# CHANGES OF AMINOACYL-tRNA SYNTHESIS IN THE LIVER OF RATS ADMINISTERED CHOLESTERYL 14-METHYLHEXADECANOATE

E. KOMÁRKOVÁ and J. HRADEC
*Department of Biochemistry, Oncological Institute, Prague 8, Czechoslovakia*

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## 1. Introduction

Cholesteryl 14-methylhexadecanoate (CMH) stimulates the activity of some enzymes required for protein synthesis in vitro [1]. This stimulating effect on enzymes present in the soluble fraction of the cell has been investigated in our laboratory during the past years [2, 3]. Evidence has been obtained that the presence of this compound in the molecules of aminoacyl-tRNA synthetases [4] as well as in peptide elongation factors [5] may be essential for the normal activity of these enzymes.

However, there is a complete lack of knowledge whether administration of this cholesteryl ester to living animals affects the activity of enzymes required for protein synthesis that are present in the cell sap. Such an effect would be expected with a compound that is claimed to participate physiologically in protein synthesis [4].

This paper describes the effect of CMH administration to rats on the activity of leucyl-tRNA synthetase in the liver tissue, and on the quantitative distribution of this cholesteryl ester in the pH 5 enzyme fraction.

## 2. Materials and methods

Random-bred Wistar rats of both sexes, kept on a standard diet and weighing 150–180 g, were injected intraperitoneally with 0.1 ml of olive oil containing quantities of CMH as indicated. Control rats were injected with the same volume of olive oil only. Animals were killed at intervals indicated. Each experimental series consisted of 3 control and 5 experimental rats and experimental groups used for the study of enzymic activities after various intervals involved 3–4 such series. Rat liver pH 5 enzymes were prepared by our usual procedure [6]. Enzyme preparations of one experimental series were incubated at the same time. Incubation mixtures contained 0.05 M tris-HCl, pH 7.5; 5 mM $MgCl_2$; 2.5 mM ATP; 1 mM 2-mercaptoethanol; 200 $\mu$g of rat liver tRNA (prepared as described by Brunngraber [7]; 1 mg of pH 5 enzyme protein; and 555 $\mu$moles of $^{14}$C-L-leucine (90 mCi/mmole) in a total volume of 0.4 ml. After incubation at 37° for 10 min the reaction was terminated by the addition of ice-cold 5% trichloroacetic acid (TCA). The TCA-insoluble fraction was collected on nitrocellulose filters and the radioactivity was assayed by liquid scintillation counting. Quantitative determination of CMH in pH 5 enzymes was done by the method of Hradec [8]. 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. *L-Leucyl-tRNA synthetase activity*

Administration of CMH to rats was followed by a sudden drop of synthetase activity in their liver tissue. Significantly lower activities of this enzyme were found 24 hr after the injection of 10 (table 1) or 100 $\mu$g of CMH (table 2). After administration of 1 mg of CMH, the lowest enzymic activity was found 12 hr after the injection ($p < 0.001$) (fig. 1).



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Table 1
Activity of L-leucyl-tRNA synthetase in the liver of rats administered 10 μg of CMH.

| Time after injection (hr) | Enzymatic activity (Mean ± S.D.) | $p$[a] |
|---|---|---|
| 12 | 266.5 ± 19.2 | 0.001 |
| 24 | 52.9 ± 13.1 | 0.001 |
| 36 | 149.2 ± 20.6 | 0.5 |
| 48 | 214.2 ± 40.4 | 0.1 |
| 72 | 125.5 ± 7.9 | 0.4 |

The enzymatic activity is expressed as pmóles of $^{14}$C-L-leucine incorporated into the TCA-insoluble portion of the incubation mixture under standard assay conditions. In the control mixtures 151.1 ± 9.0 pmoles of L-leucine were incorporated.

[a] When compared with the control values of the same experimental series.

Table 2
Activity of L-leucyl-tRNA synthetase in the liver of rats administered 100 μg of CMH.

| Time after injection (hr) | Enzymatic activity (Mean ± S.D.) | $p$ |
|---|---|---|
| 12 | 126.9 ± 9.6 | 0.3 |
| 24 | 63.7 ± 20.0 | 0.001 |
| 36 | 112.2 ± 7.9 | 0.2 |
| 48 | 498.6 ± 71.8 | 0.02 |
| 72 | 185.8 ± 21.6 | 0.1 |

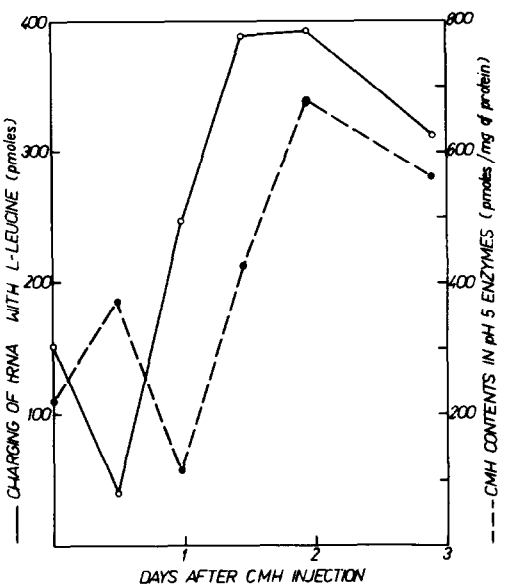


Fig. 1. Effect of CMH administration on the activity of L-leucyl-tRNA synthetase and CMH contents in pH 5 enzymes of rat liver. Animals were administered 1 mg of CMH and the activity of the synthetase (o) was determined by the standard assay procedure. CMH contents (•) were estimated by the method of Hradec [8]. In control mixtures (from rats administered olive oil only) 151.1 ± 9.0 pmoles of $^{14}$C-L-leucine were incorporated into the TCA-insoluble fraction of the sample; pH 5 enzymes from control rats contained 219 ± 65 pmoles of CMH/mg of protein.

During the period 36–72 hr after administration of CMH, the activity of leucyl-tRNA synthetase increased to values which were significantly greater than those found in the preceding period. However, only after the administration of 1 mg of CMH was the synthetase activity significantly higher ($p < 0.001$) than that of control animals of the same group; the L-leucyl-tRNA synthetase activity remained significantly increased ($p < 0.01$) even 72 hr after the administration of 1 mg CMH. With lower doses of CMH, enzymic activity after 72 hr was not significantly enhanced.

3.2. *CMH content in pH 5 enzymes*

The administration of olive oil to rats was followed by no significant changes in CMH content of pH 5 enzymes. Administration of CMH resulted in a significant increase of CMH content in this fraction ($p < 0.05$) at 12 hr after the injection. At 24 hr after administration, the CMH level in pH 5 enzymes of experimental rats was not significantly different from that in control animals ($p < 0.2$). However, 36–72 hr after CMH administration, a significant increase of CMH content in pH 5 enzymes was observed ($p < 0.02$ (fig. 1).

Neither administration of olive oil nor CMH induced any changes in the CMH content of whole liver tissue during the entire period of the experiment.

## 4. Discussion

There seems to be a correlation between the activity of L-leucyl-tRNA synthetase and the relative CMH content in pH 5 enzymes even though no purified enzymes were used in this study. Administration of CMH induced no changes in the content of this compound in the whole liver tissue. This may indicate

that the action of CMH is directed to specific subcellular fractions. This is not surprising since a strict specificity may exist between the chemical structure and the activity of cholesteryl esters in protein synthesis [9].

The initial increase of CMH content in pH 5 enzymes probably represents an accumulation of this compound into this fraction. The decrease of enzymic activity at this stage may be due to the presence of relatively high quantities of CMH, which have been shown to partially inhibit aminoacyl-tRNA synthesis [4]. Such an explanation cannot explain the increase of both the CMH content and the synthetase activity at later periods. It must be concluded that the administration of CMH alters the metabolism of this compound physiologically present in animals. However, nc definite conclusions in this respect can be reached until more about the metabolism of this compound in animals is known.

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**References**

[1] J. Hradec, Biochim. Biophys. Acta 47 (1961) 149.
[2] J. Hradec and L. Dolejš, Biochem. J. 107 (1968) 129.
[3] J. Hradec and Z. Dušek, Biochem. J. 110 (1968) 1.
[4] J. Hradec and Z. Dušek, Biochem. J. 115 (1969) 873.
[5] J. Hradec, Z. Dušek, E. Bermek and H. Matthaci, Biochem. J., in press.
[6] J. Hradec, Biochem. J. 105 (1967) 251.
[7] E.F. Brunngraber, Biochem. Biophys. Res. Commun. 8 (1962) 1.
[8] J. Hradec, J. Chromatog. 32 (1968) 511.
[9] J. Hradec and J. Sommerau, FEBS Letters 9 (1970) 161.