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Excess Supplementation of Certain Amino Acids to Medium and Its Inhibitory Effect on Toxin Production by *Clostridium tetani*

Previously (1964) we briefly reported that the excess supplementation of sodium glutamate to medium (1 percent final concentration) markedly suppressed toxin production of *Clostridium tetani*, while it exerted a rather favorable influence on growth. Since we felt that, before approaching the mechanism involved, it was necessary to know whether or not the inhibitory action is a specific property of this particular amino acid, a comparative study was made of various amino acids in terms of their effects on tetanus toxin formation. The present communication describes the results of the study demonstrating that glutamic and aspartic acids, their amides and histidine greatly inhibit the toxin production of a strain of *Cl. tetani* without affecting its growth unfavorably.

A substrain (Biken) of the Harvard A 47 strain of *Clostridium tetani* and the P-II medium described by Kubota et all (1960) were used throughout this study. An overnight culture of this organism in liver-liver broth was first inoculated into P-II medium and incubated at 35°C for 8 hours. After incubation, 0.2 ml aliquots of the culture were transfered into 20 ml portions of P-II medium in test tubes supplemented with different amino acids at final concentrations of 1 per cent. On the 6th day of incubation at 35°C, the cultures were centrifuged, filtered and the toxin content of the aseptic filtrates thus obtained were determined according to a standard method. The toxin content was thus expressed as L + /10 /ml using Japanese white mice, stock dd, weighing about 12 grams raised in closed colony. Bacterial growth was measured as the viable count on Zeissler blood agar plates.

The results are shown in the accompanying table. This table shows that all the amino acids tested, except lysine and arginine had an inhibitory effect on toxin production by *Cl. tetani* with or without an unfavorable effect on bacterial growth. The greatest inhibitions of toxin production without unfavorable influences on growth were obtained with glutamic acid, aspartic acid, glutamine, asparagine and histidine. Thus, in media supplemented with these five amino acids at final concentrations of 1 per cent, less than 10-30 L + /10 /ml of toxin was produced by this organism while the toxin production in the control medium was 400 L + /10 /ml. A very low titer of toxin was also obtained in medium supplemented with serine. In this case, however, bacterial growth was considerably suppresed and this presumably resulted in a reduction in toxin production. The table also shows that the amino acids tested (other than serine) can be roughly divided into the following three groups according to the degree of their inhibition of toxin production: 1) Amino acids showing no inhibition; arginine and lysine 2) Amino acids showing some inhibition; glycine, threonine, proline, oxyproline, alanine, phenylalanine, leucine, isoleucine, tryptophane,

Table. Effect of excess amino acid supplementation to medium* on toxin production by *Clostridium tetani*

Amino acid supplemented 1%	Toxin produced L+/10/ml	Maximal growth Viable count $\times 10^8$
L-arginine	350	8.1
L-lysine	400	7.7
L-serine	20	0.9
L-threonine	150	7.8
L-proline	150	12.0
L-oxyproline	250	9.0
L-alanine	250	6.1
L-pheneylalanine	150	6.7
L-leucine	250	9.0
L-isoleucine	200	5.9
L-tyrptophane	100-150	3.7
L-methionine	150	6.9
L-valine	250	7.8
glycine	100-150	2.5
L-glutamic acid	10	7.0
L-aspartic acid	30	7.2
L-glutamine	30	-**
L-asparagine	10	8.7
L-histidine	20	-**
-- (control)	400	5.0

* P-II medium described by Kubota et al. (1960)

** Not counted. The trubidity was similar to that of the control.

methionine and valine 3) Amino acids having a potent inhibitory activity; glutamic acid, aspartic acid, glutamine, asparagine and histidine. Thus there may be some relationship between the ionic state of these amino acids and their inhibitory action on toxin production. Histidine, an exceptional amino acid belonging to the last group, may be converted to glutamic acid by this organism and thus shows an inhibitory effect. However, further studies are necessary on this and also on the question of which amino acid plays the primary role in this toxin inhibiton. Research is now in progress and the results will be reported elsewhere.

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Isamu Tsunashima*
Kiyoshi Sato*
Ko Shoji**
Masahiko Yoneda**
Tsunehisa Amano**

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* *Kannonji Institute, Research Foundation for Microbial Diseases of Osaka University, Kannonji, Kagawa, Japan*
** *Resarch Institute for Microbial Diseases, Osaka University, Osaka, Japan.*