The frequency of labeling patterns with ^{3}H-thymidine in nuclei of DDT-treated and nontreated salivary gland cells of *Drosophila melanogaster*

	Number of nuclei examined	Number of nuclei labeled	Total labeling frequency of the nuclei (%)	Frequency of various labeling patterns (%) 2C	3C	3D	2D	1D
Control	492	195	39.6	4.3	7.7	6.5	8.5	12.6
Experimental	494	123	24.8	0.8	4.0	3.2	7.1	9.7

experimental group, the frequencies of various labeling patterns were as follows: 0.8% 2C, 4.0% 3C, 3.2% 3D, 7.1% 2D and 9.7% 1D (table).

Examining the data in the table and the histogram (fig.) the following points can be made: 1. the labeling frequency with ^{3}H-thymidine in the experimental group is lower than that in the control group; that is, DDT pretreatment reduces it by 14.8%; 2. although this reduction has been observed in all types of labeling patterns, it is highest in the 2C pattern. Statistical analysis of the data with Student's t-test showed this reduction to be significant only in the 2C-type labeling pattern ($P < 0.05$).

Discussion. The results of this study show that an effect of DDT is more severe on the initial stage of DNA replication than it is on the other stages of the replication cycle; that is, DDT inhibits the initiation of DNA replication in polytene chromosomes of salivary gland cells.

Any of the following hypotheses could account for the inhibitory effect of DDT: 1. DDT may introduce cross-links at A-T sites; 2. it may reduce the activity of the DNA polymerase or RNA polymerase which play a role in the synthesis of priming RNA during the initial stage of DNA replication; 3. it is also possible that the inhibition could have been a consequence of a nonspecific metabolic depression. Indeed; the inhibitory effects of DDT and its analogues on some metabolic enzymes in *Estigmene acreae*[6] and *Drosophila*[12] have been reported by some authors. The hypotheses suggested are presently being tested in our laboratory.

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DNA synthesis in the nuclei of differentiating muscle fibers of the silkworm, *Bombyx mori* L.

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Summary. Incorporation of ^{3}H-thymidine (^{3}HTdr) into the nuclei of myofibril-containing myofibers of larvae of the silkworm, *Bombyx mori*, was shown by means of light microscope (LM) and electron-microscope (EM) autoradiography. The number of DNA-synthesizing myonuclei attains 42% 12–18 h after each molt. Thus in the developing silkworm DNA replication and myofibrillogenesis are coexisting and not mutually exclusive processes as is the rule in vertebrate somatic myogenesis.

Key words. DNA synthesis; EM autoradiography; ^{3}H-thymidine; muscles; *Bombyx mori.*

Materials and methods. Light-microscope autoradiography was used to investigate intersegmental muscles in larvae from stage I to stage V. For this purpose 1 μCi/g of ^{3}HTdr (Soviet production, specific activity 5.2 Ci/mM) was injected with microcapillary into the larvae. After 2 h the latter were fixed for 12 h in a modified Bouin's fluid. 5-μm-thick paraffin sections were covered with M-type emulsion (Soviet production), and developed after 20 days of exposure at 4 °C.

For EM autoradiography 30 μCi/g of ^{3}HTdr of the same activity was injected into stage II larvae 6 h after their molting. 2 h later the larvae were fixed at 4 °C, at first for 10 h in 2.5% glutaraldehyde prepared in veronal-acetate buffer at pH 7.4, and then for 1 h in 1% OsO_4 under the same conditions. Ultrathin sections of the material embedded in araldite were obtained on a LKB III ultratome, contrasted[6] with lead citrate, covered with emulsion and exposed during 3 months at 4 °C. The autoradiographs were analyzed in a microscope JEM 7A at 50 kV.

Results and discussion. LM autoradiography. Data on ^{3}HTdr incorporation into the nuclei of silkworm muscle fibers have been obtained[1,2] earlier by means of light-microscope (LM) autoradiography. In the present study the DNA synthesis was examined in myofiber nuclei of *Bombyx mori* larvae starting immediately after their emergence through five moltings (fig. 1). In larvae of each stage replication is interrupted by molting and resumes over an interval of 0–6 h after the last molt. The index of ^{3}HTdr pulse labeled myonuclei (LI) increases rapidly, attaining the maximum (42%) in the interval from 12–18 h after each molting. This is characteristic of all stages except stage I, when the above mentioned LI level is reached 6 h after emergence. Then the LI decreases gradually below 1% at the end of each stage, but 6–12 h before each molting we observe a small LI increase in larvae of all stages (except stage V).

EM autoradiography. Since in insects beneath the basal membrane of myofibers we find, along with myonuclei, the nuclei of

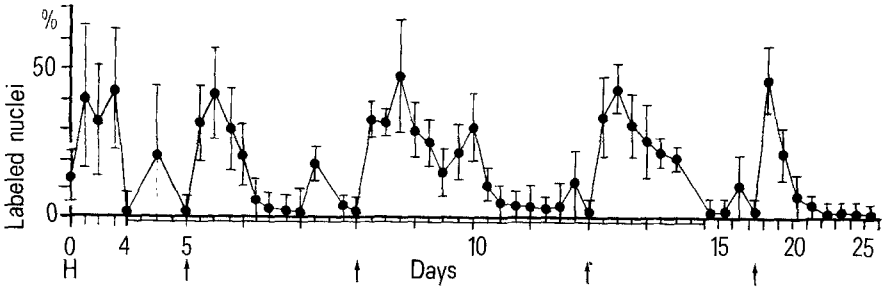

Figure 1. Index of kinetics of ^{3}HTdr pulse myofiber labeled nuclei in *Bombyx mori* larvae of stages I to V. Abscissae: age of larvae (days). Interval from 5 to 14 days is divided into 6-h intervals (scale is extended 4-fold). H, hatching of larvae from the egg; arrows: molting of larvae. Ordinates: number of ^{3}HTdr labeled nuclei (in %).

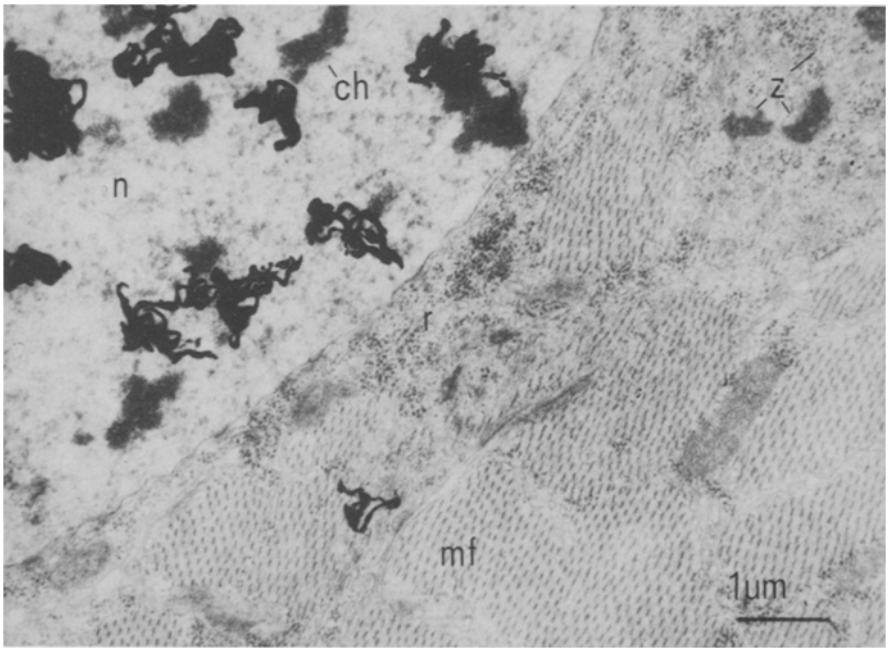

Figure 2. Labeled nucleus within myofiber fragment of a *Bombyx mori* larva. 2 h after ^{3}HTdr injection. mf, myofilaments surrounded by ribosomes (r); z, Z-body; n, nucleus; ch, chromatine.

myosatellites[3] and also of Schwann[4] and tracheal cells[5] it was necessary to localize the DNA synthesizing nuclei within myofibers unequivocally with the aid of EM autoradiography. The analysis of autographs on ultrathin sections gives results which are similar to those obtained by LM autoradiography. 6 h after molting of stage II larvae 42% of nuclei in myofibril-containing myofibers are labeled with ^{3}HTdr. In myonuclei the ^{3}HTdr label is localized preferentially above blocks of more condensed chromatin (fig. 2). The nucleoli unlabeled as a rule, but nucleolar associated chromatin is frequently labeled. The ultrastructure of the myofiber regions in the vicinity of both labeled and unlabeled myonuclei is practically the same. This concerns the structure and the degree of differentiation of myofibrils, on the one hand, and the T-system, sarcoplasmic reticulum and mitochondria, on the other. Sarcomeres in both cases display A and I bands and Z-discs, each thick myofilament being surrounded by 9–12 thin threads. Mitoses of myonuclei have never been observed. Together with the progressive increase in the nuclear size this may be indicative of polyploidization of some of the myonuclei.

The results obtained have proved unequivocally the localization of DNA-synthesizing nuclei within the myofibers proper of the *Bombyx mori* larvae. As seen from the LI kinetics (fig. 1) the DNA synthesis in muscle fibers in accomplished cyclically, which seems to be associated with fluctuations of the hormonal content during larval development.

Thus, unlike the somatic myogenesis in vertebrates[8], and in a similar way to cardiac myocyte[9] or smooth muscle cell[10] differentiation, DNA synthesis and myofibrillogenesis are compatible in developmental myofibers of the silkworm. This may be regarded as a distinguishing peculiarity of myogenesis in holometabolic insects and raises the question of the further fate of DNA-synthesizing muscle nuclei.

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