not accounted for by a loss of calmodulin sensitivity nor to a drop in Ca^{2+} affinity and thus must be due to a lowering of the turnover rate of the pump, or more likely to a reduction in the number of pump sites per cell. A drastic increase of the Ca^{2+} affinity at the external membrane surface (inside IOVs) can be ruled out as a cause from the high steady state IOV Ca^{2+} concentration reached in cows' vesicles (fig. 1B).

The leak flux for Ca^{2+} in cattle cells has not been measured very accurately, but it seems to be extremely low[7], so that it is not surprising that a rather ineffective Ca-pump suffices to keep the intracellular Ca^{2+} concentration at a level which is not hazardous to cellular function.

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The effect of DDT on DNA replication in the larval salivary gland cells of *Drosophila melanogaster*

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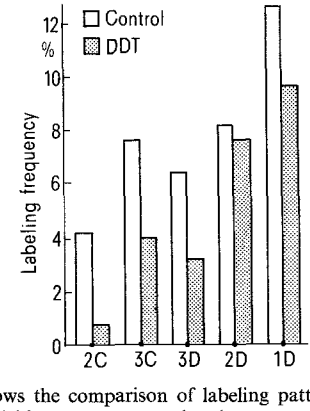
Summary. The inhibitory effect of DDT on the initial stage of the DNA replication process in polytene chromosomes of larval salivary gland cells of *Drosophila melanogaster* was investigated and possible mechanisms for the inhibition are discussed.
Key words. Drosophila melanogaster; salivary gland, larval; DNA replication; DDT; chromosomes, polytene.

Earlier reports on the Dipteran polytene chromosomes confirmed the replication process to be a temporally ordered sequence[1-4]. It has also been reported that some antibiotics[4,5] and toxic substances affect the sequential order of DNA replication. Here an attempt is made to determine how DDT (a pesticide) affects the DNA repliction in polytene chromsomes of third instar larval salivary gland cells of *Drosophila melanogaster*.

Materials and methods. In the experiments, the Oregon strain of *Drosophila melanogaster* was used. The stocks were obtained from the Biology Institute of Hacettepe University, Ankara. The salivary glands of the larvae were excised under Drosophila Ringer solution (pH = 7.2). One of the glands dissected from each larva was incubated in a drop of Drosophila Ringer containing ^{3}H-thymidine (1.0 mCi/ml, specific activity 42 Ci/mMol., Radiochemical Centre, Amersham, England) for 10 min as a control, and the other gland was first treated with 0.25 ppm DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane][6] solution for 5 min then incubated in a drop of Drosophila Ringer containing ^{3}H-thymidine, for 10 min (experimental group). DDT was dissolved in acetone, and control experiments did not show any effect of acetone on DNA replication. Later, each of the batches was fixed, stained with lactic-acetic orcein[7] and squashed on slides. These slides were carried through the routine autoradiographic procedures[8].

The differences in labeling frequency of the nuclei between the control and experimental groups was tested with Student's t-test[9].

Results. In both experimental and control slides, those polytenic cells in S-phase of DNA replication were labeled with ^{3}H-thymidine. The polytene chromosomes in the third instar larval salivary gland cells showed five types of labeling pattern with ^{3}H-thymidine. These are, in sequence; 2C (light continuous), 3C (heavy continuous), 3D (heavy discontinuous), 2D (middle discontinuous) and 1D (lightly discontinuous) labeling patterns. It has generally been accepted[10,2,5,11] that these patterns represent the initial stage (2C), middle stage (3C-3D-2D) and the terminal (1D) stage of the DNA replication cycle. In the control group, a total of 492 nuclei have been examined and 195 out of these were seen to be labeled (table). Therefore, the labeling frequency of the controls with ^{3}H-thymidine was calculated to be 39.6%. A detailed analysis of the frequencies of various labeling patterns was as follows: 4.3% 2C, 7.7% 3C, 6.5% 3D, 8.5% 2D and 12.6% 1D. On the other hand, 494 nuclei were examined in the experimental group and only 123 (24.8%) of these were labeled with ^{3}H-thymidine. In the



The figure shows the comparison of labeling patterns with ^{3}H-thymidine of the nuclei in DDT-pretreated and nontreated salivary gland cells of *Drosophila melanogaster*.

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