Inhibition of *E. coli* by D-Serine and the Production of Serine-resistant Mutants

BY BERNARD D. DAVIS AND WERNER K. MAAS[1]

In the course of determining the amino acid requirements of nutritionally deficient bacterial mutants[2,3] it was observed that DL-serine inhibits the growth of wild-type *Escherichia coli* ("Waksman" strain, ATCC 9637). Testing of the separate components of DL-serine was made possible through the generous gift of a highly purified sample of D-serine by Dr. V. du Vigneaud, and of L-serine by Drs. S. Moore and W. H. Stein. It was found that the effect is caused by the D isomer, which perceptibly delays growth in concentrations as low as 5 γ/ml.; L-serine is inactive at even 40 times this concentration. All the other naturally occurring amino acids were tested at a concentration of 200 γ/ml. and not found inhibitory; of these, alanine, aspartic acid, threonine, tyrosine, phenylalanine, methionine, leucine, isoleucine, valine, lysine and histidine were tested as DL mixtures. L-Aspartic acid is not inhibitory by itself, but when present in concentrations as low as 2 γ/ml. it enhances the effect of D-serine. The behavior of L-asparagine is quite different from that of aspartic acid; approximately 100 times as high a concentration of L-asparagine is required to produce a similar augmentation of inhibition. A difference in a bacterial response to asparagine and aspartic acid has also been reported[4,5] in studies on the utilization of these compounds as nutrilites by *Leuconostoc mesenteroides*.

The inhibitory effect is completely antagonized by glycine or L- or DL-alanine in concentrations of 25 to 100%, and partly antagonized at concentrations as low as 1%, of that of D-serine. Partial antagonism is also shown, at equimolar concentrations or higher, by L-histidine or L-leucine, and slight antagonism by most other naturally occurring amino acids. The only ones found to have no antagonistic effect were aspartic acid, L-cystine, L-cysteine, L-hydroxyproline, and, curiously, L-serine.

DL-Serine permits a number of cell divisions at the normal rate before inhibition appears. The organism eventually overcomes the inhibition, producing the same plate count in the presence of DL-serine as in its absence. On minimal medium agar,[3] containing only glucose, ammonium lactate, and salts, DL-serine produces a delay in appearance of visible colonies which increases with increasing concentration. At the highest concentration tested, 1 mg./ml., the delay exceeds forty-eight hours. It seems important to point out that the growth following the delay does not depend upon the type of enzymic adaptation which has been observed with many other delayed bacterial responses; it seems rather to involve an alteration by the bacteria, of unknown nature, in the surrounding medium. The evidence for this conclusion is two-fold: (a) bacteria grown out in the presence of high concentrations of DL-serine, when reinoculated into DL-serine-containing plates, grow no more rapidly than bacteria taken from a serine-free medium; (b) the appearance of visible colonies in the presence of DL-serine is markedly accelerated by increasing the inoculum size.

In addition to physiologic adaptation, which permits delayed growth of the whole population, the bacteria also produce spontaneous serine-resistant mutants which grow approximately as rapidly, in the presence or absence of DL-serine, as the parent strain does in its absence. The mutation frequency is increased by ultraviolet irradiation to as high a value as 10^{-3}.

Serine-resistant mutants have also been developed from mutant strains which require either L-serine or glycine for growth; the growth requirement has not been affected by the second mutation. In view of this fact, as well as the failure of L-serine to affect D-serine inhibition, it is clear that the mechanism of inhibition by D-serine has no close relation to the metabolism of L-serine.

Much of the literature on the metabolism of D and L amino acids has been reviewed recently.[6] D-Serine has been reported to have a nephrotoxic action in rats[7] which is antagonized[8] by the amino acids observed here to protect *E. coli*, as well as by pyruvate, which we have not found to protect *E. coli*. D-Serine has also recently been independently observed to inhibit formation of tetanus toxin.[9] *E. coli* is particularly suitable for studying the mechanism of a cytotoxic action which may be quite general; in addition, mutation to serine-resistance may be regarded as a model for drug-resistance, involving a simpler compound than the known chemotherapeutics. These phenomena are therefore under further investigation.

(1) Staff member, U. S. Naval Medical Research Institute.
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Inhibition of Tetanus Toxin Formation by D-Serine[1]

BY J. HOWARD MUELLER AND PAULINE A. MILLER

The fact has been commented on previously[2] that serine specifically depressed the formation of tetanus toxin under certain experimental conditions. At the time, this was considered as repre-

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