EFFECT OF CHOLESTERYL 14-METHYLHEXADECANOATE ON THE RNA POLYMERASE ACTIVITY OF RAT LIVER NUCLEI *IN VIVO* AND *IN VITRO*

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1. Introduction

Cholesteryl 14-methylhexadecanoate (CMH) affects the activity of several enzymes required for protein synthesis. It enhances *in vitro* the charging of tRNA with amino acids [1] and evidence has been presented that this ester is probably a normal constituent of aminoacyl-tRNA synthetases and essential for their normal function [2]. The same holds true for peptide elongation factors in rat liver [3]. The activity of aminoacyl-tRNA synthetases is also affected by the administration of CMH to living animals [4]. Moreover, administration of this compound to rats is followed by changes in ribosomal peptide synthesis indicating that not only the translation but also the transcription of genetic information may be affected by CMH [5].

The DNA-dependent RNA polymerase (nucleoside-triphosphate: RNA nucleotidyl transferase, E.C.2.7.7.6.) is the key enzyme involved in the expression of the genetic information. Multiple forms of this enzyme apparently exist in nuclei of eukaryotic cells, some of them synthesizing mRNA while the others produce other species of RNA [6]. These different enzymes seem to be bound to different subcellular structures [7].

In the present experiments the effect of CMH was tested on the RNA polymerase activity of solubilized rat liver nuclei and evidence is presented suggesting that the ester affects this enzymatic activity in a similar way to that of enzymes required for translational processes [3, 5].

2. Materials and methods

Wistar rats were divided into experimental groups and administered 1 mg of CMH as described elsewhere [4]. In *in vitro* experiments CMH was added into incubation mixtures as in our earlier experiments [2]. Nuclei from rat liver were isolated and solubilized by the method of Mertelsmann [8] and their RNA polymerase activity assayed as described by Kaufmann [9]. The Student's *t* test was used for the evaluation of statistical significance of differences between experimental and control animals of the same group.

3. Results

3.1. *Changes of RNA polymerase activity after CMH administration*

Administration of CMH to rats was followed by an enhancement of RNA polymerase activity in liver nuclei. This stimulation became significant at 24 hr after the injection. After this period, a gradual decrease of the enzymatic activity was found and at 48 hr after the administration of CMH the RNA polymerase activity in the liver of experimental animals was significantly lower than that in control rats. The enzymatic activity returned to normal values on the third day after the injection of CMH. A summary of these results is presented in table 1.

Table 1
RNA polymerase activity in the liver of rats administered 1 mg of CMH.

Time after injection (hr)	Enzymatic activity (mean ± S.D.)	p^a
12	3.37 ± 0.161	0.3
24	5.30 ± 0.400	0.05
36	4.72 ± 0.018	0.8
48	1.74 ± 0.296	0.001
72	4.34 ± 0.700	0.7

Incubation mixtures for the assay of RNA polymerase activity contained in a final volume of 0.1 ml 50 pmoles of ^{3}H-UTP, 2.0 mg of rat liver nuclei and all other components described by Mertelsmann [8] and Kaufmann [9]. The enzymatic activity is expressed as pmoles of ^{3}H-UTP incorporated into RNA under standard assay conditions. In the control mixtures 4.08 ± 0.086 pmoles of UTP were incorporated.
[a] When compared with the control values of the same experimental series [4].

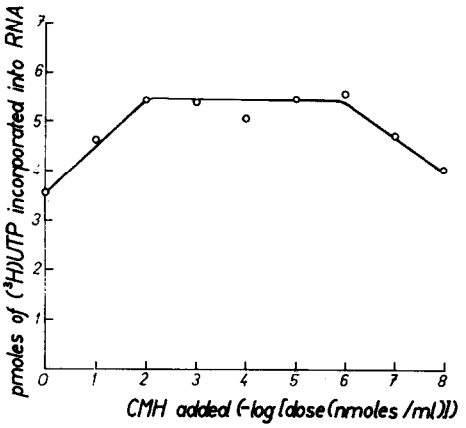

Fig. 1. Effect of different quantities of CMH added into incubation mixtures on the activity of RNA polymerase.

3.2. *Stimulation of RNA polymerase by CMH in vitro*

When added into incubation mixtures for RNA polymerase assay, CMH significantly stimulates the activity of this enzyme. This effect is dependent upon the quantity of the ester added. The most effective dose apparently lies in the range of $10^{-3}-10$ pmoles of CMH/ml (fig. 1).

The enzyme saturation curve in systems containing limiting quantities of UTP indicates that the saturation level lies significantly higher in mixtures containing CMH and that larger amounts of this nucleotide are utilized for RNA synthesis in the presence of the ester (fig. 2).

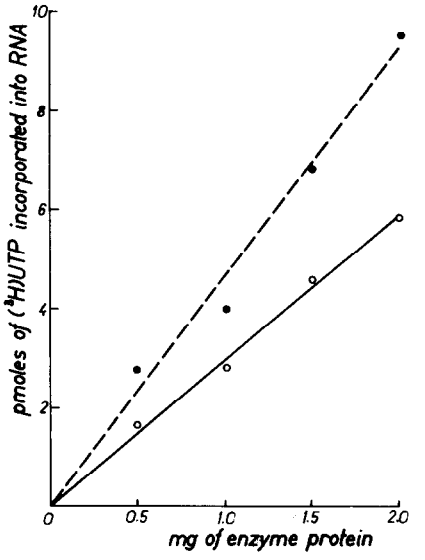

Fig. 2. Enzyme saturation curves for incubation mixtures without CMH (o——o) and with CMH added (•– – –•) (1 pmole/ml of incubation mixture). Mixtures contained limiting quantities of ^{3}H-UTP (50 pmoles) and all other components described in legends for table 1.

4. Discussion

Similarly, as with other enzymatic systems required for protein synthesis tested so far [4, 5], the early effect of CMH administration to rats is an enhancement of RNA polymerase activity in their liver which is followed by a period of decreased enzymatic activity afterwards. This biphasic alteration of enzyme activities seems to be characteristic for CMH.

The early stimulation of RNA polymerase activity may be induced by an accumulation of the administered amount of CMH in the liver tissue. Such an accumulation in certain subcellular fractions has been demonstrated for pH 5 enzymes and cell sap in our earlier experiments [4, 5]. On the other hand, the subsequent inhibition of enzymatic activities may be due to a depletion of CMH in these subcellular frac-

tions. An accumulation of unusually high quantities of CMH following the administration of this substance may probably inhibit the normal synthesis of this compound and lead by this mechanism to a decrease of CMH in certain subcellular fractions.

Unlike the other enzymatic systems tested so far [1–3], where the stimulating effect of CMH was strictly dose-dependent, a rather broad range of CMH doses stimulates the activity of RNA polymerase.

The stimulation of RNA polymerase activity by CMH may have important implications for the whole protein synthesis. This enzyme synthesizes mRNA and the availability of this RNA species may obviously represent one of the rate-limiting steps in protein synthesis. Further experiments seem to be required for the elucidation of the possible regulatory role of CMH in the transcription of genetic information.

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