The Effect of Cycloheximide on Translocation and Incorporation of Leucine and Lysine in Duodenal Sacs[1] (34206)

H. TARVER AND E. THOMSON

Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, California 94122

In the present communication we report the effect of cycloheximide (actidione, CHX) on leucine and lysine translocation in sacs prepared from the intestine of rats and on the incorporation of these amino acids into intestinal protein. CHX is a glutaramide antibiotic isolated from extracts of *Streptomyces griseus* which has been shown to inhibit protein biosynthesis in a number of mammalian systems including cell cultures (1), reticulocytes (2), liver proteins (3, 4). It is believed to act at the transfer step in protein synthesis subsequent to the formation of the complex between mRNA, amino acyl-RNA and the ribosome (5). Piperno and Oxender (6) reported results suggesting the requirement for a specific binding protein in the uptake of branched chain amino acids by *E. coli*. Actinomycin, another inhibitor of protein synthesis inhibited amino acid absorption from jejunal loops from rats (7).

Materials and Methods. Everted gut sacs were prepared from the proximal duodenum of 250-g female Sprague-Dawley rats according to the procedures of Wilson and Wiseman (8). The sacs were filled with 0.3 ml of Krebs–Ringer's–bicarbonate buffer (9) with glucose (0.20%) and with either L-leucine or lysine added to a final concentration of 100 μM. The sacs were placed in 25.0 ml of media and incubated for 30 min at 37° in an atmosphere of 95% O_2–5% CO_2. The mucosal fluid differed from the serosal fluid in that the former contained the radioactive amino acid. Each series of experiments consisted of three groups: (A) injected intraperitoneally with 1.0 mg of cycloheximide in 1.0 ml of 0.9% NaCl 60 min prior to sacrifice and sac preparation; (B) injected with the saline vehicle only and cycloheximide (100 μ/ml was added to both the mucosal and serosal fluids; and (C) controls were injected with saline only.

After the incubation, each sac was washed with 100 ml of cold saline and the serosal fluid was flushed out with 10.0 ml of saline. The intestinal tissue was dried to a constant weight and the residue was digested in NaOH by heating at 80° for 30 min. Rates of protein synthesis were estimated from rates of incorporation of ^{14}C-leucine of ^{14}C-lysine (CalBiochem) into extracts of intestinal tissue insoluble in trichloroacetic acid (TCA). Free amino acid levels in the tissue and serosal fluid were estimated in the TCA-soluble phase. The TCA-insoluble material was extracted with ethanolic ether (3:1) and solubilized in 88% formic acid. Both TCA phases were assayed for radioactivity by liquid scintillation. Efficiency was determined by automatic external standardization.

Results and Discussion. Our results show that the incorporation of both leucine and lysine into protein (TCA-insoluble material) of intestinal tissue of rats is inhibited by CHX whether it is present in the medium or is given by injection prior to sacrifice. They are in accord with the results of other investigators mentioned in the introduction which demonstrate that CHX is an inhibitor of protein synthesis and of the translocation of amino acids, a process which likewise involves protein synthesis. However, our results (Table I) are not all in accord with this conclusion. There is an anomalous stimulation of translocation of both leucine and lysine when CHX is present in mucosal and serosal fluid, whereas after intraperitoneal injection

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TABLE I. The Effect of Cycloheximide on Translocation of Leucine and Lysine in Intestinal Sacs and on Incorporation into Protein.[a]

	L-Leucine-^{14}C[b]			L-Lysine-^{14}C[b]		
		Intestinal tissue			Intestinal tissue	
	Serosal fluid TCA soluble	TCA soluble	TCA insoluble	Serosal fluid TCA soluble	TCA soluble	TCA insoluble
Controls[c]	9.0 ± 2.0	83 ± 2	1.9 ± 0.6	7.3 ± 1.8	97 ± 9	4.1 ± 0.3
Antibiotic in medium[d]	13.7 ± 3.5	99 ± 4	0.8 ± 0.0	10.4 ± 0.4	102 ± 13	2.6 ± 0.0
Antibiotic by injection[e]	3.7 ± 0.4	72 ± 3	0.9 ± 0.0	5.1 ± 0.8	73 ± 7	2.1 ± 0.3

[a] Data is given as mμmoles of amino acid translocated and incorporated per 100 mg of dry tissue per 30 min. Five animals were used to obtain each value which is given as the mean ± standard error.

[b] Amino acid concentration: 100 μ*M*.

[c] Controls injected with saline.

[d] Cycloheximide (100 μg/ml in saline) in both mucosal and serosal fluids.

[e] Cycloheximide: 1 mg in 1 ml of 0.9% NaCl injected intraperitoneally 60 min prior to sacrifice and sac preparation.

there is an inhibition of the appearance of both amino acids in the serosal fluid as well as in the TCA-soluble material in the intestinal tissue.

Summary. Cycloheximide (CHX) inhibits the incorporation of leucine and lysine into the proteins of the intestinal tract of rats regardless of whether the CHX is present in the incubation medium or whether it is given by injection to the animals prior to sacrifice. Translocation from mucosa to serosa is also inhibited when CHX is given by injection to the intact animal but when present in the medium it acts as a stimulant. Both amino acids appear more rapidly in serosal fluid and intestinal tissue in the presence of the inhibitor than in its absence.

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