

LEVELS OF DNA SYNTHESIS IN EHRlich'S ASCITES TUMOR CELLS IN CULTURE, TREATED WITH DIFFERENT CONCENTRATIONS OF A CHALONE-CONTAINING PREPARATION

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The effect of the overwhelming majority of biologically active substances is now known to be dose-dependent. Chalone is of great interest from this point of view because they are directly involved in the regulation of reproduction of cells, including tumor cells [3].

Dose-dependence relative to its action on the kinetics of culture density and mitotic activity was established previously [6] for the chalone-containing preparation (CCP) from Ehrlich's ascites tumor (EAT) cells. Dose dependence of the effect of CCP on the level of DNA synthesis in EAT in culture has not yet been investigated. The investigation described below was carried out to study this problem.

EXPERIMENTAL METHOD

EAT was maintained by periodic passage through noninbred male albino mice. A culture of EAT cells served as the test object. For the experiments, EAT cells were extracted from the peritoneal cavity of a mouse on the 9th day after transplantation. The cells were cultured by the method in [2].

To stabilize its pH, HEPES was added to the culture in a final concentration of 20 mM. CCP was obtained by the method of alcoholic fractionation [4]. The protein concentration in the solution of CCP was determined by Lowry's method [7]. To determine the level of DNA synthesis the culture was treated with ^3H -thymidine ($1.5 \mu\text{Ci/ml}$) 4 h after addition of CCP. The action of CCP was assessed 5 h after its addition to the culture. For this purpose DNA was precipitated on nitrocellulose filters (pore diameter 250μ) and incorporation of thymidine was estimated by liquid-scintillation radiometry on a "RackBeta" counter (LKB, Sweden). The significance of the difference between parameters was calculated by Student's test. Differences were considered to be significant at the $p \leq 0.05$ level.

EXPERIMENTAL RESULTS

The chalone-containing preparation had a marked inhibitory effect on DNA synthesis in the EAT culture. This effect is distinctly dose-dependent (Table 1). Small doses of CCP (under $140 \mu\text{g/ml}$) had a sufficiently weak effect on DNA synthesis in the tumor cells (Fig. 1). A further increase in CCP concentration led to marked potentiation of its inhibitory action by 91-98%.

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TABLE 1. Incorporation of ^3H -Thymidine by EAT Cells in Culture During Exposure to Different Concentrations of CCP

Final concentration of CCP in culture, μg protein/ml	cpm/1000 cells ($\text{M} \pm \text{m}$)	Percent inhibition compared with control
0	26.5 ± 1.0	
14	24.9 ± 0.93	7.1 ($p > 0.05$)
20	22.1 ± 1.7	17.5 ($p > 0.05$)
28	21.1 ± 1.0	21.3 ($p > 0.05$)
47	19.8 ± 1.5	26.1 ($p > 0.05$)
70	20.1 ± 0.6	24.6 ($p > 0.05$)
140	11.4 ± 0.6	57.5 ($p < 0.05$)
280	2.4 ± 0.4	91.0 ($p \ll 0.01$)
700	0.4 ± 0.1	98.0 ($p \ll 0.01$)
1400	0.7 ± 0.1	97.0 ($p \ll 0.01$)
2600	0.3 ± 0.1	98.0 ($p \ll 0.01$)
3300	0.3 ± 0.1	98.9 ($p \ll 0.01$)

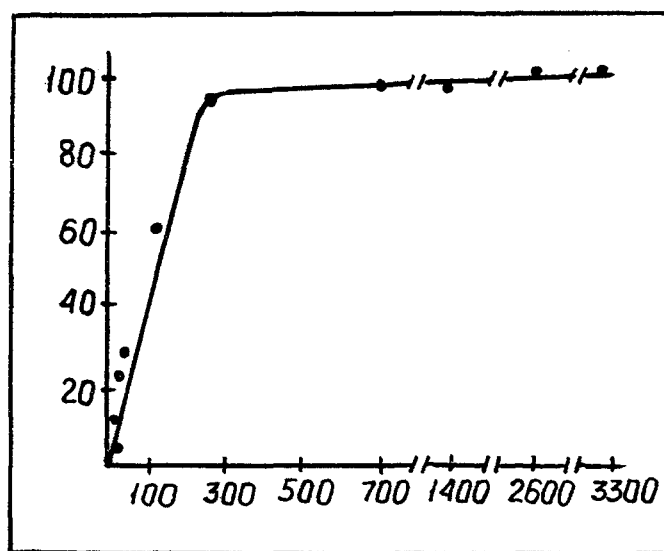


Fig. 1. Inhibition of DNA synthesis in culture of Ehrlich's ascites tumor cells exposed to different concentrations of CCP. Abscissa, CCP concentration in culture (μg protein/ml). Ordinate, % inhibition of DNA synthesis.

Data in the literature on dose-dependence of the effect of various biologically active substances (including chalcones) are quite diverse and contradictory [1, 5, 8]. In particular, the dose-effect curve of biologically active peptides has been shown to be dome shaped, i.e., starting from a certain time an increase in dose leads to weakening of the effect [1].

Considering existing views on the mechanism of action of chalcones, which assume binding of chalone molecules with specific receptors [1, 3], the existence of a saturation effect for their dose-dependence might be expected.

Our results described above fully support this hypothesis.

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