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# Alteration in plasma and cellular enzyme and protein levels after lethal and non-lethal doses of cycloheximide in the rat

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The antibiotic cycloheximide, which inhibits cytoplasmic protein synthesis in a wide variety of mammalian systems both *in vitro* and *in vivo* [1], has been employed extensively in studies of metabolic regulation in mammalian tissues. Most studies with intact rats have been conducted over a short time period (several hr) and with lethal doses (5–200 mg/kg, body wt) [2–4]. Long-term metabolic changes after administration of the inhibitor have not been studied extensively [2, 4]. With lethal doses of cycloheximide, Young *et al.* [2] observed changes in blood parameters suggesting severe metabolic acidosis; the animals exhibited gastrointestinal dysfunction, internal bleeding and hypotension, and death occurred between 9 and 12 hr. Daskal *et al.* [5] observed with doses of 5–200 mg/kg that both the cytoplasm and nuclei of rat liver cells were markedly affected with the formation of perichromatin granules in the nucleus and compact lamellar complexes in the cytoplasm [5]. Ultra-structural changes induced by lethal doses of cycloheximide were dose- and time-dependent.

With doses of cycloheximide less than 2.7 mg/kg body wt, which is the $LD_{50}$ in rats [6], Verbin *et al.* [7] and Hwang *et al.* [8] reported that the inhibition of protein synthesis at 2 hr after administration was associated with ultra-structural changes in liver cells, but no necrotic lesions. Membranous whorls induced by cycloheximide decreased in both size and frequency by 6–12 hr and essentially disappeared by 36 hr, after which cytoplamic organization seemed relatively normal [8]. These results indicate that at sublethal doses of cycloheximide the structural alterations were minimal and reversible [7].

We have reported that in rats with sublethal doses of cycloheximide there was a stimulatory phase of protein synthesis following the inhibition [9], with differences in the degree of stimulation between proteins synthesized by free and membrane bound polysomes, suggesting a specific adaptation to the effect of the inhibitor.* Thus, it was considered valuable to investigate the effect of lethal and non-lethal doses of cycloheximide on changes in specific cellular enzymes and the protein concentration in plasma and liver to evaluate possible tissue alterations which would lead to a specific adaptive change in protein synthesis. The enzymes selected were aspartate aminotransferase (AAT) (EC 2.6.1.1.), creatine phosphokinase (CPK) (EC 2.7.3.2.), and lactate dehydrogenase (LDH) (EC 1.1.1.27).

All studies were performed on 210 ± 10 g male Wistar rats, fed and watered *ad lib.* Animals were always sacrificed at 10:00 a.m. to avoid possible diurnal variations. Cycloheximide (Sigma Chemical Co.) in saline was administered i.p. at zero time. At various times after drug administration, rats in groups of two were anesthetized with ether and blood was drawn by cardiac puncture. Hemolyzed plasma was always discarded. After exsanguination, a 6-g sample of liver was used to prepare the cytosol fraction (supernatant of the 105,000 $g_{max}$ centrifugation). Enzymes were determined using automated procedures according to Technicon Methodology (Technicon Instruments Corp., Tarrytown, N.Y.). Plasma LDH isozymes were determined by the method of Davis [10]; protein was determined by the method of Lowry *et al.* [11] using crystalline bovine serum albumin as the standard. Plasma albumin and fibrinogen were separated by paper electrophoresis (Spinco model R Paper Electrophoresis, Beckman Instruments, Inc., Fullerton, Calif.). All enzyme activities were expressed in International Units (I.U.), which is that amount catalyzing the transformation of 1 μmole substrate/min at 37°. Plasma enzyme activities are expressed as I.U./liter and liver enzyme activities as I.U./g of tissue.

Alterations in plasma and cytosol levels of AAT, CPK and LDH 2 hr after administration of various doses of cycloheximide are presented in Table 1. Previous studies

* J. J. Ch'ih and T. M. Devlin, manuscript in preparation.

Table 1. Changes of plasma and liver cytosol enzyme activities after cycloheximide administration*

| Time post cycloheximide (hr) | Dosage of cycloheximide (mg/kg) | N | Plasma | | | Liver cytosol | | |
|---|---|---|---|---|---|---|---|---|
| | | | AAT | CPK (I.U./liter plasma) | LDH | AAT | CPK (I.U./g liver) | LDH |
| Control | 0 | 9 | 64 ± 6 | 349 ± 21 | 108 ± 18 | 29 ± 1 | 13.5 ± 0.3 | 153 ± 10 |
| 2 | 2 | 10 | 83 ± 6† | 488 ± 66† | 100 ± 20 | 26 ± 2 | 12.9 ± 0.8 | 147 ± 13 |
| 2 | 5 | 4 | 151 ± 23† | 655 ± 85† | 175 ± 43† | 28 ± 3 | 12.2 ± 0.7 | 147 ± 15 |
| 2 | 10 | 4 | 183 ± 12† | 794 ± 100† | 300 ± 44† | 19 ± 2† | 13.9 ± 2.5 | 118 ± 18 |
| 2 | 20 | 4 | 300 ± 23† | 1015 ± 67† | 726 ± 115† | 19 ± 3† | 13.1 ± 0.6 | 120 ± 6† |
| Control | 0 | 9 | 64 ± 6 | 349 ± 21 | 108 ± 18 | 29 ± 1 | 13.5 ± 0.3 | 153 ± 10 |
| 1 | 2 | 7 | 112 ± 23† | 587 ± 42† | 162 ± 37 | 32 ± 3 | 12.9 ± 0.6 | 174 ± 8 |
| 2 | 2 | 10 | 83 ± 6† | 488 ± 66† | 100 ± 20 | 26 ± 2 | 12.9 ± 0.8 | 147 ± 13 |
| 12 | 2 | 4 | 99 ± 5† | 395 ± 52 | 170 ± 5† | 43 ± 1† | 16.0 ± 0.5† | 191 ± 14† |
| 24 | 2 | 6 | 107 ± 11† | 406 ± 60 | 144 ± 30 | 30 ± 2 | 16.8 ± 1.5† | 157 ± 27 |
| 48 | 2 | 9 | 87 ± 6† | 398 ± 88 | 156 ± 25 | 30 ± 2 | 13.4 ± 0.5 | 171 ± 11 |

* For experimental details see text. Values presented are the mean ± S.E.M.; N is the number of experiments. Each experiment consisted of two rats. Control values are repeated for convenience in comparing results.

† These values are considered significantly different from controls, with a $P = 0.05$ or less.

have demonstrated that maximum inhibition of protein synthesis occurs at this time. At 2.0 mg/kg of cycloheximide, the plasma AAT and CPK increased by approximately 30 and 40 per cent, respectively, but there was no significant change (P value > 0.05) in the plasma level of LDH or in the cellular levels of the three enzymes. Increasing activities of all three enzymes appeared in the plasma with increasing doses. Plasma AAT, CPK and LDH activities were increased 500, 300 and 700 per cent at 20 mg/kg of cycloheximide, whereas liver cytosol AAT and LDH activities were decreased only 30 and 20 per cent respectively. No apparent change in liver cytosol CPK activity was observed. The rise in plasma CPK suggests damage to tissues other than liver, presumably muscle. Two hr after lethal doses, or 1 hr after a non-lethal dose of cycloheximide, LDH isozyme patterns indicated increases of all fractions (data not presented), confirming cellular damage of other tissues.

Changes in these activities at various times after a single injection of a non-lethal dose of cycloheximide (2 mg/kg), but a dose which inhibits liver and kidney protein synthesis greater than 80 per cent at 2 hr [9], are presented in Table 1. At 1 hr there was a marked increase of plasma AAT and CPK activities (P < 0.05); LDH also appeared to increase but this may not be significant (P < 0.2). The initial elevation of these enzyme levels implies that immediate tissue trauma occurred, releasing intracellular enzyme into the blood circulation. This initial cellular damage leading to loss of these enzymes may be due to the inhibition by cycloheximide of the synthesis of a cellular protein, with a relatively short half-life, required to maintain the integrity of the cell membrane; it is also possible that the inhibitor interferes with other cellular processes leading to an alteration in the permeability of the cell. From 12 to 48 hr post administration of cycloheximide, the plasma levels of the enzymes remained elevated. Liver cytosol enzyme activities, with the exception of AAT, fluctuated but did not show any significant change. Liver AAT activity at 12 hr and the elevated levels of plasma AAT activity observed between 12 and 24 hr were similar to the increase in AAT activity observed with other antibiotics and drugs known to be hepatotoxins (erythromycin, rifampin and tetracycline) or agents causing hepatic sensitivity (penicillin) or both [12]. These phenomena and membranous whorl formation in rat parenchymal cells at 6–12 hr post sublethal cycloheximide administration observed by Hwang *et al.* [8] may be explained as adaptive hypertrophy of the smooth endoplasmic reticulum at the site of biotransformation [13].

Cytosol and plasma protein concentrations were examined at 1–48 hr post cycloheximide (2 mg) administration (Fig. 1). At 12 hr there was an increase in cytosol protein concentration which may be due to a dehydration of the tissue [2]. A slight decrease in total plasma protein and albumin throughout the 48-hr period was observed, but a slight increase of fibrinogen was seen at 12 hr post cycloheximide administration which may be explained by the so-called "acute-phase reaction" [14, 15].

The findings reported here, as well as by others [2–5], suggest that, if lethal doses of cycloheximide are used in the intact rat, it is conceivable that not only the protein synthetic ability but many other biochemical functions of the organism would be altered. With non-lethal doses of cycloheximide, the effects of cycloheximide on cell structure [7, 8], protein synthesis [9] and other biochemical alterations must be followed by a compensatory process of recovery.

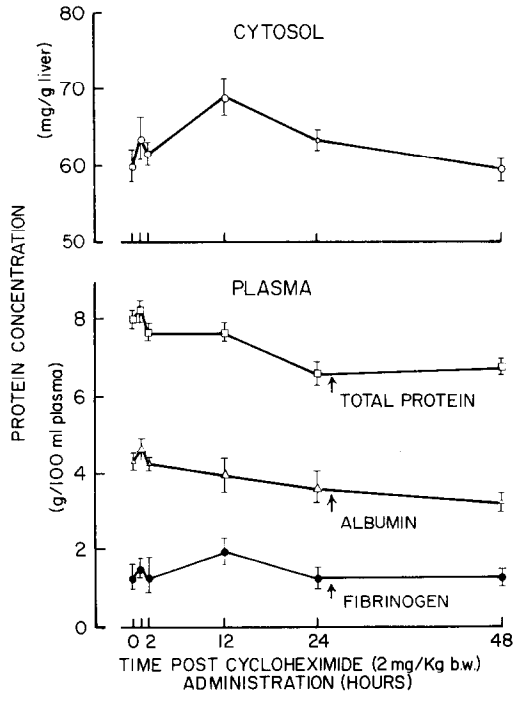


Fig. 1. Changes of plasma and liver cytosol proteins after cycloheximide administration. Proteins were determined at various times after a single injection of a non-lethal dose of cycloheximide (2 mg/kg). For details see text. Values presented are the mean ± S.E.M. of at least four experiments and each experiment consisted of two rats.

*Department of Biological Chemistry, Hahnemann Medical College and Hospital, Philadelphia, Pa.* 19102, *U.S.A.*

JOHN J. CH'IH
DOLORES M. OLSZYNA
THOMAS M. DEVLIN

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