Biochemical Pharmacology, Vol. 17, pp. 1197–1206. Pergamon Press. 1968. Printed in Great Britain

STRUCTURE–ACTIVITY STUDIES WITH CYCLOHEXIMIDE AND CONGENERS

HERBERT L. ENNIS

Laboratory of Microbial Genetics and Physiology,
St. Jude Children's Research Hospital, and
Department of Biochemistry, The University of Tenessee Medical Units,
Memphis, Tenn., U.S.A.

(*Received* 27 *November* 1967; *accepted* 25 *January* 1968)

Abstract—The antibiotic cycloheximide inhibits protein synthesis in animal cells. The effect of a number of derivatives, related compounds and stereoisomers of cycloheximide on protein synthesis in animal cells and in extracts prepared from animal cells, was studied.

Substitution of the oxygen attached to carbon-1 of the cyclohexanone ring or esterification of the hydroxyl group attached to the α-carbon of the hydroxyethyl-glutarimide side chain greatly diminishes inhibitory activity.

The absolute configuration of the cyclohexanone ring is important for activity, but a cyclic ketone is not necessary.

Compounds with a substitution by an acetoxy or hydroxyl group of the hydrogen attached to carbon-4 of the cyclohexanone ring, as in acetoxycycloheximide and streptovitacin A, respectively, inhibit protein synthesis better than cycloheximide.

The results obtained in the present investigation using animal cells are in general agreement with previous studies using yeast cells.

CYCLOHEXIMIDE (β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide), a glutarimide antibiotic produced by *Streptomyces griseus*, inhibits protein synthesis in intact animal and yeast cells and in extracts derived from these cells, but has no effect on bacterial systems.[1–10] The antibiotic inhibits transfer of amino acid from aminoacyl-tRNA to polypeptide. Although cycloheximide has been shown to inhibit the breakdown of polyribosomes[11, 12] and possibly to block the initiation of new polypeptide chains[13] and peptide bond formation,[14] conclusive evidence on the exact mode of action of the antibiotic is still lacking.

Some work has been done to elucidate the structural basis of the inhibition of protein synthesis and growth by cycloheximide in animal cells.[3, 12, 15, 16] However, more work has been done on this subject with yeast.[17–19] The present investigation is concerned with the structure of cycloheximide as it relates to its biological activity. A number of stereoisomers, derivatives, and related compounds were tested for their ability to inhibit protein synthesis in intact animal cells and in cell-free extracts derived from animal cells. The results obtained in the present investigation using animal cells are in general agreement with previous studies using yeast cells,[17] although they differ in some small details.

MATERIALS AND METHODS

Structural properties of compounds tested

A. Cycloheximide and stereoisomers (*see* Fig. 1). *l*-Cycloheximide *(β-[2(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide, melting point (m.p.) 115–117°, possesses four asymmetric centers. The substituents on the cycloheximide ring are 2-equatorial, 4-axial, 6-equatorial.

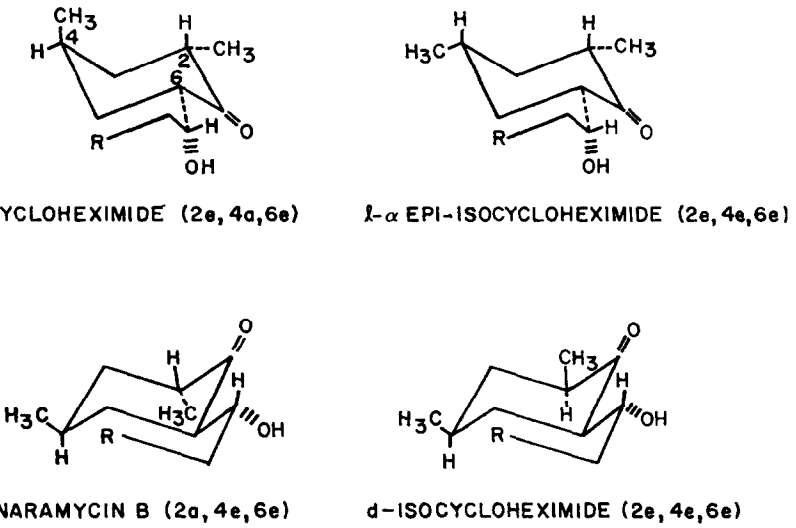


FIG. 1. Conformational structures of *l*-cycloheximide and stereoisomers. R = glutarimide.

d-Isocycloheximide,† m.p. 97–99°, is different from cycloheximide in the absolute configuration of the cyclohexanone ring, and the methyl group at position 4 in the ring is equatorial.

d-Naramycin B,‡ m.p. 112–113°, differs from cycloheximide in the absolute configuration of the cyclohexanone ring, and the methyl group at position 2 is axial and at position 4, equatorial. This preparation contained 68% *d*-naramycin B and 32% cycloheximide.

dl-Neocycloheximide,† m.p. 190°, possesses the substituents on the cyclohexanone ring 2-equatorial, 4-equatorial and 6-axial.

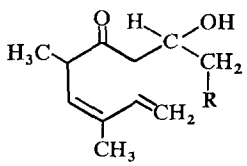
dl-α-Epi-isocycloheximide,‡ m.p. 153°, has the same orientation of groups around the asymmetric centers of the cyclohexanone ring as *d*-isocycloheximide (2e, 4e, 6e). The *l*-isomer differs from *l*-cycloheximide only in the orientation of the methyl group at position 4.

B. Related compounds (*see* Fig. 2). These compounds included: inactone,† m.p. 116°, is a mixture of the two conformations shown in Fig. 2 and contains only two asymmetric centers located at positions 2 and 4, and the ring is unsaturated between

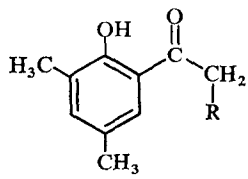
* A gift from Drs. O'Connell and Whitfield, The Upjohn Co., Kalamazoo, Mich.

† A gift from Dr. Johnson, The Dow Chemical Co., Wayland, Mass.

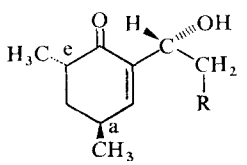
‡ A gift from Dr. Siegel, University of Kentucky, Lexington, Ky.



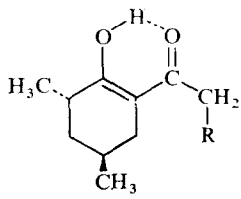
Streptimidone



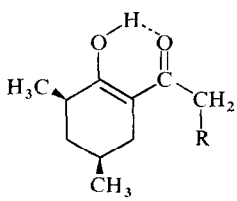
Actiphenol



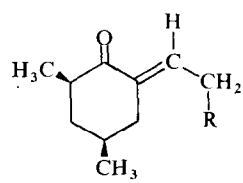
Inactone



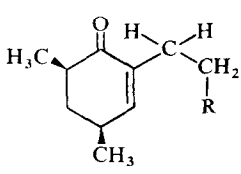
Dehydrocycloheximide



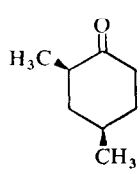
Dehydroisocyloheximide



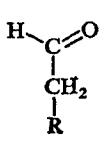
Anhydrocycloheximide



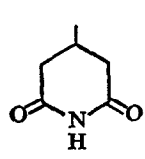
Epianhydrocycloheximide



2,4-dimethyl-cyclohexanone



3-Glutarimidyl-acetaldehyde



—R

FIG. 2. Structural formulae of compounds related to *l*-cycloheximide. The structure of inactone given is in equilibrium with 2a, 4e.

positions 5 and 6; actiphenol,* m.p. 200–201°, contains a substituted phenol instead of the cyclohexanone ring and there are no asymmetric centers; streptimidone,† m.p. 72°, possesses an aliphatic ketone; dehydrocycloheximide,* m.p. 173–175°, possesses a hydrogen bond between the oxygen at carbon-1 of the cyclohexanone ring and the oxygen attached to the α-carbon of the hydroxyethylglutarimide side chain; anhydrocycloheximide,*‡ m.p. 135–137°, formed by the dehydration of cycloheximide, lacks a hydroxyl group and is unsaturated between carbon-6 of the cyclohexanone ring and the α-carbon of the side chain; *dl*-dehydroisocycloheximide,* m.p. 151–152°, differs from dehydrocycloheximide in the orientation of the methyl group at carbon 2 in the cyclohexanone ring; *dl*-epi-anhydrocycloheximide,* m.p. 83–85°, lacks the hydroxyl group and is unsaturated between carbons 5 and 6 of the cyclohexanone ring; 2,4-dimethylcyclohexanone* (93% *cis*; 7% *trans*) and 3-glutarimidyl-acetaldehyde,* m.p. 120–122°.

C. Derivatives and analogs (*see Fig.* 3). The following compounds possessing the same steric configuration as *l*-cycloheximide but differing in substitutions at various positions in the cyclohexanone ring or hydroxyethylglutarimide portions of the molecule, were used: cycloheximide oxime,‡ m.p. 188–190°; cycloheximide acetate,‡ m.p. 149–151°; *Ψdl*-cycloheximide-I-acetate,* m.p. 140°; cycloheximide semicarbazone,‡ m.p. 168–169°; cycloheximide thio-semicarbazone,‡ m.p. 166–167°; cycloheximide benzoylacetate,‡ m.p. 148–149°; *N*-methyl-cycloheximide acetate,§ m.p. 140–141°; α-dihydrocycloheximide,* m.p. 163°; β-dihydrocycloheximide,* m.p. 164°; dihydrocycloheximide diacetate,‡ m.p. indefinite; acetoxycycloheximide,‖ m.p. 140°; and streptovitacin A,‡ m.p. 156–159°.

All compounds that possessed significant toxicity were obtained from samples which had been analyzed and found to contain less than 5 per cent contamination with cycloheximide.

No stereochemical work has been done on acetoxycycloheximide and streptovitacin A, but the side chain of the 4-methyl group orientations are probably 4-equatorial-acetoxy and 4-equatorial-hydroxy cycloheximide respectively, since naramycin B, isocycloheximide and neocycloheximide, which have different orientations at this group, have little biological activity.[20]

Siegel *et al.*[17] and Sisler and Siegel[21] give a comprehensive bibliography of articles concerning the isolation, synthesis and properties of the compounds used in the present study and the interested reader is referred to these papers.

Determination of protein synthesis in intact cells

L cells growing in suspension culture were used for measurements on intact cells. Actively growing cells were washed once with warm Eagle's suspension medium (Microbiological Associates, Bethesda, Md.) and resuspended in fresh, warm medium in a number of separate flasks at approximately 4–5 × 10^5 cells/ml. Replicate 10-ml suspensions of cells in 50-ml stoppered flasks were incubated at 80 rpm for 30–60

* A gift from Dr. Johnson, The Dow Chemical Co., Wayland, Mass.
† A gift from Dr. Machamer, Parke, Davis & Co., Detroit, Mich.
‡ A gift from Drs. O'Connell and Whitfield, The Upjohn Co., Kalamazoo, Mich.
§ A gift from Dr. Siegel, University of Kentucky, Lexington, Ky.
‖ A gift from Dr. McBride, Chas, Pfizer and Co. Inc., Maywood, N.J.

min at 37° in a New Brunswick gyratory water bath shaker. Next, the compounds tested were added and the cells were incubated for an additional 10 min to allow the drugs to penetrate the cells. ^{14}C-leucine (0·1 μc/ml) was added and the flasks were shaken for 60 min more. Eight ml of cells was then mixed with 5 ml Earle's salt solution and centrifuged at 1000 *g* for 10 min at room temperature. The cells were suspended in 2 ml water and 2 ml 1 N NaOH was added. The resulting solution was incubated at 37° for 30 min. Duplicate 1-ml samples were neutralized with HCl, 2 ml 10% trichloroacetic acid was added, and the precipitates were filtered and washed with 5% trichloroacetic acid on Millipore filters (0·45 μ, pore size; Millipore Filter Co., Bedford, Mass.). The filters were mounted on aluminum planchets, dried and counted with a Nuclear-Chicago thin-window low-background counter at 24 per cent efficiency.

The concentration (M) of antibiotic required to inhibit by 50 per cent the incorporation of ^{14}C-leucine into trichloroacetic acid-insoluble material was determined from curves plotted on semi-log paper. Inhibition (per cent) is expressed relative to cycloheximide. The figure given is an average of 3–5 separate determinations for each antibiotic.

Determination of protein synthesis in cell-free extracts

The cell-free system was derived from rat liver.[8] The effect of the compounds on the synthesis of hot trichloroacetic acid-precipitable peptide directed by both endogenous messenger ribonucleic acid and polyuridylic acid was determined. The reaction mixture contained in a volume of 1 ml: 100 μmole Tris-HCl, pH 7·8; 6 μmole disodium creatine phosphate; 20 μg creatine kinase; 2 μmole disodium ATP; 0·12 μmole trisodium GTP; 100 μmole KCl; 1·3 μmole (0·5 μc) ^{14}C-phenylalanine; antibiotics, as indicated; rat liver extract S-30 fraction (Dublin male rats), approximately 0·5 mg RNA and 3·8 mg protein; 300 μg polyuridylic acid when used. The S-30 extract was preincubated before use. After 60 min of incubation at 37°, 0·5 ml samples were added to 2·5 ml of 10% trichloroacetic acid containing nonradioactive phenylalanine (1 mg/ml). Each suspension was heated at 100° for 10 min, and filtered through a Millipore filter (0·45 μ, pore size). The retained precipitate was washed, mounted on an aluminum planchet, dried and counted. The inhibition was determined as described in the methods used for intact cells. The uninhibited reaction incorporated 250 $\mu\mu$mole phenylalanine per ml into hot acid-insoluble material.

Although protein synthesis in both intact cells and cell-free extracts was determined, it is probably better to look at the effects of the compounds studied on protein synthesis in intact cells than on cell-free protein synthesis because the intact cells are more sensitive to the action of the antibiotics than the cell-free system. Only the data for intact cells are tabulated. The few inhibitions observed with the cell-free extract are given in the legend to Table 2.

Biochemical and radioactive materials

Disodium ATP, trisodium GTP, disodium creatine phosphate, and creatine phosphokinase were products of Calbiochem, Los Angeles, Calif. and of Sigma Chemical Co., St. Louis, Mo. Polyuridylic acid was purchased from Miles Chemical Co., Elkhart, Ind. ^{14}C-L-leucine (23·3 mc/m-mole) and ^{14}C-L-phenylalanine (285 mc/m-mole) were purchased from New England Nuclear Corp., Boston, Mass.

RESULTS AND DISCUSSION

This investigation of structural requirements necessary for inhibition of protein synthesis by a variety of steroisomers, derivatives and other compounds related to cycloheximide has provided the following information.

1) Both the configuration of the cyclohexanone ring and the orientation of the methyl groups around the asymmetric centers are important for maximum activity (Table1).

TABLE 1. EFFECT OF STEREOISOMERS OF CYCLOHEXIMIDE AND RELATED COMPOUNDS ON PROTEIN SYNTHESIS IN INTACT CELLS*

Compound	Intact cells	
	50 per cent Inhibition (M)	per cent Inhibition relative to cycloheximide
l-Cycloheximide	$2{\cdot}9 \times 10^{-7}$	100·0
d-Isocycloheximide	$8{\cdot}8 \times 10^{-7}$	32·9
d-Naramycin B†	$2{\cdot}4 \times 10^{-6}$	12·1
dl-α-Epi-isocycloheximide	$5{\cdot}0 \times 10^{-6}$	5·8
dl-Neocycloheximide	‡	—
Streptimidone	$5{\cdot}8 \times 10^{-7}$	50·0
Inactone	$5{\cdot}6 \times 10^{-6}$	5·2
Actiphenol	$> 10^{-4}$	< 0·4
Dehydrocycloheximide	$> 10^{-4}$	< 0·4
dl-Dehydroisocycloheximide	$> 10^{-4}$	< 0·4
Anhydrocycloheximide	$> 10^{-4}$	< 0·4
dl-Epi-anhydrocycloheximide	‡	—
2,4-Dimethyl-cyclohexanone	$> 10^{-4}$	< 0·4
3-Glutarimidyl-acetaldehyde	$> 10^{-4}$	< 0·4

* In the cell-free system, all compounds possessed less than 10 per cent the inhibitory activity of cycloheximide. *Note*: Because of solubility and technical considerations in assay, the highest concentration of any given antibiotic tested with intact cells was 10^{-4} M and with the cell-free system, 10^{-3} M. In those cases in which 50 per cent inhibition was not attained at these concentrations, the values of per cent inhibition relative to cycloheximide are a maximum. If it were possible to determine the exact concentration which gave 50 per cent inhibition in these cases, the per cent inhibition would be much lower than that given in the tables.

† *d*-Naramycin B, 68%; *l*-cycloheximide, 32%.

‡ Not tested.

l-α-Epi-isocycloheximide differs from *l*-cycloheximide only in the orientation of the methyl group at position 4 of the cyclohexanone ring. This orientation is axial in cycloheximide and equatorial in the isomer. Nevertheless, this small difference has a marked effect on the biological activity of the compound. If one assumes that the *l*-isomer is the only active isomer in the racemic mixture, then *l*-α-epi-isocycloheximide possesses only about 12 per cent of the activity of *l*-cycloheximide.

The isomer, *d*-isocycloheximide, differs from *l*-cycloheximide in the orientation of the methyl group at position 4 and in the absolute configuration of the cyclohexanone ring. The orientation of the side chain is the same as in cycloheximide. In spite of this large difference in configuration, this compound is about one-third as toxic as cycloheximide.

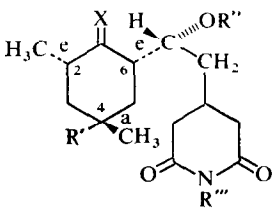
d-Naramycin B differs from *l*-cycloheximide in the orientation of the methyl groups at positions 2 and 4, and in the configuration of the cyclohexanone ring. The orientation of the side chain is the same as in cycloheximide. Thus, *d*-naramycin B differs from

d-isocycloheximide only in the orientation of the methyl group at position 2. Nevertheless, this isomer lacks activity. The observed small inhibition by this compound (Table 1) is due to contamination of this preparation by *l*-cycloheximide (see Materials and Methods).

dl-Neocycloheximide differs from cycloheximide in the orientation of the methyl group at position 4 and the side chain at position 6. This isomer possesses no biological activity. *d*-Cycloheximide is inactive against yeast,[17] but was not available for the present study.

2) The results obtained studying other related glutarimide derivatives also point to the importance of the type of cyclic moiety attached to the hydroxyethylglutarimide portion of the molecule (Table 1).

Since the ketone ring in inactone is unsaturated between carbons 5 and 6, the compound possesses only 2 asymmetric centers. In actiphenol, the cyclohexanone ring is replaced by a phenol. The structures of anhydrocycloheximide, dehydrocycloheximide and dehydroisocycloheximide are shown in Fig. 3. None of these compounds possesses significant biological activity.



Compound	Substitutions
Cycloheximide	X = =O, R′,R″,R‴ = −H
α-Dihydrocycloheximide	X = —OH-e
β-Dihydrocycloheximide	X = —OH-a
Cycloheximide Oxime	X = =N—OH
Cycloheximide Semicarbazone	X = =N—NH—C(=O)—NH_2
Cycloheximide Thiosemicarbazone	X = =N—NH—C(=S)—NH_2
Dihydrocycloheximide Diacetate	X = —O—C(=O)—CH_3, R″ = —C(=O)—CH_3
ψ-Cycloheximide-I-Acetate	X = —O—C(=O)—CH_3, R″ = O Ketone
Acetoxycycloheximide	R′ = —O—C(=O)—CH_3
Streptovitacin A	R′ = —OH
Cycloheximide Acetate	R″ = —C(=O)—CH_3
Cycloheximide Benzoylacetate	R″ = —C(=O)—CH_2—C(=O)—C_6H_5
N-Methylcycloheximide Acetate	R″ = —C(=O)—CH_3, R‴ = —CH_3

FIG. 3. Structural formulae of derivatives and analogs of *l*-cycloheximide possessing the same steric configuration as *l*-cycloheximide, but differing in substitutions at various positions in the molecule. Unless otherwise listed, the substitutions are the same as on cycloheximide.

Although the configuration of the cyclohexanone ring is important for toxicity in compounds which possess the ring structure, the ring is not absolutely necessary for activity. Streptimidone, which possesses an aliphatic side chain instead of the cyclohexanone ring, inhibits protein synthesis by about 50 per cent. However, the actual configuration of the aliphatic side chain in solution may be sufficient to give a molecule which is similar to that of cycloheximide and which possesses biological activity.

3) The position of substitutions on *l*-cycloheximide is important for the biological activity of the resulting compounds (Table 2).

Substitution of the oxygen attached to carbon-1 of the cyclohexanone ring or esterification of the hydroxyl group attached to the α-carbon of the hydroxyethylglutarimide side chain gives compounds which possess greatly diminished inhibitory activity. *N*-methylcycloheximide, which has a methyl group substituted in place of the imide nitrogen, was not available for this study. This compound is inactive in inhibiting protein synthesis in yeast,[17] and on the basis of the overall similarity of the results obtained in animal and yeast cells, *N*-methylcycloheximide is probably also inactive against animal cells.

TABLE 2. EFFECT OF DERIVATIVES OF CYCLOHEXIMIDE ON PROTEIN SYNTHESIS IN INTACT CELLS*

Compound	Intact cells	
	50 per cent Inhibition (M)	per cent inhibition relative to cycloheximide
l-Cycloheximide	$2{\cdot}9 \times 10^{-7}$	100·0
Acetoxycycloheximide	$1{\cdot}3 \times 10^{-8}$	2230·0
Streptovitacin A	$3{\cdot}8 \times 10^{-7}$	76·0
α-Dihydrocycloheximide	$2{\cdot}2 \times 10^{-6}$	13·2
Cycloheximide benzoylacetate	$3{\cdot}0 \times 10^{-6}$	10·0
Cycloheximide oxime	$4{\cdot}4 \times 10^{-6}$	6·6
Cycloheximide acetate	$3{\cdot}5 \times 10^{-5}$	0·8
Cycloheximide thiosemicarbazone	$3{\cdot}7 \times 10^{-5}$	0·8
Cycloheximide semicarbazone	$5{\cdot}7 \times 10^{-5}$	0·5
Ψ-*dl*-Cycloheximide-I-acetate	$> 10^{-4}$	< 0·4
N-methyl-cycloheximide acetate	$> 10^{-4}$	< 0·4
Dihydrocycloheximide diacetate	$> 10^{-4}$	< 0·4
β-Dihydrocycloheximide	†	—

* In the cell-free system, acetoxycycloheximide and streptovitacin A possessed 5980 and 472 per cent inhibition, respectively, relative to cycloheximide. All other compounds possessed less than 10 per cent the activity of cycloheximide. Fifty per cent inhibition by cycloheximide was observed at $5{\cdot}2 \times 10^{-4}$ M. See note to Table 1.

† Not tested.

On the other hand, acetoxycycloheximide and streptovitacin A, which have acetoxy and hxdroxyl substitutions of the hydrogen attached to carbon-4 of the cyclohexanone ring, possess much greater biological activity *in vitro* than the parent compound, cycloheximide. However, since the stereochemistry of these compounds has not been unequivocally established, one cannot definitely say that the reason for the enhanced activity compared to cycloheximide is due only to the substitutions at carbon-4.

4) The results obtained with animal cell systems are in general agreement with the previous work done with yeast.

Siegel *et al.*[17] have carried out a thorough study of the effect of these compounds related to cycloheximide on protein synthesis in intact yeast cells and in extracts derived from these cells. The results of the present investigation using animal cells are in good agreement with most of the work done by Siegel *et al.*[17] The data, however, differ in three minor respects. α-Epi-isocycloheximide appears to have little effect on protein synthesis in animal cells, but is active against yeast. Acetoxycycloheximide is an excellent inhibitor *in vitro* in both yeast and animal cells, but does not possess activity *in vivo* in yeast. This antibiotic probably does not get into yeast cells (or may be detoxified), whereas animal cells are quite permeable to this compound.[8] Likewise, streptovitacin A does not appear to get into intact yeast cells and is ineffective *in vivo*. This antibiotic is about 75 per cent as active as cycloheximide in intact animal cells and therefore would probably enter animal cells quite well. However, the streptovitacin A is about five times more active in inhibiting protein synthesis in cell-free extracts of animal cells, indicating perhaps that intracellular levels of the drug do not attain the maximum concentration possible in animal cells.

By using cytotoxicity of KB cells growing in culture as a criterion for growth inhibition, Smith *et al.*[15, 16] found that streptovitacin A possessed three times the activity of cycloheximide. The present study would therefore indicate that cytotoxicity and inhibition of protein synthesis probably cannot be equated in judging the activity of this antibiotic. The reason the primary inhibition of protein synthesis by streptovitacin A may be less than the ultimate cytotoxic effect may be based on the differences in cell penetration. Since the toxicity of streptovitacin A to cell-free protein synthesis is five times that of the effect on intact cells, one might expect an increased inhibitory effect in intact cells because the longer the cells are exposed to the antibiotic the more it would penetrate and act.

Acknowledgements—I wish to express my sincere thanks to the following individuals for their most generous gifts of the various antibiotics and derivatives of cycloheximide employed in this study: Dr. F. Johnson, The Dow Chemical Co., Wayland, Mass.; Dr. H. E. Machamer, Parke, Davis & Co., Detroit, Mich.; Dr. T. J. McBride, Chas. Pfizer & Co., Maywood, N. J.; Drs. P. W. O'Connell and G. B. Whitfield, the Upjohn Co., Kalamazoo, Mich.; and Dr. M. R. Siegel, University of Kentucky, Lexington, Ky. In addition, I would like to thank Dr. M. R. Siegel for allowing me to read an unpublished manuscript and Dr. F. Johnson for his explanation of the stereochemistry of cycloheximide and isomers.

This investigation was supported by Public Health Service Grant GM 12359-03 from the Division of General Medical Sciences, by Grant GB 3521 from the National Science Foundation, and by the American Lebanese Syrian Associated Charities (ALSAC).

REFERENCES

1. A. J. Whiffen, J. N. Bohonos and R. L. Emerson, *J. Bact.* **52**, 610 (1946).
2. A. J. Whiffen, *J. Bact.* **56**, 283 (1948).
3. C. W. Young, P. F. Robinson and B. Sacktor, *Biochem. Pharmac.* **12**, 855 (1963).
4. D. Kerridge, *J. gen. Microbiol.* **19**, 497 (1948).
5. M. R. Siegel and H. D. Sisler, *Biochim. biophys. Acta* **87**, 70 (1964).
6. M. R. Siegel and H. D. Sisler, *Biochim. biophys. Acta* **87**, 83 (1964).
7. M. R. Siegel and H. D. Sisler, *Biochim. biophys. Acta* **103**, 558 (1965).
8. H. L. Ennis and M. Lubin, *Science, N.Y.* **146**, 1474 (1964).
9. W. J. Cooney and S. G. Bradley, in *Antimicrobial Agents and Chemotherapy* (Eds. M. Finland and G. M. Savage), p. 237. Am. Soc. Microbiol., Detroit, Mich. (1961).

10. L. L. BENNETT, V. L. WARD and R. W. BROCKMAN, *Biochim. biophys. Acta* **103**, 478 (1965).
11. F. O. WETTSTEIN, H. NOLL and S. PENMAN, *Biochim. biophys. Acta* **87**, 525 (1964).
12. B. COLOMBO, L. FELICETTI and C. BAGLIONI, *Biochim. biophys. Acta* **119**, 109 (1966)
13. S. LIN, R. D. MOSTELLER and B. HARDESTY, *J. molec. Biol.* **21**, 51 (1966).
14. L. FELICETTI, B. COLOMBO and C. BAGLIONI, *Biochim. biophys. Acta* **119**, 120 (1966).
15. C. G. SMITH, *Proc. Soc. exp. Biol. Med.* **100**, 757 (1959).
16. C. G. SMITH, W. L. LUMMIS and J. E. GRADY, *Cancer Res.* **19**, 843, 1959.
17. M. R. SIEGEL, H. D. SISLER and F. JOHNSON, *Biochem. Pharmac.* **15**, 1213 (1966).
18. A. P. GROLLMAN, *Proc. natn. Acad. Sci. U.S.A.* **56**, 1867 (1966).
19. B. K. LEE and D. WILKIE, *Nature, Lond.* **206**, 90 (1965).
20. F. JOHNSON, N. A. STARKOVSKY and W. B. GUROWITZ, *J. Am. chem. Soc.* **87**, 3492 (1967).
21. H. D. SISLER and M. R. SIEGEL, in *Antibiotics, Mechanism of Action* (Eds. D. GOTTLIEB and P. D. SHAW), vol. 1, p. 283. Springer-Verlag, New York (1967).