INHIBITORY EFFECT OF CYCLOSERINE ON SOME ENZYMIC ACTIVITIES RELATED TO VITAMIN B₆

KOZO YAMADA, SHUNJI SAWAKI AND SUMIHIKO HAYAMI

*Department of Internal Medicine, School of Medicine,
Nagoya University, Showa-ku, Nagoya*

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Makino *et al.* (1) reported that convulsions of mice and rats provoked by an injection of 2-methyl-6-amino-5-hydroxymethylpyrimidine (OMP) were prevented by vitamin B₆ and that OMP had an antagonistic action to pyridoxal phosphate (PAL-PO₄) in the action of tyrosine-decarboxylase of bacteria (2).

It is also a known fact that antitubercular agents, *i.e.*, isonicotinic acid hydrazide (INAH) and thiosemicarbazide manifest a similar convulsive effect, and that the former is inhibited by certain concentrations of vitamin B₆ (3) whereas pyridoxamine (4) alone has an inhibitory effect to the latter. Yoneda *et al.* (5) Boone *et al.* (6) and Dewey *et al.* (7) found that both of these agents had an antagonistic action to vitamin B₆ in microorganisms. The reduction in vitamin B₆-enzyme system is reported in vitamin B₆-deficient animals (8) and is considered to be a cause of nervous symptoms (convulsions). From these findings, a close relation between vitamin B₆ and convulsions is suggested.

On the other hand, convulsions or epilepsy-like attacks (9, 10) have been reported as a side effect of a new antitubercular agent, D-4-amino-3-isoxazolidinone, *i.e.*, cycloserine¹ (CS) and have been experienced quite frequently. This suggests similar actions of OMP and CS to the organism. It was therefore examined if the convulsions provoked by CS in mice can be prevented by vitamin B₆ and found, contrary to the expectation, that vitamin B₆ had no significant preventing effect, possibly suggesting the causes of convulsions to be complicated. Using bacteria the mode of action of CS on B₆-enzyme systems was then examined. The present report describes the results of the experiment.

EXPERIMENTAL

Materials and Methods

Test Material—Fresh cells of *Escherichia coli-communior* were washed three times with distilled water and were treated with cold acetone. The

¹ The preparation of Eli Lilly Co. was used.

dry powder thus obtained was treated with $M/45$ borate buffer, pH 8.5, according to Tayler and Gale (11). The cell-free enzyme solution thus obtained was used as amino-acid decarboxylase. As tryptophanase, acetone-dried bacteria prepared according to Wood *et al.* (12) were used. For determining amino-acid decarboxylase activity CO_2 evolved was estimated manometrically according to Gale (13). Indole formation was determined photometrically according to Wood *et al.* (12) and the results were compared with the effect of INAH reported by Yoneda *et al.* (4). For demonstrating the complex formation of CS with pyridoxal² (PAL) both absorption spectrum and separation by paper chromatography were applied.

RESULTS AND DISCUSSION

As shown in Figs 1–2 and Table I, the inhibitory effect of CS on the

FIG. 1 Inhibitory Effect of CS and Recovery Effect of PAL to the Activity of Arginine Decarboxylase of *E. coli*.

Reaction temperature ; 37.5° , main compartment, 0.5 ml *E. coli* cell-free enzyme solution and in addition, various substances are added to final concentrations given in the figure.

Side bulb, 0.5 ml of $M/10$ arginine. Citrate-phosphate buffer (pH 4.5) of McIlvaine is added up to 2.1 ml. Gas space, air.

activities of amino-acid decarboxylase and tryptophanase of *E. coli* was manifest in lower concentrations than that of INAH and a straight line relationship was observed within a certain limit between the logarithms of the CS concentrations and the inhibition percentages. The inhibition was recovered by the addition of PAL, whereby both PAL and PAL-PO_4 ³ were effective,

² The preparation of Nutritional Biochemicals Corporation was used.

³ The preparation of California Foundation for Biochemical Research was used.

FIG. 2 Inhibitory Effect of CS and Recovery Effect of PAL to the Activity of Glutamic Decarboxylase of *E. coli*

Reaction system is the same as in Fig. 1. 0.5 ml of M/10 sodium glutamate was used. Concentrations of various substances are given in final concentrations.

and OMP failed to inhibit in such concentration, presumably due to the difficulty in OMP-PO₄ formation in the reaction system. Substrate can be added after five minutes, *i. e.*, it was not necessary to care for the exact contact time of the inhibiting agents with bacterial cells as reported by Yoneda *et al.* It is natural that the inhibition by CS and the recovery by PAL are more marked in cell-free enzyme solution than in the suspension of the bacillus.

TABLE I
Inhibitory Effect of CS and Recovering Effect of PAL on the Activity of Tryptophanase of *Escherichia coli*

To 0.5 ml of suspension of *E. coli*, 0.1 ml of CS solution of varying concentrations and 1.9 ml of M/15 phosphate buffer (pH 8.0) are added and distilled water is added up to 3.0 ml. After incubating at 38° for 10 minutes, 0.4 ml of M/160 tryptophane is added, after 10 minutes at 38°, the reaction is stopped by 1g/ml trichloroacetic acid. After extraction with toluene, Ehrlich's aldehyde reagent is added and after 10 minutes, the optical densities were determined photometrically at 530 mμ.

Final concentration of CS	Final concentration of PAL	Amount of indole formed	Inhibitory effect	Recovery effect
<i>M</i>	<i>M</i>	<i>γ</i> /mg dry cells	<i>per cent</i>	<i>per cent</i>
1/200		4 (25) ^a	90 (37) ^a	
1/400		11	72	
1/800		16	60	
1/1600		20	50	
0		40	0	
1/400	1/800	25		35
1/400	1/1000	19		20
1/400	1/2000	15		10

^a Parenthesized figures indicate the inhibitory effect when M/200 INAH was used.

As a presumable mode of inhibition, it is considered that either CS competes with PAL-PO₄ for the apo-enzyme of bacteria or CS forms a complex

with vitamin B₆. The latter is more probable than the former from the following facts:

- (a) The mixture of CS and PAL has a more intensive fluorescence (greenish blue).
 (b) Optical density changes as shown in Fig. 3.

FIG. 3 *Ultra-Violet Absorption Spectrum of CS, PAL and the Mixture*
 Both CS and PAL are M/3200. In the mixture the same volumes of CS and PAL were added.

FIG. 4 *Differentiation of CS, PAL and the Mixture by Paper chromatography*
 Developing solvent system (descending): A, Acetic acid-butanol-water, 1:4:5.
 B, Ethanol-water, 1:1. bg, bluish-green fluorescence; bv, bluish violet fluorescence; v, violet fluorescence

(c) A fluorescent substance assumed to be a complex is separated in paper chromatogram as shown in Fig. 4.

The above findings suggest that CS inhibits the vitamin B₆-enzyme system in bacteria by forming a complex with vitamin B₆ as in the case with KCN, hydrazine or hydroxylamine rather than by acting as a so-called "biological antagonist". This resembles the report by Davison *et al.* (4) that the inhibition by INAH is due to the formation of hydrazone with PAL-PO₄ and it is supposed that a part of the side effect of CS may be due to these facts.

SUMMARY

It is considered that cycloserine inhibits the vitamin B₆-enzyme system in bacteria by forming a complex with vitamin B₆.

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