INHIBITION OF BACTERIAL GROWTH BY LEUCINE PEPTIDES*

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In previous reports (1, 2), data were presented on the growth response of a leucineless mutant (strain 679-680)1 of Escherichia coli strain K-12 to various L-leucine peptides. Measurement of the extent of bacterial growth after 24 hours incubation showed that, at low concentrations, the peptides were almost as effective in the stimulation of growth as was L-leucine; at high concentrations, however, the peptides appeared to exert an inhibitory action on the growth of the mutant (1). When the growth response of the mutant to L-leucine and to glycyl-L-leucine (G-L-L) was studied as a function of time as well as of the concentration of the test compound, it became evident that the dipeptide acted as an inhibitor of the initiation of growth, and that the duration of the lag phase of growth (i.e., the period preceding the initiation of rapid growth) increased with increasing concentrations of G-L-L. However, the extent of bacterial growth ultimately attained was approximately the same for equimolar concentrations of L-leucine and G-L-L (2).

In the present study, a comparison has been made of the relative inhibitory action of a number of leucine peptides on the growth of the leucineless mutant. The methods used in these experiments have been described previously (2). Nearly all of the L-leucine present in a peptide becomes available to the organism for growth; for convenience, therefore, the concentration of the leucine peptides will be given in terms of the amount, in micromoles per 10 ml., of leucine which may be derived from these peptides. Thus, 0.05 μM of L-leucyl-L-leucine (L-L-L-L) will be given as 0.10 μM, and this may be compared directly with 0.10 μM of L-leucine or L-leucylglycine (L-LG).

Peptides of L-Leucine As Inhibitors—In most cases, the growth curves for equivalent concentrations of L-leucine and of L-leucine peptides varied

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† James Hudson Brown Fellow of the Yale University School of Medicine.
1 Strain 679-680 is a double mutant which requires an exogenous source of both leucine and threonine. The present discussion is concerned only with the leucine requirement, and, for convenience, the organism is designated as a leucineless mutant.
significantly only in their position on the time axis (cf. also (2)). Under the conditions of these experiments, the maximal growth (measured turbidimetrically with an Evelyn colorimeter) attained by the mutant in minimal medium containing L-leucine or L-leucine peptides corresponded to an optical density of 0.250. A concentration of approximately 1.0 \( \mu M \) of L-leucine per 10 ml. of medium was sufficient to cause this growth, and the growth curves at concentrations of L-leucine up to 30 \( \mu M \) were identical with that for the 1.0 \( \mu M \) level. Curves for levels below 1.0 \( \mu M \) had maxima at values below 0.250, depending on the concentration of L-leucine in the medium.

Since the time at which the turbidity of a culture reached half its particular maximal value could be estimated accurately from the growth curve, this point, designated \( t_1 \), was used as the basis for a comparison of the relative inhibitory action, at a given concentration, of a series of leucine peptides. If the growth curves for equivalent concentrations of L-leucine and of L-leucine peptides can be superimposed by a shift parallel to the abscissa, the value of \( t_1 \) for the peptide may be compared directly with the value of \( t_1 \) for L-leucine itself. The difference between these two values of \( t_1 \) (\( \Delta t_1 \)) is a measure of the extent to which the peptide lengthens the lag phase. In practice, growth curves were obtained simultaneously for L-leucine and for an L-leucine peptide at concentrations (in leucine equivalents) of 0.1, 0.4, 0.8, 2.4, and 4.8 \( \mu M \), and the \( \Delta t_1 \) was estimated to the nearest half hour for each concentration. Although the \( t_1 \) for a specific concentration of a test compound might vary by 2 or 3 hours from one experiment to another, the value of \( \Delta t_1 \) was quite reproducible.

In Table I are given the data for the L-leucine peptides which have been investigated. At concentrations up to, and including, 2.4 \( \mu M \), all the peptides gave growth curves which could be superimposed on the curve for the equivalent concentration of L-leucine. At the concentration of 4.8 \( \mu M \) of peptide leucine, however, the growth curves for some peptides had a less steep slope than those at the lower concentrations. Although the lag period at 4.8 \( \mu M \) was somewhat longer than at 2.4 \( \mu M \), the \( \Delta t_1 \) is not a true measure of the inhibitory action of these peptides at the 4.8 \( \mu M \) level. The change in slope of the growth curves indicated, therefore, that, at the higher concentration, the peptides also caused a reduction in the rate of logarithmic growth. Furthermore, the maxima of these curves were usually lower than the expected value of 0.250, which suggests poor utilization of peptide leucine for growth. If, as suggested previously (2), rapid growth in media supplemented with an inhibitory peptide is possible only after the concentrations of peptide have been reduced, possibly by hydrolysis, to a level which is no longer inhibitory, the slower growth rate
at 4.8 µM of peptide leucine may be a reflection of the fact that enough unchanged peptide was present at all times to interfere with normal growth.

**Table I**

**Inhibition of Growth of Leucineless Mutant by Leucine Peptides**

The time, in hours, required for attainment of half maximal growth at any given concentration of the test compound is denoted by \( t_4 \). \( \Delta t_4 \) is calculated by subtracting \( t_4 \) for a given concentration of L-leucine from \( t_4 \) for the equivalent concentration of peptide. The values of \( t_4 \) for L-leucine were 0.1 µM, 18.0 ± 1.6 (7); 0.4 µM, 20.5 ± 1.3 (8); 0.8 µM, 22.0 ± 1.3 (8); and 2.4 µM, 22.0 ± 1.4 (8). The precision is indicated by the average deviation; the number of experiments is shown in parentheses.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>0.1 µM</th>
<th>0.4 µM</th>
<th>0.8 µM</th>
<th>2.4 µM</th>
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<tr>
<td>L-Leucylglycine</td>
<td>+3.0</td>
<td>+6.5</td>
<td>+8.5</td>
<td>+18.0</td>
</tr>
<tr>
<td></td>
<td>±0.4 (4)</td>
<td>±1.1 (4)</td>
<td>±0.9 (4)</td>
<td>±2.2 (4)</td>
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<td>+11.5</td>
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<td>±2.3 (3)</td>
<td>±2.0 (3)</td>
<td>±3.0 (3)</td>
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<tr>
<td>L-Leucylglycyl-L-leucine</td>
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<td>+11.5</td>
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<td>±5.0 (3)</td>
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<td>±0.5 (2)</td>
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<td>+10.5 (1)</td>
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<td>±0 (2)</td>
<td>±0 (2)</td>
<td>±0.3 (2)</td>
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<tr>
<td>Glycyl-L-leucylglycyl-L-leucine</td>
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<td>+4.0</td>
<td>+7.5</td>
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<tr>
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<tr>
<td>Glycylglycyl-L-leucylglycine</td>
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<td>+3.5</td>
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<tr>
<td></td>
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<td>±0.3 (2)</td>
<td>±0.3 (2)</td>
<td>±0.5 (2)</td>
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<tr>
<td>L-Leucyl-L-tyrosine†</td>
<td>+2.0</td>
<td>+3.0</td>
<td>+3.5</td>
<td>+4.5</td>
</tr>
<tr>
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<td>±1.8 (3)</td>
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<td>L-Prolyl-L-leucine</td>
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<td>−1.0</td>
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<td>+0.5</td>
</tr>
<tr>
<td></td>
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<td>±0.3 (2)</td>
<td>±0.3 (2)</td>
<td>±0 (2)</td>
</tr>
</tbody>
</table>

* Slope of curve less steep than that for equivalent concentration of L-leucine.
† Freshly prepared solutions of this peptide gave only slight inhibition, but somewhat greater inhibition was noted with solutions that had been left at room temperature for several days. This difference was not observed with the other peptides tested.

The data in Table I show a considerable variation in the inhibitory action of the peptides which have been tested. Although, in most cases, \( \Delta t_4 \) rises as the initial concentration of the peptide is increased, glycyl-
glycyl-L-leucylglycine (GG-L-LG) and L-leucyl-L-tyrosine were only slightly inhibitory, and L-prolyl-L-leucine gave no significant inhibition, at all the concentrations tested. The results with GG-L-LG indicate that neither the presence of glycine residues nor the lengthening of the peptide chain necessarily favors the inhibitory action of leucine peptides. This is further supported by the strong inhibitory action of L-L-L-L. In the series of peptides containing L-leucine and glycine, the greatest inhibition (at 2.4 μM of peptide leucine per 10 ml.) was observed with those peptides in which L-leucine is a terminal amino acid. Glycyl-L-leucylglycyl-L-leucine (G-L-LG-L-L) represents a special case among the leucine peptides examined for their inhibitory action; a representative series of growth curves for this peptide is shown in Fig. 1. At low concentrations, the inhibitory effect of the compound was small and the growth rate was slow. Furthermore, at levels of 0.05 and 0.1 μM of peptide leucine, the leucine was utilized poorly. Not until the concentration reached 0.8 μM was the growth rate normal, and only at the higher concentrations was the inhibitory effect marked.

Changes in the aeration of the culture or in the pH of the medium caused little effect on the inhibitory action of the L-leucine peptides. An experiment with G-L-L (0.1 to 2.4 μM) in which the tubes were shaken vigorously during the incubation period gave approximately the same values for ΔΔt as those obtained when the tubes were incubated in a stationary position. Alteration of the pH of the medium from the usual value
of 6.9-7.0 to 6.0 or to 7.4 did not cause any marked change in $\Delta t_1$ for G-L-L or L-leucylglycylglycine (L-LGG) (0.1 to 4.0 $\mu$M).

It has been reported (2) that the inhibitory action of G-L-L on the leucineless mutant was not reversed by L-leucine. L-Leucine also will not reverse the inhibition caused by L-LG, glycyl-L-leucylglycine (G-L-LG), glycyl-L-leucyl-L-leucine (G-L-L-L-L), or GG-L-LG. Tests made in media containing 0.4 $\mu$M of each peptide and 0.4 to 24.0 $\mu$M of L-leucine showed that the inhibitory action of all these peptides was not altered in any way by the presence of L-leucine in the medium. In addition, the growth of strain K-12 and of a threonineless strain (No. 679), both of which can synthesize leucine from the constituents of the minimal medium, also was inhibited by G-L-L. These findings indicate that the inhibition described in the present communication cannot be related to the inability of the leucineless mutant to synthesize leucine.

Plate counts made at intervals of 2 hours from the time of inoculation of the leucineless mutant into a medium containing 0.4 $\mu$M of n-leucine or 0.4 $\mu$M of G-L-L showed that the dipeptide was not bactericidal and that it served only to delay the initiation of active cell multiplication. Preliminary experiments have shown that some inhibition always results when G-L-L (2.4 $\mu$M) is added to culture tubes at any time between inoculation and the onset of active cell multiplication; i.e., during the first 12 to 15 hours of incubation. Other experiments in which G-L-L or G-L-L-L-L (2.4 $\mu$M) was added to cultures containing L-leucine (2.4 $\mu$M) at the time when cell multiplication had just begun indicated that the initial rate of growth was somewhat reduced compared to the growth rate in the absence of the peptides. Under these conditions, therefore, the peptides still were able to interfere with normal growth.

**Peptides of n-Leucine**—In addition to the L-leucine peptides listed in Table I, the following n-leucine peptides were examined for their possible inhibition of bacterial growth: glycyl-n-leucine, glycyl-n-leucylglycine, and glycyl-L-leucyl-n-leucine (G-L-L-D-L). The compounds were tested first, at concentrations up to 24.0 $\mu$M, for their ability to serve as a source of leucine for growth. They also were tested, at the same concentrations but in the presence of 0.4 $\mu$M of n-leucine, for their ability to spare the L-leucine requirement and to inhibit the growth of the leucineless mutant on L-leucine. The results of all tests were negative. In the tests for inhibition, the values for $\Delta t_1$ at the level of 24.0 $\mu$M of peptide leucine were $-0.5$ to $+1.5$ hours.

The complete inactivity of G-L-L-D-L was especially striking. Under the experimental conditions employed in this study, the mutant strain obviously cannot cleave the tripeptide to give G-L-L, which has both growth-promoting and inhibitory activity; any d-leucine which might
have been formed would not have been utilized for growth (1). The enzymes involved in the conversion (hydrolysis) of G-L-L-L-L to a compound utilizable in place of L-leucine, therefore, must have a high degree of specificity. Furthermore, the inhibition caused by the L,L diastereoisomer also must involve some mechanism which is highly specific with respect to the configuration of the inhibitor.

**Leucine Peptides As Growth Factors**—In Table II are given the concentrations, in micromoles of peptide leucine per 10 ml., required to produce half maximal growth of the leucineless mutant. These values were estimated from concentration curves obtained by plotting the maximal density ultimately attained in the presence of each test concentration of peptide against the concentration used. In contrast to the other concentration curves, which were of the usual form (1, 2), the curve for G-L-L-G-L-L was S-shaped; at 0.1 and 0.2 µM only about half of the peptide leucine appeared to be used, while at higher concentrations almost all the leucine became available for growth.

In the range of concentrations giving half maximal growth, there were no marked differences among the various L-leucine peptides in their ability to substitute for L-leucine. As noted above, D-leucine peptides had no detectable growth-promoting action, even when they were present in concentrations 500 times greater than the concentrations of analogous L-peptides which gave measurable growth.

### Table II

**Relative Growth-Promoting Activity of Leucine Peptides for Leucineless Mutant**

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Amount* required for half maximal growth† µM per 10 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>0.30</td>
</tr>
<tr>
<td>L-Leucylglycine</td>
<td>0.33</td>
</tr>
<tr>
<td>L-Leucylglycylglycine</td>
<td>0.48</td>
</tr>
<tr>
<td>L-Leucylglycyl-L-leucine</td>
<td>0.48</td>
</tr>
<tr>
<td>L-Leucyl-L-leucine</td>
<td>0.38</td>
</tr>
<tr>
<td>L-Leucyl-L-tyrosine</td>
<td>0.50</td>
</tr>
<tr>
<td>Glycyl-L-leucine</td>
<td>0.36</td>
</tr>
<tr>
<td>Glycyl-L-leucylglycine</td>
<td>0.33</td>
</tr>
<tr>
<td>Glycyl-L-leucyl-L-leucine</td>
<td>0.30</td>
</tr>
<tr>
<td>Glycyl-L-leucylglycyl-L-leucine</td>
<td>0.42</td>
</tr>
<tr>
<td>Glycylglycyl-L-leucylglycine</td>
<td>0.36</td>
</tr>
<tr>
<td>L-Prolyl-L-leucine</td>
<td>0.41</td>
</tr>
</tbody>
</table>

* Expressed in leucine equivalents.
† Estimated from concentration curves drawn by plotting the maximal optical density at each concentration of the test compound against the concentration used. At half maximal growth, the optical density is 0.125.
Since practically all of the L-leucine in peptide combination ultimately became available for growth, it must be concluded that the mechanism whereby the organism overcomes the inhibitory action of a peptide does not destroy that portion of the L-leucine residue required for growth. Complete hydrolysis of the peptides to yield L-leucine seems to be the most probable mechanism both for the reversal of the inhibition and for the process by which peptide leucine is made available for growth.

Growth-Promoting Action of Acetyl-L-leucine—Previous experiments (1) showed that acetyl-L-leucine was not an active growth factor for the leucineless mutant, although it spared the requirement for L-leucine. The amount of sparing action was found to be dependent upon the initial amount of L-leucine present in the medium. The data on which these conclusions were based were obtained by measurement of bacterial growth after 24 hours incubation. The activity of acetyl-L-leucine has been reinvestigated by following bacterial growth as a function of time.

Fig. 2 contains a representative series of growth curves showing the sparing action of acetyl-L-leucine when 0.4 \( \mu \text{M} \) of L-leucine and increasing amounts of the acetyl derivative were present in the medium. Other tests for sparing action were carried out with mixtures of 0.1 or 0.2 \( \mu \text{M} \) of L-leucine and up to 40 \( \mu \text{M} \) of acetyl-L-leucine. As is shown in Fig. 2, the growth curves were diphasic. During the first phase, the curves obtained...
with the mixtures rose rapidly and were exactly like that for L-leucine alone. In the second phase, the rate of growth, except in the presence of very high concentrations of acetyl-L-leucine, was reduced and the slopes of the curves appeared to depend upon the concentration of acetyl-L-leucine originally present. Of all the mixtures tested, only that composed of 0.4 \( \mu M \) of L-leucine and 30 \( \mu M \) of acetyl-L-leucine gave a growth curve in which there was no obvious change in slope. With mixtures containing 0.2 \( \mu M \) of L-leucine, the presence of as much as 40 \( \mu M \) of the acetyl compound still gave a definitely diphasic curve, although the change in slope was not very great. With a mixture of 0.1 \( \mu M \) of L-leucine and 40 \( \mu M \) of acetyl-L-leucine, the slope of the second phase was no more than half that of the first.

Similar diphasic curves were obtained by allowing the organism to grow almost to the maximum in the presence of 0.4 \( \mu M \) of L-leucine and then adding acetyl-L-leucine. Addition of at least 30 \( \mu M \) of the acetyl derivative was required to produce a second rapid rise in the growth curve, although only 0.2 \( \mu M \) of additional L-leucine gave the same effect. It may be concluded, therefore, that, when mixtures of L-leucine and its acetyl derivative are present initially, the first phase of the curve represents growth on the L-leucine supplied in the medium, and the second phase growth on acetyl-L-leucine, which is gradually converted either to L-leucine or to another compound which serves in place of L-leucine.

The utilization of acetyl-L-leucine for growth was not only slow but was also very inefficient since no more than a small fraction of the potential L-leucine became available for growth. From the final maxima of the diphasic curves, rough estimates were made of the percentage of acetyl-L-leucine which might have been converted to L-leucine to produce the extra growth obtained in the second phase. With mixtures containing 0.1 \( \mu M \) of L-leucine and 1 to 40 \( \mu M \) of acetyl-L-leucine, the estimated L-leucine equivalence of the acetyl compound was 1 per cent. In the presence of 0.2 \( \mu M \) of L-leucine and 1 to 40 \( \mu M \) of acetyl-L-leucine, the value was 2 per cent, and with 0.4 \( \mu M \) of L-leucine and 1 to 30 \( \mu M \) of acetyl L-leucine it was 3 per cent. Clearly, the fraction of acetyl-L-leucine which becomes available for growth, as well as the rate at which it is used, depends upon the amount of L-leucine originally present in the medium and, thus, upon the number of cells in the culture at the time the supply of L-leucine is exhausted and growth on acetyl-L-leucine begins.

The variation in the slopes of the growth curves during the second phase, showing an increased growth rate with increasing concentrations of acetyl-L-leucine, and the poor utilization of the L-leucine supplied as the acetyl derivative are similar to the results obtained with L-leucinamide (2). These two compounds probably are made available for growth by
enzyme systems which are relatively inactive compared to those involved in the utilization of the unsubstituted L-leucine peptides.

Test Compounds

Carbobenzoxyglycinhydrazide—10 gm. of carbobenzoxyglycine were dissolved in 100 ml. of methanol containing 3.65 gm. of HCl. The solution was allowed to stand at room temperature for 36 hours, the solvent was removed in vacuo, and the residual syrup was dissolved in 50 ml. of methanol. 3 ml. of hydrazine hydrate were added; after 24 hours at room temperature, there had crystallized 7.2 gm. of the product; m.p. 116–117°.

C₁₉H₁₆O₄N₄ (223.2). Calculated, N 18.8; found, N 18.5 (Dumas)

Carbobenzoxyglycyl-L-leucinhydrazide—6.4 gm. of carbobenzoxyglycinhydrazide were dissolved in a mixture of 50 ml. of water, 5 ml. of glacial acetic acid, and 3 ml. of concentrated hydrochloric acid, and converted to the azide in the usual manner by the addition of aqueous NaNO₂ (2.1 gm.). The azide was extracted with ethyl acetate, and the ethyl acetate solution was washed with water and dilute aqueous bicarbonate. After being dried over Na₂SO₄, the azide solution was added to an ethyl acetate solution of L-leucine methyl ester (prepared from 10 gm. of the hydrochloride), and the solution was left at room temperature for 24 hours. The mixture was then washed successively with dilute hydrochloric acid, dilute aqueous bicarbonate, and water, and then dried over Na₂SO₄. On evaporation of the solution in vacuo, an oil (6 gm.) resulted which, on trituration with a few ml. of ethyl acetate, gave a crystalline material (0.25 gm.). This proved to be N,N'-bis(carbobenzoxyglycyl)hydrazine; after recrystallization from ethanol, it melted at 138°.

C₂₉H₂₅O₅N₄. Calculated. C 57.9, H 5.3, N 13.5
414.4 Found. " 57.9, " 5.7, " 13.3

The remainder of the coupling product was dissolved in 60 ml. of absolute ethanol, 1.5 ml. of hydrazine hydrate were added, and the solution was allowed to stand at room temperature. After 36 hours, 4.2 gm. of the carbobenzoxydipeptide hydrazide had crystallized. After recrystallization from ethanol-ether, it melted at 134°.

C₁₉H₂₄O₅N₄ (336.3). Calculated, N 16.7; found, N 16.6 (Dumas)

Glycyl-L-leucylglycine—2 gm. of carbobenzoxyglycyl-L-leucinhydrazide were converted to the corresponding azide in the usual manner. The ethyl acetate solution of the azide was added to an ethyl acetate solution of glycine ethyl ester (prepared from 5 gm. of the hydrochloride). From
the reaction mixture, 1.8 gm. of the carbobenzyxtripeptide ester were obtained; m.p. 109°.

\[ C_{20}H_{19}O_4N_3 (407.4). \text{ Calculated, N 10.3; found, 10.2} \]

1 gm. of the methyl ester was saponified in the usual manner to yield a syrupy carbobenzyxtripeptide, which was subjected to hydrogenolysis with palladium-black as the catalyst. Yield of tripeptide, 0.4 gm.; \([\alpha]_D^{22} = -41.8°\) (1.9 per cent in water).

\[ C_{16}H_{10}O_4N_3 (245.3). \text{ Calculated, N 17.1; found, N 16.9} \]

This tripeptide has been synthesized previously by a slightly different method (3); the optical rotation was \([\alpha]_D^{24} = -41.2°\) (2.5 per cent in water).

**Glycyl-L-leucyl-L-leucine**—The reaction between carbobenzyoxyglycyl-L-leucinazide (prepared from 2 gm. of the hydrazide) and L-leucine methyl ester (prepared from 2.5 gm. of the hydrochloride) was conducted in the usual manner. 2 gm. of the carbobenzyxtripeptide ester were obtained; m.p. 131-132°.

\[ C_{23}H_{35}O_6N_3 (449.5). \text{ Calculated, N 9.3; found, N 9.4} \]

Saponification of 1.3 gm. of this ester yielded a crystalline carbobenzyxtripeptide (0.7 gm.; m.p. 137°) which was converted to the free peptide by catalytic hydrogenolysis. Yield, 0.4 gm.; \([\alpha]_D^{22} = -66.7°\) (1.7 per cent in NHCl).

\[ C_{14}H_{25}O_4N_3. \text{ Calculated. C 55.8, H 9.0, N 13.9} \]
\[ 301.3 \text{ Found. “ 55.5, “ 9.0, “ 13.8} \]

**Glycyl-L-leucyl-D-leucine**—This compound was prepared in a manner similar to that employed for the synthesis of the diastereoisomer. Yield of the carbobenzyxtripeptide ester, 1.9 gm.; m.p. 117–118°.

\[ C_{25}H_{30}O_6N_3 (449.5). \text{ Calculated, N 9.3; found, N 9.4} \]

Saponification of 1 gm. of the ester gave 0.6 gm. of the carbobenzyxtripeptide (m.p. 144–145°) which was converted to the free peptide (0.3 gm.) by catalytic hydrogenolysis. \([\alpha]_D^{22} = 0°\) (1.5 per cent in NHCl).

\[ C_{14}H_{27}O_4N_3. \text{ Calculated. C 55.8, H 9.0, N 13.9} \]
\[ 301.3 \text{ Found. “ 55.6, “ 9.1, “ 13.9} \]

**L-Leucylglycyl-L-leucine**—Carbobenzoxy-L-leucinazide (prepared from 2 gm. of the hydrazide) and glycyl-L-leucine methyl ester (prepared from 1.5 gm. of glycyl-L-leucine) were allowed to react in the usual manner. 2 gm. of the carbobenzyxtripeptide ester were obtained; m.p. 89–91°.

\[ C_{27}H_{39}O_6N_3 (449.5). \text{ Calculated, N 9.3; found, N 9.3} \]
Saponification of the ester gave a syrupy carbobenzoxytripeptide which was subjected to catalytic hydrogenolysis. Yield of free peptide, 0.4 gm.; $[\alpha]_{D}^{20} = +5.6^\circ$ (2.5 per cent in $\pi$ HCl).

$$C_{14}H_{27}O_{3}N_{3} (221.3). \quad \text{Calculated, N 13.9; found, N 13.9}$$

Glycyl-L-leucylglycyl-L-leucine—Carbobenzoxyglycyl-L-leucinazide (prepared from 2 gm. of the hydrazide) and glycyl-L-leucine methyl ester (prepared from 1.5 gm. of glycyl-L-leucine) were allowed to react in the usual manner. 2.4 gm. of the carbobenzoxytetrapeptide ester were obtained; m.p. 88–90°.

$$C_{25}H_{35}O_{7}N_{4} (506.5). \quad \text{Calculated, N 11.0; found, N 10.8}$$

Saponification of 1 gm. of the ester gave a syrupy carbobenzoxytetrapeptide which was subjected to catalytic hydrogenolysis. Yield of free peptide, 0.5 gm.; $[\alpha]_{D}^{20} = -52.6^\circ$ (1.5 per cent in $\pi$ HCl).

$$C_{16}H_{30}O_{5}N_{4} (358.3). \quad \text{Calculated, N 15.6; found, N 15.7}$$

Most of the other leucine peptides employed in this study were synthesized by methods described previously. For the following compounds the bibliographic references are L-leucylglycine (3), L-leucylglycylglycine (4), L-leucyl-L-leucine (5), glycyl-D-leucine (6), glycyl-D-leucylglycine (3), glycylglycyl-L-leucylglycine (3), and L-prolyl-L-leucine (7). The L-leucyl-L-tyrosine and glycyl-L-leucine were Hoffmann-La Roche preparations.

**DISCUSSION**

The suggestion has been offered previously (8, 9) that certain of the peptide antibiotics may inhibit bacterial growth by interference with the incorporation of essential amino acids into the bacterial peptides and proteins. This working hypothesis was based on the finding that penicillin inhibits the utilization of glycine for the growth of strain SF, but has no effect when glycine is offered to the organism as a component of a peptide, *e.g.* L-leucylglycine. A similar conclusion as to the possible rôle of penicillin in blocking protein synthesis has recently been drawn from studies with *Staphylococcus aureus* (10). Such inhibition of protein synthesis by peptide antibiotics may well represent another general type of antimetabolite action, in which the introduction of one or several key amino acids into peptide linkage is selectively blocked. It may be assumed that this action is exerted upon the enzymatic step or steps directly concerned with the formation of a peptide bond rather than upon one of the energy-yielding processes to which peptide synthesis must be coupled. Clearly, the inhibition of the latter reactions by an antibiotic would also prevent peptide synthesis, which is an endergonic process. Such a mechanism...
may be involved in the action of aureomycin, which has recently been reported to block aerobic phosphorylation (11).

The data presented in the present communication suggest that the bacteriostatic effect of synthetic leucine peptides on the growth of the leucineless mutant is due to a selective inhibition of the incorporation of L-leucine into peptide linkage. This inhibition may be overcome by cleavage of the inhibitory peptides by the bacterial peptidases. It would seem, therefore, that, by suitable variation of the chemical structure of the inhibitory peptides, synthetic antibiotic agents might be prepared which would combine the structural features necessary to block the incorporation of L-leucine into peptide linkage with those features which prevent rapid hydrolysis by bacterial enzymes (9).

SUMMARY

A study has been made of the growth response of a leucineless mutant of Escherichia coli to a number of leucine peptides. Most of the peptides of L-leucine exerted an inhibitory effect upon the initiation of logarithmic growth; in general, the duration of the lag phase of growth increased with increasing concentrations of peptide in the medium. However, approximately the same amount of bacterial growth was ultimately attained in the presence of equivalent concentrations of L-leucine and of L-leucine peptides. None of the peptides of D-leucine had growth-promoting or growth-inhibiting activity for the leucineless mutant. Acetyl-L-leucine, which spared the requirement of the mutant for L-leucine, was utilized poorly as a growth factor in place of the amino acid.

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