23 Sakurai, S., Ikekawa, N., Ohtaki, T., Chino, H., Science *198* (1977) 627.
24 Johnson, P., Cook, I.F., Rees, H.H., and Goodwin, T.W., Biochem. J. *152* (1975) 303; Rees, H.H., Davies, T.G., Dinan, L.N., Lockley, W.J.S., and Goodwin, T.W., in: Progress in Ecdysone Research, p. 125. Ed. J.A. Hoffmann. Elsevier/North-Holland Biomedical Press, Amsterdam 1980.
25 Davies, T.G., Lockley, W.J.S., Boid, R., Rees, H.H., and Goodwin, T.W., Biochem. J. *190* (1980) 537; Davies, T.G., Dina, L.N., Lockley, W.J.S., Rees, H.H., and Goodwin, T.W., Biochem. J. *194* (1981) 53.
26 Ohnishi, E., Ohtaki, T., and Fukuda, S., Proc. Japan Acad. *47* (1971) 413.
27 Ohnishi, E., Sericologia *21* (1981) 14.
28 Ohnishi, E., Mizuno, T., Chatani, F., Ikekawa, N., and Sakurai, S., Science *197* (1977) 66.
29 Ohnishi, E., Mizuno, T., Ikekawa, N., and Ikeda, T., Insect Biochem. *11* (1981) 155.
30 Ikekawa, N., Fujimoto, Y., Takasu, A., and Morisaki, M., J. chem. Soc. chem. Commun. *1980*, 709.
31 Kaplanis, J.N., Weirich, G.F., Svoboda, J.A., Thompson, M.J., and Robbins, W.E., in: Progress in Ecdysone Research, p. 163. Ed. J.A. Hoffmann. Elsevier/North-Holland Biomedical Press, Amsterdam 1980.

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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.