STUDIES ON THE NUTRITION AND PHYSIOLOGY OF *PASTEURELLA PESTIS*

IV. Utilization of Peptides During the Growth of *Pasteurella pestis*

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The amino acid requirements for the growth of *Pasteurella pestis* have been studied by a number of workers (Rao, 1939; Doudoroff, 1943; Herbert, 1949; Hills and Spurr, 1952; Rockenmacher *et al.*, 1952; and Higuchi and Carlin, 1958). Very little is known, however, regarding the role of peptides in the nutrition of this organism. Peptides have been reported to be superior to free amino acids in the nutrition of a number of microorganisms (Fruton and Simmonds, 1949; Hendlin, 1954; Kihara and Snell, 1955; and Wright, 1956). Higuchi and Carlin (1957) reported that *P. pestis* was capable of utilizing peptides in a partially hydrolyzed casein medium. Their results suggested that a study of the peptide nutrition of this organism might be of value.

METHODS AND MATERIALS

Organism. The strain of *P. pestis* employed in this investigation was the "Alexander" strain originally isolated in 1949 from a human case of bubonic plague in New Mexico (Link, 1950).

Medium and cultural conditions. The basal medium (table 1) containing the amino acids and salt constituents (and test media containing peptides) was adjusted to pH 7.5 and sterilized by autoclaving for 15 min at 121 C. D-Xylose and the vitamin mixture were autoclaved separately and added aseptically to the medium. The addition of vitamins apparently was not necessary for good growth under the conditions employed, but was included in order to insure that no deficiency occurred. The peptides employed in these studies were obtained from Nutritional Biochemicals Corporation and Mann Research Laboratories.

Twenty-five ml of media contained in a 500-ml Erlenmeyer flask were inoculated with 1 ml of a washed suspension of cells from a 24-hr culture grown in a casein hydrolyzate medium (Higuchi and Carlin, 1957) to give an initial count of approximately 10^9 cells per ml. Cultures were incubated at 27 ± 1 C on a reciprocating shaker operating through a 3 inch stroke at 100 cycles per minute.

Measurement of growth. Growth was measured nephelometrically in a Coleman model 9 Nepho-Colorimeter using an arbitrary turbidity standard for reference. A reading of 50 turbidity units obtained with a 1:10 dilution of a culture in a standardized 18-mm culture tube corresponded to an optical density value of approximately 0.5 determined with a colorimeter at 650 mμ wave length.

RESULTS

Several of the amino acids employed in the basal medium could be omitted singly without seriously limiting the growth of the organism. Methionine, phenylalanine, and cysteine, however, were essential for even minimal growth. The peptides tested, therefore, contained these essential amino acids in order that their effects could be readily observed. Glycine peptides were also examined because of their marked stimulation of the rate of growth of *P. pestis*.

Methionine peptides. The requirement for DL-methionine is shown in figure 1. Both D- and L- forms of free methionine were utilized. In a separate experiment, D-, L-, and DL-methionine at 0.0016 M concentration yielded turbidity values of 172, 184, and 184, respectively, after 30 hr of incubation. D-Methionine at an excessively high concentration (0.0048 M), however, was inhibitory. Under conditions of methionine deficiency, the peptide glycyl-DL-methionine gave approximately half of the amount of growth expected on the basis of its DL-methionine content (figure 1). It appeared therefore that only the L-methionine peptide was utilized.

Phenylalanine peptides. Phenylalanine was essential for the growth of the Alexander strain as shown in figure 2. The L- and DL- forms of

TABLE 1

Composition of basal medium

Component	Conc
	mmoles/L
L-Glutamic acid	82.0
DL-Phenylalanine	5.0
DL-Methionine	3.2
DL-Valine	13.7
DL-Leucine	2.9
DL-Lysine·HCl	2.2
L-Proline	7.0
DL-Threonine	2.7
DL-Isoleucine	7.3
L-Cysteine·HCl	4.0
Glycine	13.3
K_2HPO_4	25.0
NH_4 acetate	10.0
Na gluconate	10.0
Citric acid·H_2O	10.0
$MgSO_4 \cdot 7H_2O$	2.5
$FeSO_4 \cdot 7H_2O$	0.1
$MnSO_4 \cdot H_2O$	0.01
Thiamin·HCl	0.003
Ca pantothenate	0.002
Biotin	0.002
D-Xylose	66.7

free phenylalanine were utilized to approximately the same extent. At 0.000313 M concentration, glycyl-DL-phenylalanine and DL-phenylalanylglycine each gave approximately one half of the amount of growth obtained with an equimolar amount of glycyl-L-phenylalanine. This was another indication that only peptides containing the L-isomer were utilized by *P. pestis*. DL-Phenylalanylglycine apparently was utilized more rapidly than glycyl-DL-phenylalanine. The final total growth obtained, however, was approximately the same with equimolar amounts of each compound. These data indicated that the position of the glycine moiety in these peptides affected the rates of utilization.

A study of the utilization of the dipeptide, DL-alanyl-DL-phenylalanine, was of interest because it contains two asymmetric amino acids which results in a mixture of four peptides. On the assumption that peptides containing D-amino acids were not utilized by *P. pestis*, only one of the alanylphenylalanines (L-alanyl-L-phenylalanine) in the mixture was available for growth. Almost twice as much growth was obtained, however, than could be expected on the assumption that equal amounts of each form of the dipeptides were present. Control media containing L-alanine and L-phenylalanine were employed for comparison.

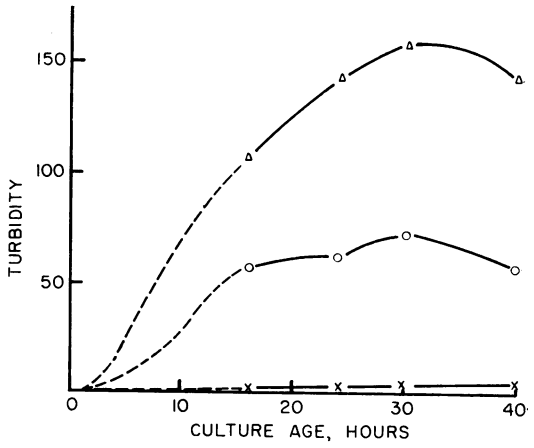

Figure 1. Effect of methionine and a methionine peptide on the growth of *Pasteurella pestis*. X——X, none; △——△, DL-methionine; and ○——○, glycyl-DL-methionine. Each compound at 0.0016 M.

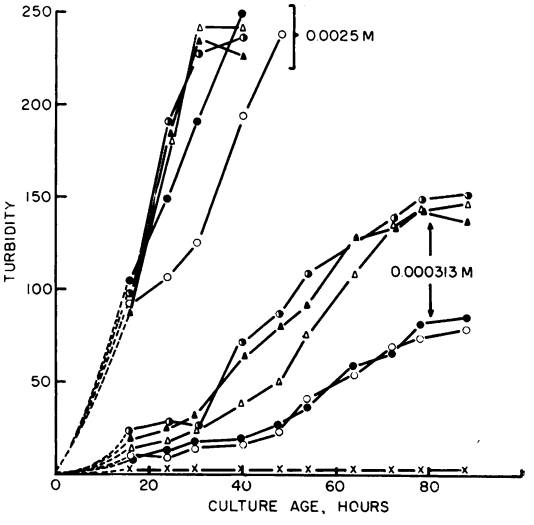

Figure 2. Effect of phenylalanine and phenylalanine peptides on the growth of *Pasteurella pestis*. X——X, none; △——△, DL-phenylalanine; ▲——▲, L-phenylalanine; ◑——◑, glycyl-L-phenylalanine; ○——○, glycyl-DL-phenylalanine; and ●——●, DL-phenylalanylglycine.

Glycine peptides. Although glycine was not essential for growth of *P. pestis*, its presence promoted more rapid initiation of growth (figure 3).

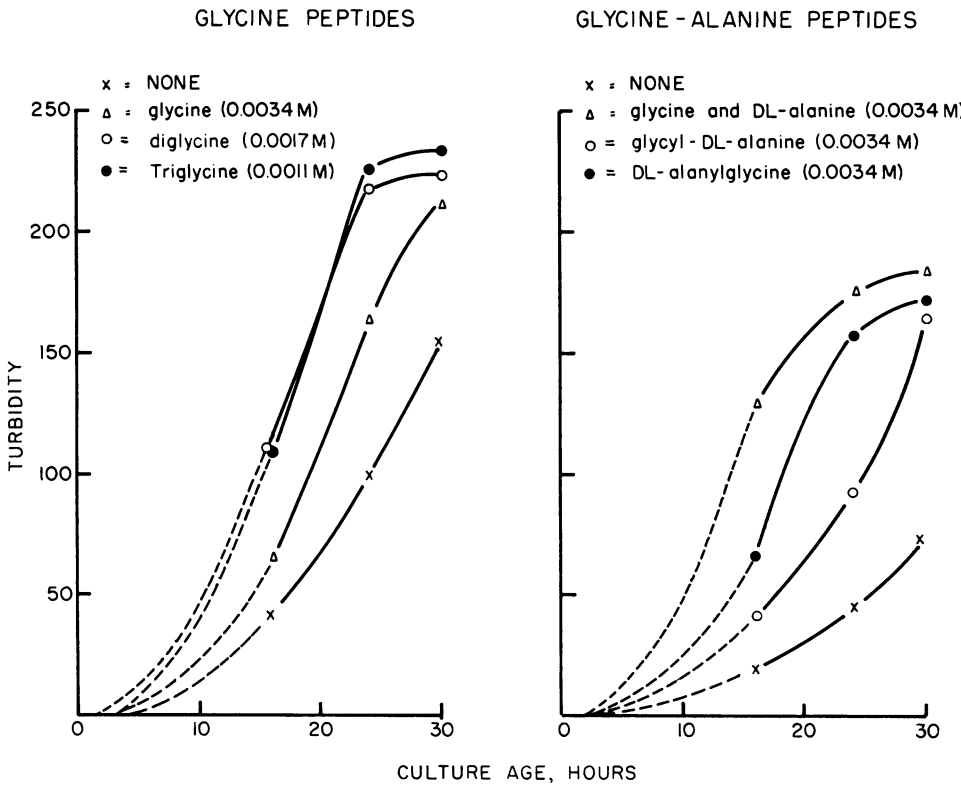

Figure 3. Effect of glycine peptides on the growth of *Pasteurella pestis*

Furthermore, the peptides diglycine and triglycine, produced greater stimulation than did free glycine (figure 3). The total growth obtained after 30 hr incubation, however, was approximately the same in all cultures. Tetraglycine also gave results which were similar to those obtained with di- and triglycine. Peptides such as glutathione, diketopiperazine, and hippuric acid, which also contain glycine, failed to stimulate growth.

Free glycine was oxidized rapidly when incubated with aerated resting cell suspensions of *P. pestis* in phosphate buffer. Glycine peptides were utilized more slowly under these conditions. Paper chromatograms of the reaction mixtures during the incubation period revealed that diglycine and triglycine were able to supply glycine to the cells over a longer period of time because they were apparently degraded more slowly than free glycine.

DL-Alanylglycine was superior to glycyl-DL-alanine for stimulation of growth (figure 3). This difference indicated that glycyl dipeptides are less adequate as nutrients than those peptides containing glycine in the terminal carboxyl position.

DISCUSSION

The results obtained with the dipeptides containing the essential amino acids, either methionine or phenylalanine, indicated that only the peptides containing L-linkages were utilized for growth; Simmonds *et al.*, (1951) and Kihara and Snell (1952) also have reported that peptides containing D-amino acids were apparently not utilized by their test organisms. Not many workers in the field have tested peptides containing D-amino acids in nutritional studies with microorganisms. Examples are known, however, where peptides containing D-amino acids have been shown to be stimulatory for growth or for certain other physiological processes in bacteria (Taylor, *et al.*, 1950; Peters *et al.*, 1953; Jordan and San Clemente, 1955; and Mueller and Miller, 1956).

The amount of growth obtained with DL-alanyl-DL-phenylalanine was greater than expected, but this was probably due to the presence

of a higher proportion of L-alanyl-L-phenylalanine in the mixture than of some of the other isomers. Alternative possibilities exist, however; either that the L-alanyl-L-phenylalanine isomer was more stimulatory for growth than the constituent free amino acids or, that the organism was able to utilize other isomers.

The fact that free glycine was rapidly decomposed by resting cell suspensions of *P. pestis* whereas glycine in the peptide form was more slowly utilized, may provide an explanation for the superiority of glycine peptides for the growth of *P. pestis*. Demain and Hendlin (1958) have reported recently that a strain of *Bacillus subtilis* gave better growth in the presence of glycine peptides than in the presence of free glycine.

The observation that dipeptides containing glycine in the primary amino position are less adequate as nutrients than those dipeptides containing glycine in the terminal carboxyl position was analogous to results reported by other workers who had noted the effect of the position of the constituent amino acids in peptides on the growth of bacteria (Ågren, 1947; Krehl and Fruton, 1948; and Stone and Hoberman, 1953). The position effect is perhaps due to differences in the rates of hydrolysis of these peptides by bacterial enzymes.

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SUMMARY

The utilization of certain peptides for growth of *Pasteurella pestis* has been studied. Of the dipeptides tested which contained the essential amino acids methionine or phenylalanine, it appeared that only peptides of L-amino acids were utilized. DL-Peptides supported only one half of the growth obtained with the L-isomers.

Glycyl dipeptides of alanine and of phenylalanine were apparently less available to the organism than the corresponding dipeptides with the glycine moiety in the terminal carboxyl position.

Diglycine and triglycine were more stimulatory for rapid growth of *P. pestis* than free glycine.

With the limited number of compounds tested, the growth of *P. pestis* was not enhanced by supplying amino acids (except glycine as diglycine or triglycine) in peptide form.

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