Thyroidectomy in male rats resulted in a significant increase in serum TSH. Administration of T4 to Tx rats reduced serum TSH levels to a level with no difference to those of intact rats. These results are conceivable and in consistent with earlier reports[20-22]. However, thyroidectomy had no effect on the basal release of TSH, and reduced the release of TSH in response to TRH by rat AP in vitro. These results favor a view that the negative feedback control of TSH secretion by thyroid hormones might be mainly at the level of the hypothalamus. This concept is supported by Berelowitz et al.[20], who suggested that the elevated TSH levels seen in primary hypothyroidism may result in part from a decrease in the tonic inhibitory effect of hypothalamic somatostatin. However, our data do not rule out the possibility that T4 may also act at the anterior pituitary level as well.

A deficient effect of T4 replacement in vivo on the TSH release in response to TRH in vitro is similar to those on the prolactin release in the present study. These results could be explained by the loss of TRH receptors in both thyrotrophs[12] and lactotrophs[11,13] of the rat AP after chronic treatment of thyroid hormones. The deficiency of the dose of T4 therapy may be another possibility even the same dose of T4 is sufficient to restore the prolactin concentration in serum of Tx rats. Whereas the different results of the total hormone production between prolactin and TSH in the AP of Tx rats replaced with T4 reflect a possibility of different mechanisms in regulating the hormone synthesis between prolactin and TSH in vivo.

In summary, the present results suggest that the inhibitory effects of thyroidectomy on AP and serum prolactin in male rats are mediated at least in part by the reduction of the total production and basal release of prolactin and the responsiveness of prolactin to TRH.

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7-Dehydrosterols in prothoracic glands of the silkworm, *Bombyx mori*

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Summary. Sterol analysis of prothoracic glands of the silkworm, *Bombyx mori* revealed the presence of 7-dehydrocampesterol and 7-dehydrositosterol together with 7-dehydrocholesterol. It was also found that the amounts of these 7-dehydrosterols were increased in proportion to the ecdysone titer.

Key words. 7-Dehydrocholesterol; 7-dehydrocampesterol; 7-dehydrositosterol; *Bombyx mori;* ecdysone; prothoracic gland.

Usually, 7-dehydrocholesterol represents only a few percent of the total sterol content in most insects[2], but high titers of this sterol have been found in the prothoracic glands and in eggs[3,4]. The importance of 7-dehydrocholesterol has been described in relation to the biosynthesis of ecdysteroids[5]. The sterols of the silkworm, *Bombyx mori* were reported to contain cholesterol (**1a**), campesterol (**1b**), and sitosterol (**1c**) along with very small amounts of fucosterol[6], isofucosterol[6], desmosterol[7] and fucosterol epoxide[8]. We describe here the identification of 7-dehydrocampesterol (**2b**) and 7-dehydrositosterol (**2c**) in addition to 7-dehydrocholesterol (**2a**), in prothoracic glands. The peak titers of these 7-dehydrosterols occur on days 5 and 9 of the 5th larval instar.

Prothoracic glands (3200) of 5th instar larvae of *B.mori* were extracted with chloroform-methanol (2:1). The extracts were applied to precoated TLC plates (Merck, Kiesel-gel 60 F254) and developed with benzene-acetone (10:1). The UV absorbing band corresponding to 7-dehydrocholesterol was scraped off and extracted with ethyl acetate. Gas-liquid chromatography (GLC) analysis of the extract after TMS (trimethylsilyl imidazol) derivatization detected cholesterol, campesterol and sitosterol as well as three additional sterols, which have slightly longer retention times than the respective Δ^5 (fig. 1). These sterols were identified as 7-dehydrocholesterol (**2a**), 7-dehydrocampesterol (**2b**), and 7-dehydrositosterol (**2c**) by the use of GC-MS analysis[9].

We then analyzed the sterol fractions (eluted with hexane-ethyl acetate (4:1) on a column of silica gel) isolated from hemolymph (2 ml), prothoracic glands (10 pairs) or fat body (2 g wet wt) of the 5th instar larvae (day 2 of wandering). GLC analysis of these fractions after TMS derivatization revealed cholesterol, campesterol and sitosterol in all three tissues, whereas no 7-dehydrosterols were detected in the samples of hemolymph and fat body.

Encouraged by these observations, we then went on to measure the changes in the amounts of 7-dehydrosterols in the protho-

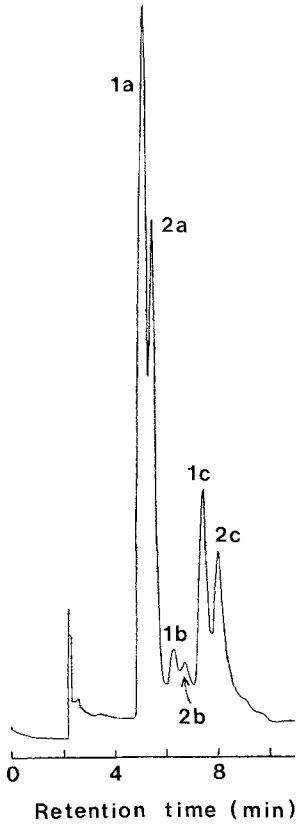


Figure 1. GLC profile of the sterol fraction in prothoracic glands of *B.mori*.
Analyzed by a Shimadzu LKB-9000 GC-MS spectrometer using a 1.5% OV-1 containing a glass column (2 m × 4 mm i.d.) at oven temperature 272 °C and He flow rate 30 ml/min. The sterols except for **2b** and **2c** were identified by reference to authentic samples (retention times and mass spectrum).

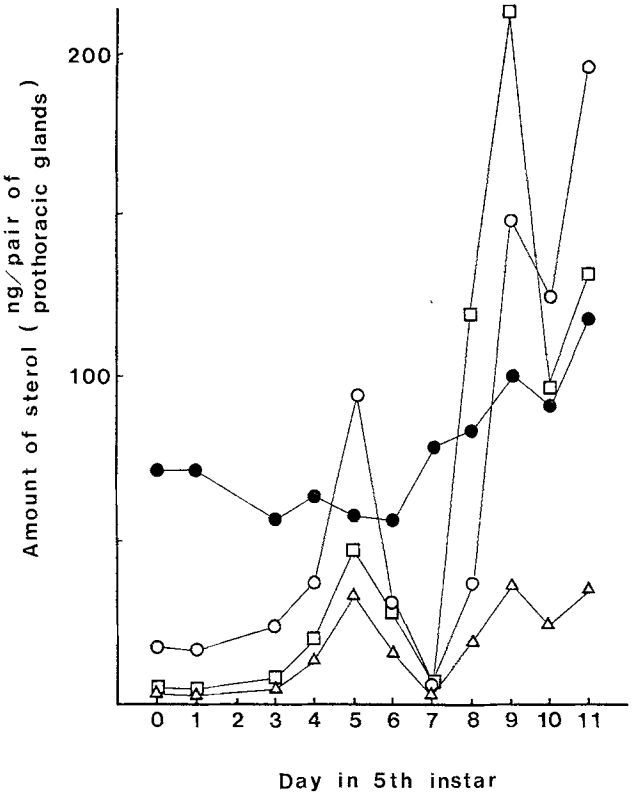
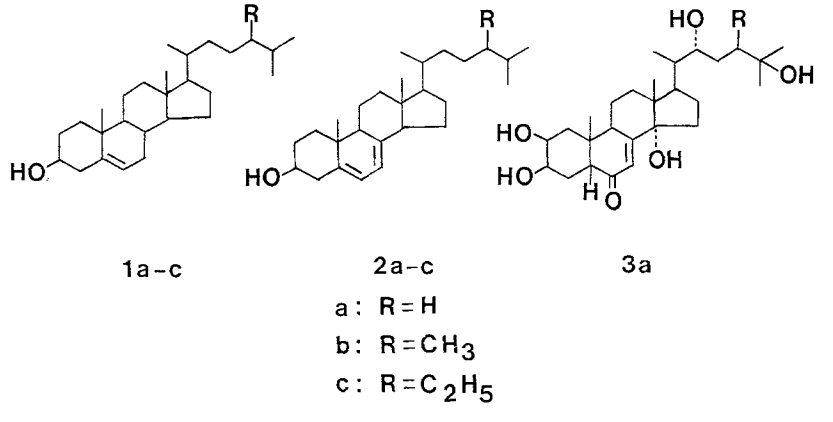


Figure 2. Sterol changes in prothoracic glands of *B.mori*. ●: cholesterol, ○: 7-dehydrocholesterol, △: 7-dehydrocampesterol, □: 7-dehydrositosterol. The levels of sitosterol (11 ± 2 ng) and campesterol (5 ± 1 ng) are not presented in the figure. Days 0 and 6 correspond to the ecdysis and gut purge, respectively. The data was obtained with a Shimadzu DF 9020 GC-MS spectrometer using a capillary column (Shimadzu Hicap CBP-1, 25 m × 0.33 mm i.d.), equipped with a solventless inlet system. The sterol amounts were calculated based on the peak area using a known amount of cholesterol or 7-dehydrocholesterol as a standard. Massfragmentgraphic analysis was also carried out for some samples (data not shown), which was in good agreement with the data above.



racic glands during the 5th instar of silkworms reared on an artificial diet (Silkmate II, Nihon-Nohsan). The results are shown in figure 2. It can be seen from the figure 2 that the amount of 7-dehydrocholesterol has its peak titer around day 5 and day 9, although the level of cholesterol does not change significantly during this period. More interestingly the changes in **2b** and **2c** parallel that of **2a**. The peak titer of 2a (day 9) is coincident with both the peak ecdysone titer in hemolymph[10] and the time of maximum in vitro prothoracic gland activity during the 5th instar of *B.mori*[11]. The data appears to support the role of 7-dehydrocholesterol as an intermediate in the biosynthesis of ecdysone (**3a**) by prothoracic glands. To the best of our knowledge, the occurrence of 7-dehydrocampesterol and 7-dehydrositosterol in insects is described here for the first time[12]. It appears that the prothoracic glands convert the circulating sterols in blood into 7-dehydrosterols regardless of the

C-24 substituent. When the accumulation and fate of these 7-dehydrosterols is considered, the following possibilities come to mind: 1) prothoracic glands, like guts[13], are able to dealkylate these 7-dehydrosterols such as **2b** and **2c** into 7-dehydrocholesterol, the latter being metabolized into normal ecdysone; 2) these dehydrosterols are converted into a modified ecdysone (24-alkylecdysones, without dealkylation of the C-24 substituents; 3) **2a** is selectively metabolized into ecdysone and the remaining **2b** and **2c** are equilibrated with **1b** and **1c**. Detailed analysis of the ecdysone of *B. mori* will shed some light on these questions. It is of interest to note that makisterone, an ecdysteroid with a 24-methyl group, has been isolated from *Oncopeltus fasciatus*[14,15], *Dysdercus cingulatus*[16], *D. fasciatus*[17], *Apis mellifera*[18], and *Drosophila melanogaster*[19], although, in the cases of *Oncopeltus* and *Dysdercus*, the insect do not convert their dietary plant sterol to cholesterol[15,17].

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Possible target of Abelson virus phosphokinase in cell transformation[1]

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Summary. By fusing interphase cells to cells undergoing mitosis, the interphase chromosomes can be visualized. When analyzed in this way, chromosomes of normal mouse cells show characteristic undercondensed centromeric regions. We have found that the centromeric regions of chromosomes from Abelson virus-transformed cells are fully condensed. Abelson virus transforms mouse cells by introducing into them a virally encoded phosphokinase that is expressed constitutively. Thus, we propose that the condensation of centromeric chromatin is a result of overphosphorylation by the Abelson virus phosphokinase, and that the centromeric region is the relevant target of overphosphorylation in transformed cell growth.
Key words. Abelson virus; centromere; chromosome condensation; premature chromosome condensation; transformation.

Interphase chromosomes, unlike metaphase chromosomes, cannot be directly visualized in situ by light microscopy because they are not condensed. During mitosis, the nuclear membrane disappears, allowing the condensed metaphase chromosomes to be spread on a microscope slide after hypotonic treatment of the cells. When interphase cells are fused to metaphase cells, the interphase nuclear membrane rapidly dissolves, and the interphase chromosomes condense and can be prepared in the same way as metaphase chromosomes[4]. By varying the time between fusion and chromosome preparation, we can vary somewhat the degree of condensation of the interphase chromatin, because the factors introduced by the metaphase cell take some time to act. We fused mitotic Chinese hamster ovary (CHO-B11) cells to untransformed interphase NIH/3T3 mouse fibroblasts and to the Abelson virus-transformed NIH/3T3 cell line ANN-1[5] (fig. 1). The mitotic condensation factors from the hamster cells were allowed to act on the interphase mouse chromosomes for 30, 40, or 50 min before chromosome spreads were prepared (fig. 2). Since the hamster metaphase chromosomes are metacentric with the two sister chromatids connected, they can be easily distinguished from the single acrocentric mouse chromatids of G_1 phase cells. On chromatids of the NIH/3T3 cells (fig. 2A), the centromeric regions characteristically appear as lightly stained elongated chromosomal stretches, as is characteristic of normal mouse cells[6,7]. At each of the sampling times, about 90% of the chromosome spreads from NIH/3T3 cells showed this undercondensation of the centromeric chromatin (table 1). In the transformed fibroblast line ANN-1, on the other hand, only 52% of the cells had undercondensed centromeric chromatin 30 min after fusion, and this decreased to 35% after 40 min and 15% after 50 min (table 1). The condensed centromere (fig. 2B) could be visualized only by using the C-banding technique[8] (not shown). In contrast to the centromeric region, the condensation rate for the noncentromeric chromatin was not markedly different in the two cell types.

At 50 min after fusion, the centromeric chromatin was fully condensed in four independent Abelson virus-transformed pre-B-cell lines, whereas 70Z/3, a chemically transformed pre-B-cell line, was similar to the NIH/3T3 cell line (table 2). Interestingly, other dividing cells of lymphocyte origin, including lipopolysaccharide-stimulated B lymphoblasts, exhibited fully condensed centromeric regions after fusion; normal spleen cells, thymus cells, and transformed cells of macrophage, mast cell, monocyte, melanocyte, and mammary carcinoma origin did not.

Although we cannot be sure of the proximate cause of the phenomenon described here, it is known that premature chro-