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EFFECT OF MALATHION ON THE INITIATION AND ELONGATION STEPS OF TRANSCRIPTION *

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In isolated cell nuclei of pig thymus malathion, *S*-1,2-*bis*(ethoxycarbonyl)-ethyl-*O,O*-dimethyldithiophosphate inhibited both initiation and elongation of all three classes of nuclear RNA polymerases; proflavin was used as an inhibitor of initiation, and actinomycin, as an inhibitor of elongation.

Malathion (S-1,2-*bis*(ethoxycarbonyl)ethyl *O,O*-dimethyldithiophosphate), a phosphoorganic insecticide, is known to affect RNA synthesis in eukaryotic cells. It alters RNA synthesis in the phytohaemagglutinin-stimulated lymphocytes [1, 2]; inhibitis all three types of eukaryotic RNA polymerases in isolated cell nuclei [3], lowers synthesis of all types of RNA in human lymphocytes and isolated calf thymus nuclei [3, 4] and affects transcriptional activity of chromatin [5]. It was demonstrated that malathion affects initiation step of transcription in isolated pig thymus cell nuclei [6].

The aim of the present work was to establish specificity of the inhibitory effect of malathion on transcription.

MATERIALS AND METHODS

Transcriptionally active cell nuclei were isolated from pig thymus by the modified method of Allfrey & Mirsky [3]. Purity of the nuclei was checked under a contrast-phase microscope after staining with Unna. The nuclei were suspended in the buffer consisting of: 12% glycerol, 0.1 mM EDTA, 5 mM $MgCl_2$, 50 mM Tris/HCl, and 5 mM dithiothreitol, pH 7.8, at a density of 10^6-10^7 nuclei/ml of the buffer. The nuclei were preincubated for 1 h at 37°C

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with proflavin, and α-amanitin, or with actinomycin D at concentration of 20 μg, 5-20 μg and 200 μg/ml of cell nuclei suspension, respectively. After preincubation, cell nuclei were incubated for 1 h at 37°C with malathion (40, 70 or 100 μg/ml of cell nuclei suspension). Transcription was measured in 50 mM Tris/HCl buffer, pH 7.8, containing 12% glycerol, 0.1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.1 mM UTP, 4 μCi [^{3}H]UTP. Transcription was started by the addition of 100 μl of cell nuclei suspension to 250 μl of the above transcription mixture. The reaction was carried out at 37°C for 20 min, and stopped by adding 2 ml of 10% trichloroacetic acid on ice. The acid-insoluble fraction was sedimented on Millipore HA 0.45 μm filters, then washed with 100 ml of 3% trichloroacetic acid and ethanol. The filters were dried, placed in vials containing 5 ml of Scintiol [3], and the radioactivity was measured in the Beckman scintillation counter.

RESULTS AND DISCUSSION

Proflavin, an acridine intercalating DNA [7], and an inhibitor of initiation process in the isolated thymus cell nuclei inhibited incorporation of [^{3}H]UTP by about 30% (31.8% ± 6.6%), whereas malathion by about 50% (51.2 ± 7.2%) (Fig. 1). The same decrease of [^{3}H]UTP incorporation was observed when the nuclei were first preincubated with proflavin and then incubated with

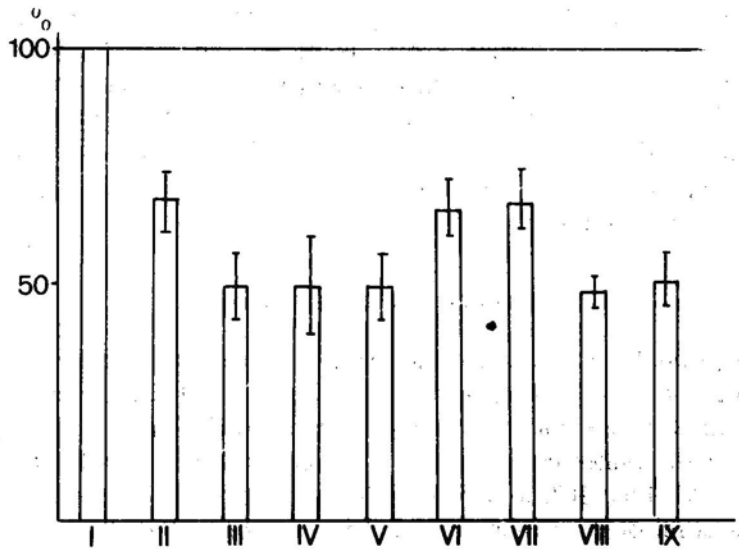

Fig. 1. Inhibition of RNA synthesis by proflavin and malathion in the isolated nuclei from pig thymus cells, mediated by α-amanitin where indicated, I, Control; II, proflavin; III, malathion; IV, proflavin, malathion; V, malathion, proflavin; VI, proflavin, α-amanitin (5 μg); VII, proflavin, α-amanitin (200 μg); VIII, proflavin, malathion, α-amanitin (5 μg); IX, proflavin, malathion, α-amanitin (200 μg)

malathion (Fig. 1, IV and V), or *vice versa*. This suggest that malathion acts not only as an inhibitor of initiation but also inhibits further steps of transcription (Fig. 1). In the cell nuclei in which the initiation step had been inhibited with proflavin and the activity of RNA polymerases II with α-amanithin (Fig. 1) RNA synthesis was lowered by about 30% (33.5±6.1%) and remained practically unchanged (34.4±7%) on the additional inhibition of polymerase III. In both cases (Fig. 1) inhibition of [^{3}H]UTP incorporation by malathion was about 50% (51.0±2.7% and 48.7±11.2%, respectively).

It may be assumed that in the isolated cell nuclei used as a model, transcription of some genes occur which were initiated prior to inhibition, were non susceptible to proflavin, but susceptible to malathion acting on further transcription steps. It is known from our previous results [3] that malathion affects synthesis of polymerases resistant to α-amanitin [3]. In the presence of actinomycin D, an inhibitor of elongation, [^{3}H]UTP incorporation was inhibited to 40% of the control value. Malathion at the concentration of 40 μg/ml was ineffective whereas higher concentration (70 and 100 μg/ml) applied alone or together with actinomycin led to a more pronounced decrease of [^{3}H]UTP incorporation (Fig. 2).

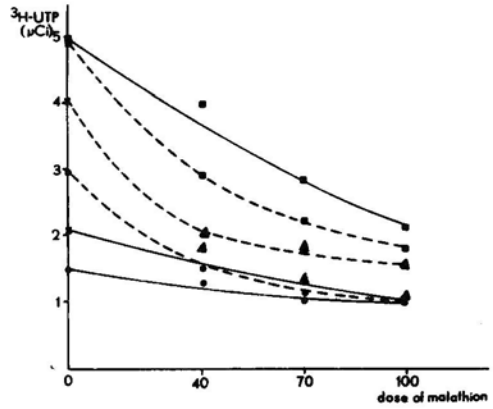

Fig. 2. Effect of malathion and actinomycin D on RNA synthesis in the pig thymus cell nuclei. Actinomycin treatment preceded addition of malathion. - - -, Malathion; ——, malathion and actinomycin D; ●, ■, ▲, series of the experiment

Actinomycin D inhibits the elongation step by intercalating between GC pairs whereas malathion preferentially methylates guanine [9]. Thus it may be assumed that both inhibitors act on the same sites of DNA molecule. Since, however, proteins can undergo intense methylation one can not exclude a nonspecific more general action of malathion on the structure of the enzymes involved in cellular metabolism [10-13].

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