STUDIES OF THE EFFECTS OF CERTAIN AMINO ACIDS AND PEPTIDES ON THE UTILIZATION OF SERINE IN *LEUCONOSTOC MESENTEROIDES* P-60

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PREFACE

The microbiological technique is a most useful tool not only for assay of vitamins and amino acids but also for the study of nutrition in microorganisms. Studies of their amino acid nutrition revealed the existence of interrelationships between dietary amino acids. During the past ten years the interrelationships between amino acids in microorganisms have received considerable attention in order to investigate nutritional aspects as well as intermediary metabolism.

For one of the interrelationships between amino acids, it has been known that the growth-promoting activity of peptides is somewhat greater than that of free amino acids under certain conditions.

The present study reported in this thesis involved one phase of peptide effects on the bacterial growth with respect to the interrelationship between a pair of amino acids.

One of the greatest difficulties encountered in this study was brought about by the high contamination of some of the peptides employed. Because of this, the interpretation of the experimental results was extremely complicated, but it is considered that the results have contributed to our knowledge of peptide activity in microorganisms.

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INTRODUCTION

An outstanding feature of studies of interrelationships among amino acids in bacterial nutrition is that they elucidate the metabolic functions of certain amino acids as well as the pathways of protein biosynthesis. Interrelationships among amino acids include not only competitive antagonism, perhaps the most well known and widely studied type of interrelationship, but also various other types of interaction. Competitive antagonism is a condition in which the utilization of an amino acid is competitively inhibited by the presence of other amino acids. This phenomenon was first recognized in early studies of the amino acid requirements of microorganisms, in which the protein hydrolysate of the medium was replaced by a mixture of amino acids (1).

Several instances of the occurrence of this phenomenon in lactic acid bacteria have been reported recently (2, 3, 4, 5). For example, when the concentration of leucine was limited, isoleucine and valine inhibited the growth of Lactobacillus arabinosus, and the degree of inhibition by isoleucine was greater than that by valine. This phenomenon may be attributed to the structural similarity of the antagonistic amino acids involved (6, 7).

An interrelationship essentially opposite to the above antagonism has been shown in the metabolism of Leuconostoc mesenteroides P-60 (8). In this interrelationship, which involved arginine and proline, a large amount of one amino acid was required for optimal utilization of growth-limiting amounts of the other. The

mechanism of this interrelationship, which was termed an interdependence, has not been established. Interconversion between arginine and proline through the intermediate, ornithine, was considered possible, but was found not to occur. A possible explanation of this relationship between the two amino acids is that the organism may have difficulty in performing "a key metabolic reaction, the formation of the arginine-proline bond" in a suboptimal condition. To test this possibility, partial hydrolysates of bacterial cells and other proteins were used as a peptide source, since the peptides of arginine and proline were not available. These hydrolysates exhibited greater growth-promoting activity than did free proline. While these studies did not exclude factors other than the peptides, three possible explanations for the growth-promoting activity of the peptides were suggested. These were 1) the direct incorporation of the peptides into cellular protein synthesis by the organism, 2) the possible destruction of the free amino acid but not of its peptides, and 3) transpeptidation reactions, which could conceivably facilitate the synthesis of those peptides needed as direct precursors of protein synthesis (9).

Wold (10) found later that the arginine-proline interdependence was not a separate, specific one, but that it existed for many other amino acid combinations and in other lactic acid bacteria in a modified Henderson and Snell medium (11). His arbitrary classification of interrelationships, based on his experimental results, includes the following types:

1. Inhibition of the utilization of a given (growth-limiting) amino acid by the presence of a high amount of another

(modifying) amino acid.

2. Inhibition of the utilization of the limiting amino acid by low amounts of the modifying amino acid.
3. Inhibition of the biosynthesis of a limiting amino acid by the presence of high amount of another amino acid.
4. Inhibition of the biosynthesis of a limiting amino acid by low amounts of other amino acid.

In his experiments he found 76 instances of type 1, 161 of type 2, of which 13 were reversible, 43 of type 3, and 18 of type 4. These interrelationships varied considerably in degree, from very slight to very striking effects of the modifying amino acid on the utilization and on the synthesis of the limiting amino acid. It is apparent that the competitive antagonism described earlier is of type 1, and that the interdependence characterized by arginine-proline is of type 2.

Results obtained in studies of a glycine-arginine interdependence, which was also classified as type 2, were the same as those obtained in the case of the arginine-proline interdependence (10, 12). It was also found that lowering the pH of the medium reduced the interdependence, and that partial hydrolysates of proteins were also more stimulatory than the free amino acid for the growth of Leuconostoc mesenteroides P-60. Several dipeptides of glycine used as a source of glycine in the glycine-arginine interdependence gave a growth response very similar to those shown with the incomplete hydrolysates.

The results of the studies reviewed thus far all appear compatible with the possibility that the existence of this type of inter-

dependence is due to the inability of the organism to synthesize peptides as precursors in protein synthesis, as was originally suggested by Sirny et al. (8).

There are, however, other reports of studies on peptide utilization that make the hypothesis less attractive. It is well-known that in many instances peptides are used less effectively than are free amino acids (13, 14, 15). The use of both naturally-occurring (16) and synthetic peptides (17, 18, 19) has even caused the inhibition of bacterial growth.

If the utilization of peptides occurs only after hydrolysis, and by physical diffusion into the cells, peptides must necessarily be less active than their component free amino acids. Glycine-containing peptides themselves have equal or less activity than free glycine for the growth of Leuconostoc mesenteroides under certain conditions, and the utilization of these peptides occurs after their hydrolysis to free amino acids (14, 15). Since hydrolysis does occur in the resting cells of most bacteria (15, 16, 21, 22, 23, 24), it may be assumed that peptides are hydrolyzed by proteolytic enzymes prior to utilization (25). Furthermore, it has been shown that the most rapidly hydrolyzed of several different peptides was the most active (21).

For some reason, however, there are many cases in which certain peptides are more efficiently utilized by bacteria than are free amino acids (26, 27, 28, 29, 30, 31, 32, 33). The proposed mechanism by which peptides are directly incorporated into bacterial cells appears unlikely for two reasons: 1) the fact that the most bacteria hydrolyze peptides prior to utilization, and 2) the

fact that the non-natural peptides may be utilized after hydrolysis (23). In two particular cases greater utilization of peptides than of the corresponding free amino acids has been explained clearly. Leucyl-tyrosine and glycyl-tyrosine far surpassed tyrosine in growth-promoting activity for Streptococcus faecalis in a medium containing high vitamin B_6, while they were not more active than tyrosine in the absence of vitamin B_6 in the medium. The results were due to the activity of tyrosine decarboxylase, which was non-functional when vitamin B_6 was absent from the medium. Tyrosine, but not its peptides, was decarboxylated by this enzyme. Peptides of tyrosine were hydrolyzed by resting cells of Streptococcus faecalis, but the failure of the tyrosine produced by hydrolysis of tyrosine-containing peptides to undergo decarboxylation by growing cells was ascribed to gradual release of tyrosine at a low concentration, and the higher affinity of protein-synthesizing enzymes than of tyrosine decarboxylase for tyrosine (22).

Another situation in which peptides resulted in higher growth-promoting activity than free amino acids involved mutation of an organism. A low supply of L-histidine caused the development of the histidine-sensitive mutant of Lactobacillus delbrueckii. The mutant culture did not grow without histidine, but did grow with low levels of histidine. At higher levels of histidine, no development of this ability occurred. The parent strain of this organism removed distinctly less histidine from a basal medium than did the mutant strain. Both strains utilized carnosine (beta-alanyl-L-histidine) equally well. Thus, the parent strain cannot

remove L-histidine from dilute solution with the same facility with which it removes the histidine peptide. Consequently, for this organism the histidine peptide showed greater growth-promoting effect (23).

There is no positive evidence which supports the view that the growth-promoting activity of peptides is due to a mechanism involving transpeptidation. It has been suggested, however, that transpeptidation reactions of leucine-containing peptides with other amino acids or other peptides, possibly catalyzed by proteolytic enzymes (34, 35, 36, 37), might conceivably yield all the peptide bonds necessary for the synthesis of the bacterial protein of the leucine-requiring mutant, Escherichia Coli (25). It has also been pointed out that the existence in the mutant of peptidases that hydrolyze the peptides was not incompatible with the utilization of peptides prior to hydrolysis.

Transpeptidation has also been proposed as explanation of the greater activity of serine-containing peptides than free serine on the growth of Lactobacillus delbrueckii. There is, however, no positive evidence supporting this suggestion. In fact, the statement has been made that it is improbable that a simple transpeptidation reaction could transfer serine in the serine-containing peptides to other peptides, whatever the linkage of the serine-containing peptide might be (24).

The ultimate goal of studies of these amino acid interdependences is to elucidate the mechanism or mechanisms, by which growth-limiting amounts of amino acids are utilized by a bacterial cell. Although the stimulatory activity of peptides under certain conditions

is beyond doubt, the question has not yet been clearly answered as to whether or not the interdependence is really due to inability of bacteria to synthesize peptide bonds from a pair of involved amino acids. Whatever the mechanisms of the interdependence and of peptide activity may be, they must be, at any rate, very closely related to each other.

For interdependences between amino acids of the arginine-proline type and arginine-glycine type, it has been previously demonstrated that when the amino acid in growth-limiting amounts is added as a peptide, the need for high amounts of the modifying amino acid is reduced or eliminated. The effect of supplying the modifying amino acid as a peptide was not investigated. In the present studies a system was selected from the previous work done by Wold (10) and an amino acid available in peptide form was used as a modifying amino acid.

GENERAL EXPERIMENTAL PROCEDURE

Assay organism.

The lactic acid bacterium used for the study was Leuconostoc mesenteroides P-60 (ATCC 8042). The organism was kept on an agar medium (Appendix A) as a stab culture, and was transferred to fresh agar medium approximately every two weeks. Following transfer, the culture was incubated at 37°C for 72 hours, and was stored in a refrigerator at 4°C.

When the organism was used for assays, it was transferred into a test tube containing 2 ml. of a sterile liquid medium (Appendix A) and was incubated at 37°C for 18 to 24 hours. After the cells were centrifuged down and the supernatant was removed, they were suspended in 25 ml. of 0.9 % KCl solution. One drop of the suspended cells was added to each assay tube from a 5 or 10 ml. sterile syringe.

Basal medium.

An all-potassium modification (9) of the uniform medium recommended by Henderson and Snell (11) was used for all the work (Appendix B).

In preparing the basal medium, the amino acids, DL-serine and L-leucine, were always omitted. For certain assays, glycine was also omitted. The pH of the media employed in this work was 7.0.

Assay techniques.

Approximately 24 hours preceding assay preparation the organism was transferred from the agar stab culture to the liquid medium,

and was incubated at 37°C for later use.

i) Preparation of assays

Assay racks, each of which contained sixty 18x150 mm. rimless tubes with six tubes per row, were employed in all the assays studied. Assays were always prepared in duplicate.

The aqueous DL-serine solution of 40 µg. per ml. was added to each row, so that six tubes in the row received respectively 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. Distilled water was added to bring the volume in each tube to 0.5 ml. Appropriate amounts of L-leucine or its peptides, and of glycine for certain assays, were added to each tube to bring the volume in each tube to 1.0 ml. The basal medium, with serine, leucine, and glycine omitted, was then added to each tube to give a final total volume of 2.0 ml. Additions of these solutions were carried out by means of a Cannon Automatic Dispenser.

ii) Sterilization

Following the dispension of all the solutions needed, the assay tubes were placed in a preheated autoclave and sterilized for 5 minutes at 15 lb. pressure at 121°C. Five minutes after these conditions were attained, the pressure was reduced to atmospheric, and the tubes removed and cooled to room temperature.

iii) Inoculation and Incubation

The previously-grown bacterial cells were collected and suspended in 0.9 % KCl solution, and one drop of the suspension was added to each tube by means of a sterilized syringe. The tubes were then placed in an incubator at 37°C. Incubation was continued for 66 to 72 hours.

iv) Titration

The growth response was measured by electrometrically titrating the acids* produced by the organism with approximately 0.05 N. KOH solution. The titration was carried out by means of a Cannon Automatic Titrator. A one normal calomel electrode as a reference and a quinhydrone electrode as an indicator were utilized for the titration. The amount of base used for titration to pH 7.3 is expressed in terms of titration counts, 100 counts of which correspond approximately to 4 ml. of the base.

*Leuconostoc mesenteroides P-60 produces equimolecular amounts of lactic acid and acetic acid in the course of its metabolism.

SPECIFIC EXPERIMENTAL PROCEDURE AND RESULTS

Preliminary Studies on the Serine-leucine Interdependence

According to the work done by Wold, the utilization of serine is inhibited by lowering the concentration of L-leucine in a basal medium. In his original observation revealing this interdependence no serine synthesis by the organism was found under the conditions he employed. However in many trials of this interdependence, the serine synthesis, which is manifested by high blanks, i.e., high growth in the absence of added serine, was unavoidable in many of the modifications of the basal medium which had to be studied. The high blanks which resulted prevented accurate interpretation of assay results of the interdependence study. It was previously shown that the serine synthesis in Leuconostoc mesenteroides P-60 was inhibited by the presence of a high amount of DL-alanine (10). The minimum amount of DL-alanine required for inhibiting serine synthesis was shown to be 4 mg. per tube instead of the 2 mg. per tube in the normal Henderson and Snell medium. Thus for this series of experiments, the medium was modified so that each tube contained 4 mg. of DL-alanine. The L-leucine levels were selected as 20, 200, and 800 µg. per tube.

The results of these preliminary experiments are shown in Figure 1, and it can be seen that the interdependence between serine and leucine does exist. However, it should be noted that the interdependence is of considerably lesser degree than the arginine-proline interdependence reported by Sirny et al. (8) or the

arginine-glycine interdependence studied by Wold (10). Thus, in this interdependence, the utilization of serine is reduced by lowering the amount of leucine in the medium, though this effect of leucine as the modifying amino acid is less striking than the known effects of certain other modifying amino acids in other interdependences. It can also be seen in Figure 1 that some serine synthesis occurred at the level of 200 µg. leucine, and that blanks were even higher at the 800 µg. leucine level. Thus it appears serine synthesis increases successively with increase of modifying amino acid, L-leucine.

Effect of L-leucine on the Utilization of Peptide-bound Serine

It has been shown, as previously described, that when a modifying amino acid is supplied in high amount, or when peptides are used as sources of a limiting amino acid, the inhibition of utilization of the limiting amino acid is reduced or eliminated. To confirm these earlier observations, experiments were conducted to compare the growth response to DL-serine with that to glycyl-DL-serine[1] in the presence of 20 µg. and 800 µg. of L-leucine. In an attempt of further inhibition of serine synthesis 6 mg. of DL-alanine were supplied to each tube instead of the 4 mg. used in the preliminary experiments. It is also known that glycine is a precursor of serine synthesized by the organism, and that the serine synthesis is stimulated by increasing concentration of

[1]Obtained from California Foundation for Biochemical Research.

glycine in the medium (10). Since glycine contained in the peptides employed might conceivably increase the serine synthesis, the concentration of total glycine (free and/or peptide-bound) was kept constant at 200 µg. per tube in all studies. It is recognized that this procedure does not completely eliminate the possibility that the peptide-bound glycine may be more stimulatory, in its effect on serine synthesis, than free glycine, even in the presence of a high amount of glycine in the medium.

The growth responses are shown in Figure 2. The utilization of glycyl-DL-serine is much more effectively accomplished than is that of free serine. The need for a high level of leucine is seen to be reduced by replacing serine with its peptide. The increases in the blanks in curves 2 and 4 are probably due to the presence of a high amount (800 µg.) of L-leucine. L-leucine, thus, appears to have slight stimulatory effect on serine synthesis.

Activity of L-leucine-containing Peptides on the Utilization of Serine

To study whether or not peptides have any different effect from free amino acids on the utilization of a limiting amino acid when they are supplied as the source of a modifying amino acid, these next experiments were conducted to compare the effect of the following compounds on the utilization of DL-serine:

	Modifying levels (µg. per tube)
L-leucine[1]	20 - 800

[1,4] Obtained from H. M. Chemical Co. Ltd.

	Modifying levels (µg. per tube)		
Glycyl-DL-leucine[2]	57.2	114.4	-
DL-leucyl-glycine[3]	57.2	114.4	-
Glycyl-L-leucine[4]	28.6	57.2	-
D-arabonyl-L-leucine ethyl ester[5]	46.5	-	-

The numbers appearing in each column are equivalent to each other with respect to their content of L-leucine. Twenty micrograms of L-leucine, which is one-tenth of the amount in the normal Henderson and Snell medium, is adequate to give maximal growth of the organism when no other nutrients are limiting. The amounts indicated in the second column were chosen possibly to reveal more strikingly whether or not peptide-bound leucine would reduce or eliminate the high requirement for L-leucine in the serine-leucine interdependence. The amount of glycine was also kept constant as previously described.

It was found that all peptides tested, except glycyl-L-leucine and D-arabony-L-leucine ethyl ester, gave somewhat greater growth response to serine than did L-leucine (Figures 3a, 3b, 3c). While the amounts of glycyl-L-leucine equivalent to 20 µg. of L-leucine supported as much growth as did 20 µg. of L-leucine, the DL-leucine-containing peptides promoted greater growth responses than did L-leucine. It can be seen that amounts of glycyl-L-leucine and DL-leucyl-glycine equivalent to 40 µg of L-leucine promoted maximal

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[2,3]Obtained from General Biochemicals, Inc.

[5]Obtained from Dr. David G. Doherty, Oak Ridge National Laboratory, Oak Ridge, Tennessee. (J. Biol. Chem. 201, 857 (1953)).

growth as did the presence of 800 µg. of L-leucine, while glycyl-DL-leucine did not. High and variable blanks can also be seen in Figure 3a.

Since these blanks could not be adequately explained in terms of what was already known about serine synthesis, possible contamination of the peptides with serine was next investigated.

For this purpose, peptides were hydrolyzed by autoclaving them in 3 N. HCl solution at 121°C at 15 lb. pressure overnight. Following hydrolysis, the hydrolyzed peptides were compared with L-leucine as to their effect on serine utilization. The results of this experiment are presented in Figure 4. It can be seen by inspection of blank tubes in Figure 4 that L-leucine was not contaminated with serine significantly. It can be safely said that glycyl-L-leucine also did not have contamination with serine. DL-leucine, glycyl-DL-leucine, and DL-leucyl-glycine, which had very much high blanks, must have been contaminated with serine, otherwise D-component of DL-leucine and of DL-leucine-containing peptides should have extremely stimulatory effect on serine synthesis. It is difficult to conceive that the optical antipode of an amino acid which is usually considered inactive for biological system has so much effect on the synthesis of other amino acid. Thus, the DL-leucine-containing peptides appeared to involve an appreciable and essentially similar degree of serine contamination.

With knowledge of this serine contamination of the peptides, the results in Figure 3a are subject to somewhat different interpretation. If the high blanks obtained with the DL-leucine-containing peptides were due only to serine contamination, the blanks for the

equal amounts of the two peptides should have yielded essentially the same values, and all blanks should have been equally high. It can be seen that this was not obtained; instead, not only were large differences obtained between blanks for equal amounts of the two peptides, but also, practically no utilization of the contaminant serine occurred in the presence of glycyl-DL-leucine. From these considerations, it appears that DL-leucyl-glycine has more stimulatory effect on the utilization of contaminant serine than does glycyl-DL-leucine. This does not prove that these peptides are more stimulatory than free leucine in their effect on the utilization of free serine, but it does suggest that there is a difference in activity between one or both of the leucine-containing peptides and free leucine. Furthermore, it appears that the mode of linkage of leucine in the peptides must be responsible for the different effect observed on the utilization of free serine.

The glycyl-L-leucine was not more active than free L-leucine on the utilization of free serine, as can be seen in Figue 3c, growth curves 1 and 3. It can be seen in the same figure that 57.2 µg. of glycyl-L-leucine, which is equivalent to 40 µg. L-leucine, gave almost identical growth curve as the one shown by 800 µg. L-leucine. However, this may not necessarily mean that glycyl-L-leucine equivalent to 40 µg. of L-leucine has as much activity as 800 µg. of L-leucine, because no pronounced interdependence was obtained between serine and leucine in this experiment.

D-arabonyl-L-leucine ethyl ester did not promote any growth. This is probably due to the structural modification of this peptide. The inactivity was possibly due to the ester group of this peptide

rather than the arabonyl group, for it has been reported that an ester form of an amino acid peptide, in contrast to unsubstituted forms of peptides, was much less active for bacterial growth (14).

DISCUSSION

The interdependence in which utilization of serine, especially peptide-bound serine, is more readily accomplished in the presence of high amount of L-leucine was confirmed. Although work has not progressed to a stage permitting the postulation of any definite mechanism of the interdependence and peptide activity, certain possible mechanisms may be considered.

Since resting cells of most bacteria hydrolyze peptides prior to utilization, it is difficult to visualize how the peptide-bound amino acid is utilized more efficiently than is the free amino acid. However, apparently the condition in which resting cells hydrolyze a peptide to component amino acids is not at all optimal for their growth, that is, the experiment is conducted in the absence of all other nutrients except the peptides. Therefore, it is logical to consider that there is some difference between resting cells and growing cells in their action on the peptides supplied. This difference may involve activities of enzymes which catalyze transpeptidation and hydrolysis. Perhaps in the presence of an ample amount of amino acids and other nutrients in a medium, the conditions may be more favorable for transpeptidation of the peptide with other amino acids than for hydrolysis. In the absence of the nutrients the peptide is necessarily hydrolyzed by perhaps a proteolytic enzyme, since the enzyme catalyzing transpeptidation would be non-functional in the absence of other amino acids. Thus in the growing cells transpeptidation may be more likely than hydrolysis. Of course, this does

not exclude the possibility that the successive reactions involving hydrolysis following transpeptidation may occur. Thus, the peptide may be utilized more readily than is a free amino acid by the organism, and the interdependence is reduced. The view is still compatible with the hypothesis that the interdependence is caused by inability of the organism to synthesize peptide bonds under suboptimal condition.

In consideration of the serine-leucine interdependence, it must be emphasized that it is not a reversible relationship, as recognized by Wold, i.e., serine is not needed in high amounts for the utilization of leucine. Thus, the mechanism to explain this interdependence may not be similar to that for a reversible interdependence of the arginine-proline type. Perhaps the organism may be unable to synthesize a particular linkage of peptides under low concentration of serine and leucine, but it may be able to form other linkage easily even under the suboptimal condition, and this may involve difference of activities of enzymes which catalyze the reaction of peptide bond formation.

Glycyl-L-leucine, which was found not to be contaminated with serine, at 20 µg. per tube with respect to L-leucine exhibits an effect essentially equal to that of L-leucine on the utilization of free serine. This may suggest that the peptide is hydrolyzed by the organism, and that the produced L-leucine necessarily gives the same activity as the added L-leucine. However, it seems conceivable that the peptide would be more active than L-leucine on the utilization of

serine at higher concentration of the peptide. This type of peptide activity would be dependent on the concentration of the peptide. In higher concentration of the peptide the chance for serine to interact or to become involved in transpeptidation with the peptide may increase. As another alternative, the peptide might perform some mechanical, rather than chemical, interaction with serine to facilitate diffusion into the cells.

Because of the serine contamination, a positive conclusion as to whether or not the DL-leucine-containing peptides have more stimulatory effect on the utilization of serine than does free leucine cannot be drawn. However, in spite of essentially same degree of serine contamination, the results from the experiments revealed that the two peptides exhibited large differences in blanks, indicating that the activity of peptides on the utilization of serine may probably depend upon the peptide linkages.

Assuming there is no effect of the D-form of leucine contained in these peptides on the utilization and the synthesis of serine, leucyl-glycine is more effective than free leucine in promoting utilization of free serine, since it was shown that DL-leucyl-glycine was more active than glycyl-DL-leucine and that glycyl-L-leucine exhibited essentially the same activity as did L-leucine. Because of this, it seems reasonable to conclude that, at least in some respects, the leucine-containing peptides are more active than free leucine in stimulating utilization of serine.

It may be further suggested that the contamination of the DL-leucine-containing peptides with serine is incredibly high, as can

be seen in Figure 4. Therefore, the D-form of leucine might have conceivably contributed to the very high growth response to free serine. However, this possibility appears quite untenable since it is known that D-leucine is not utilized by this organism, at least under conditions routinely employed.

It appears, at any rate, that unsubstituted peptides containing leucine are not less effective than free leucine with regard to utilization of serine, and that their activities are dependent on the mode of linkage in the peptides.

SUMMARY

In a modified Henderson and Snell medium, L-leucine and four leucine-containing peptides were subjected to studies concerning their effect on the utilization of growth-limiting amounts of DL-serine by a lactic acid bacterium.

These peptides in amounts equivalent to 20 µg. and 40 µg. L-leucine, were compared to 20 and 800 µg. of free L-leucine with respect to their ability to support the growth of Leuconostoc mesenteroides P-60 in the presence of low amounts of serine.

This study supports the view that leucine-containing unsubstituted peptides are not less effective than free leucine on the utilization of serine, and that the activity of the peptides is possibly dependent upon their mode of linkage in the peptide.

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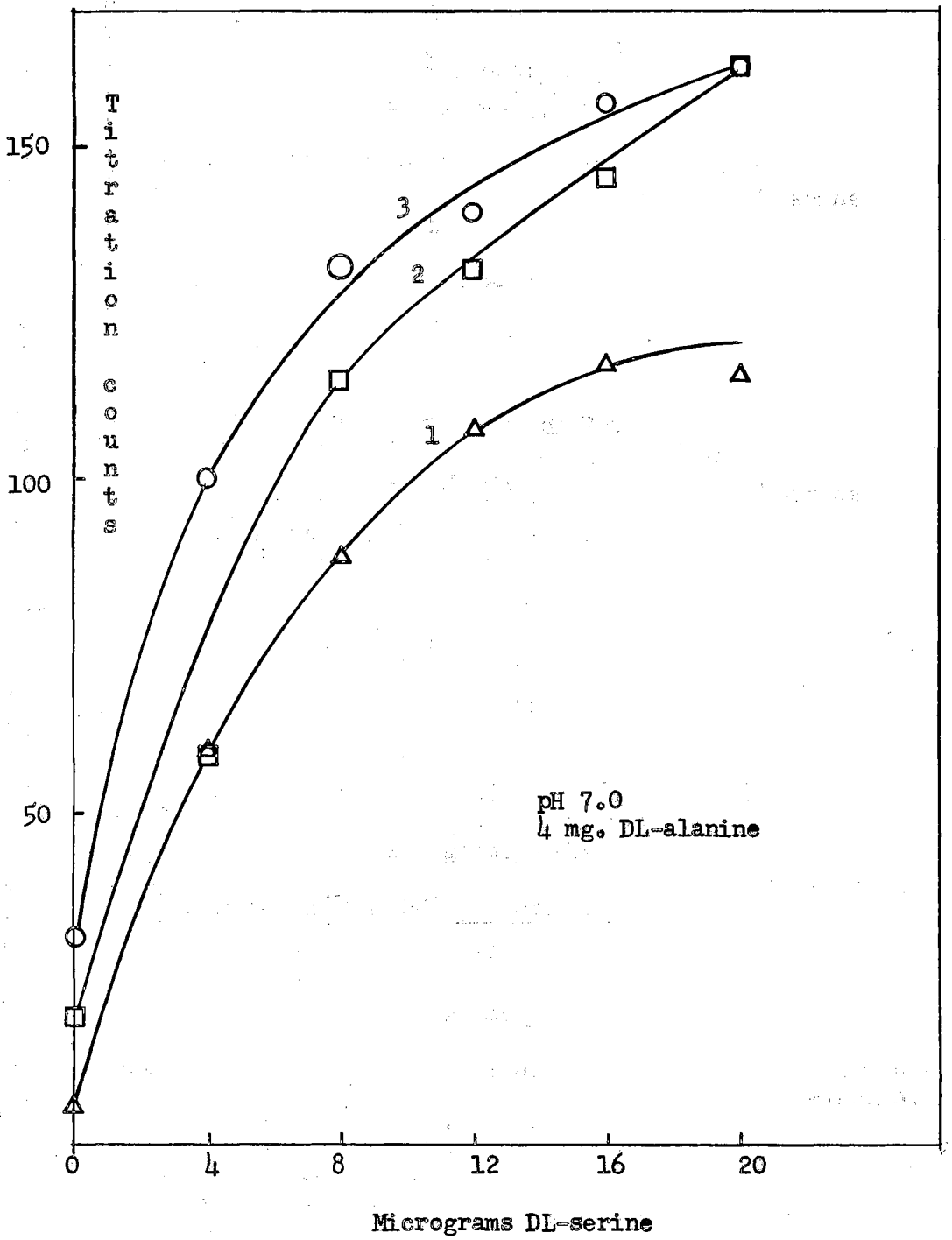
Figure 1

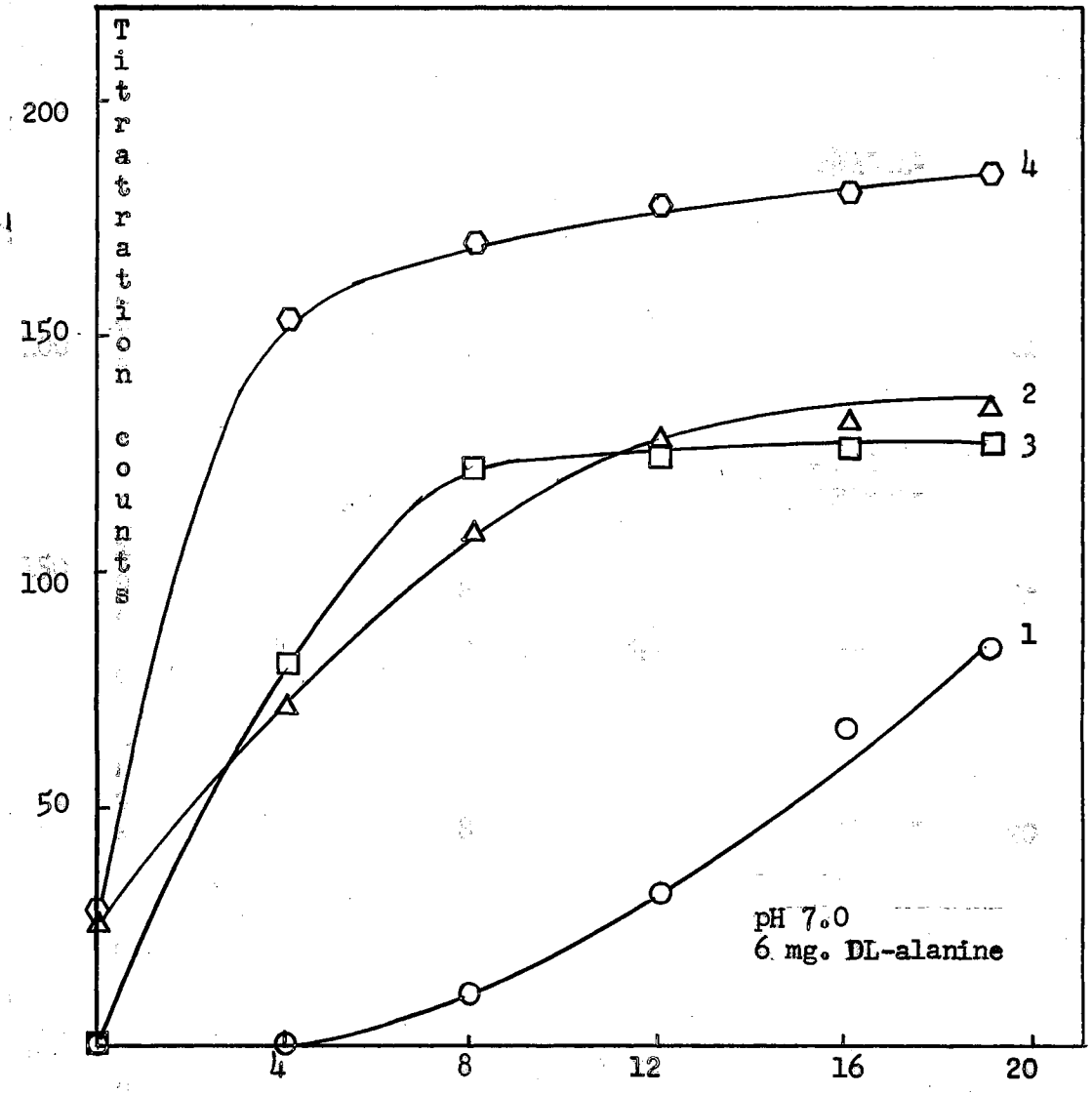
The Effect of Leucine on the Utilization of Serine



Response to serine in the presence of: (1) 20 µg. (2) 200 µg. (3) 800 µg. L-leucine

Figure 2

The Effect of Leucine on the Utilization of Glycyl-DL-serine

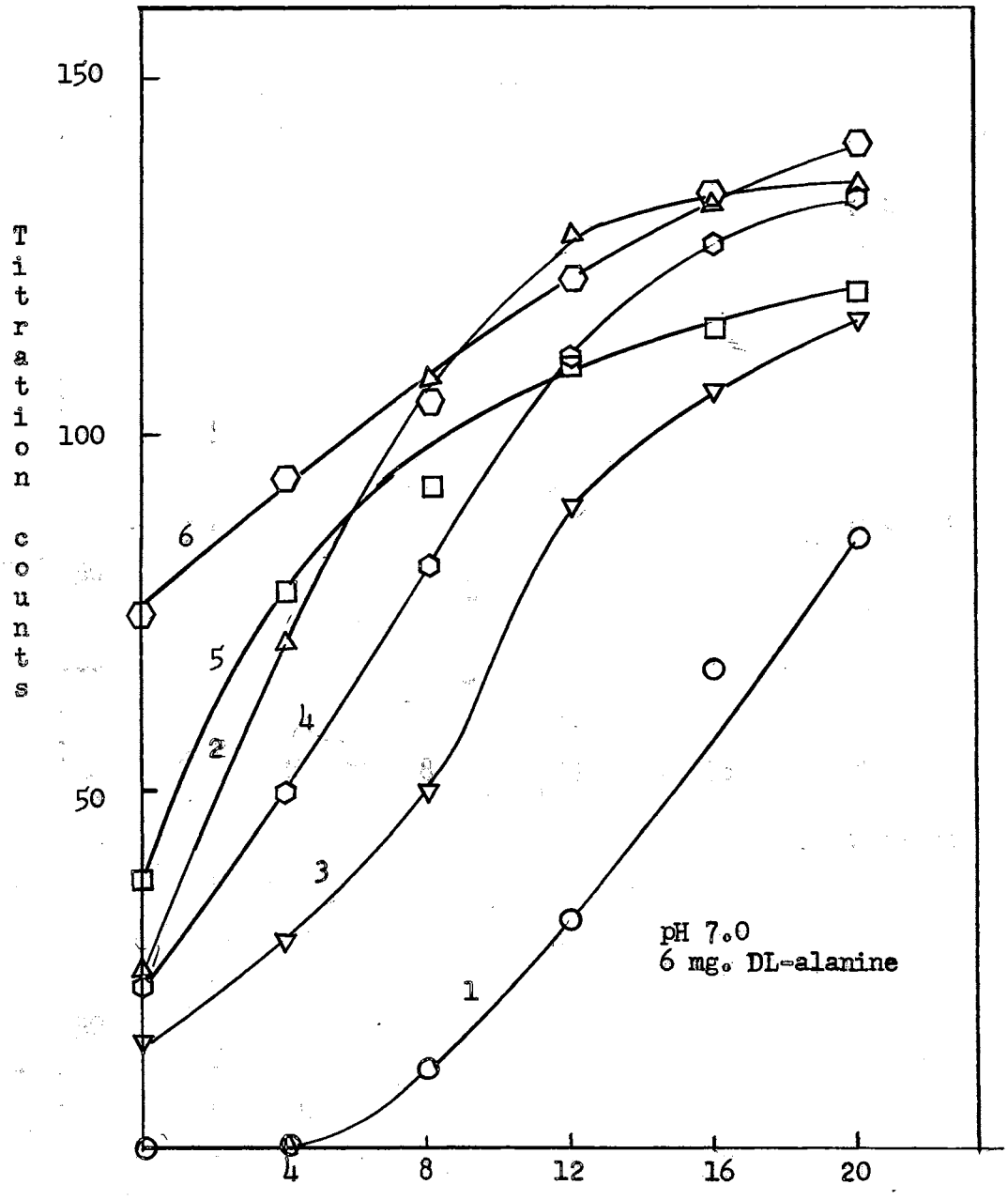


Micrograms DL-Serine, or its Equivalent

Response to DL-serine in the presence of: (1) 20 µg. (2) 800 µg. L-leucine.
Response to glycyl-DL-serine in the presence of: (3) 20 µg. (4) 800 µg. L-leucine.

Figure 3a

The Effect of Leucine-containing Peptides on the Utilization of Serine

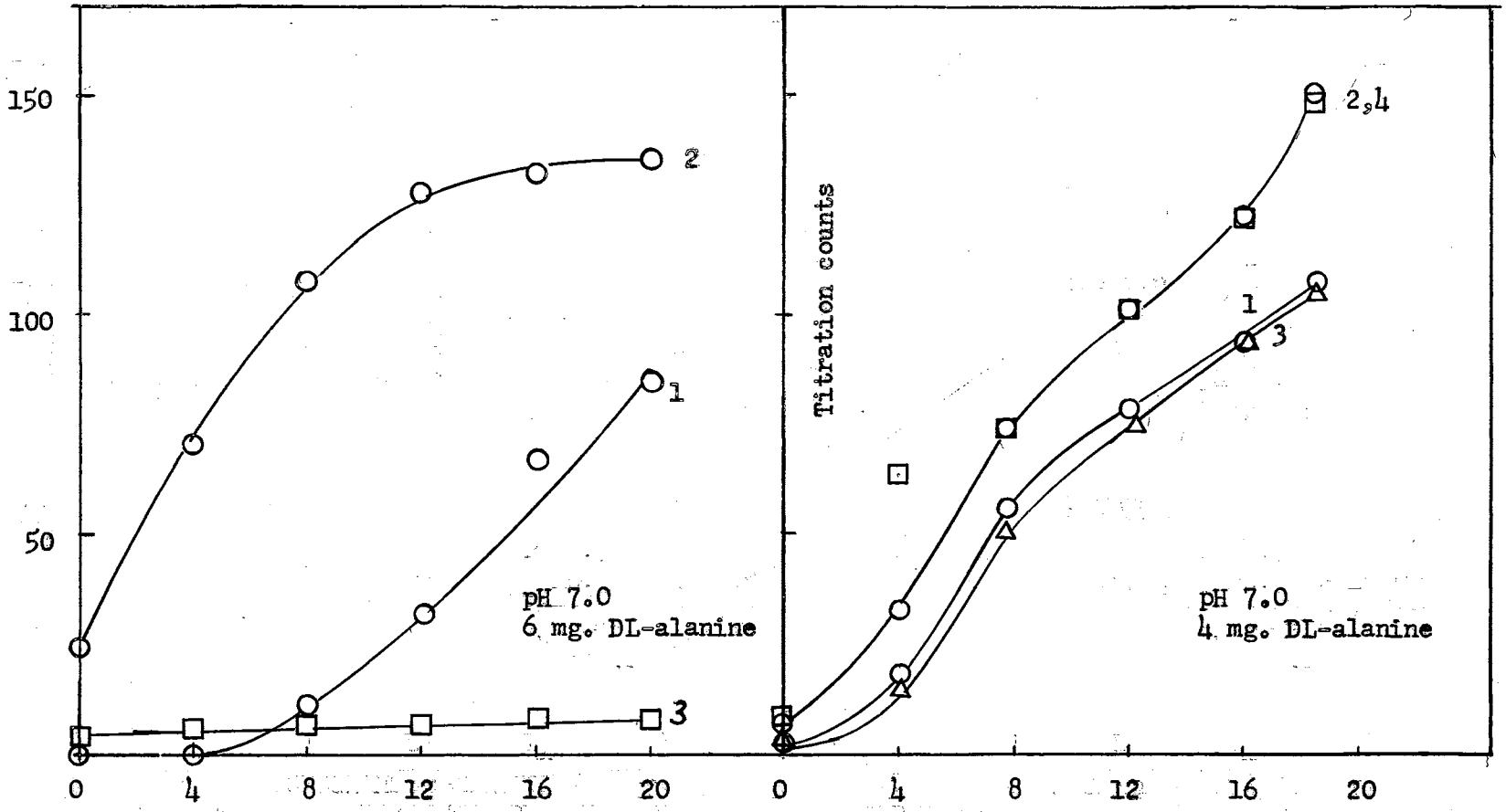


Response to DL-serine in the presence of: (1) 20 µg. L-leucine, (2) 800 µg. L-leucine, (3) 57.2 µg. Glycyl-DL-leucine, (4) 114.4 µg. Glycyl-DL-leucine, (5) 57.2 µg. DL-leucyl-glycine, (6) 114.4 µg. DL-leucyl-glycine.

Figure 3b

Figure 3c

The Effect of Leucine-containing Peptides on the Utilization of Serine

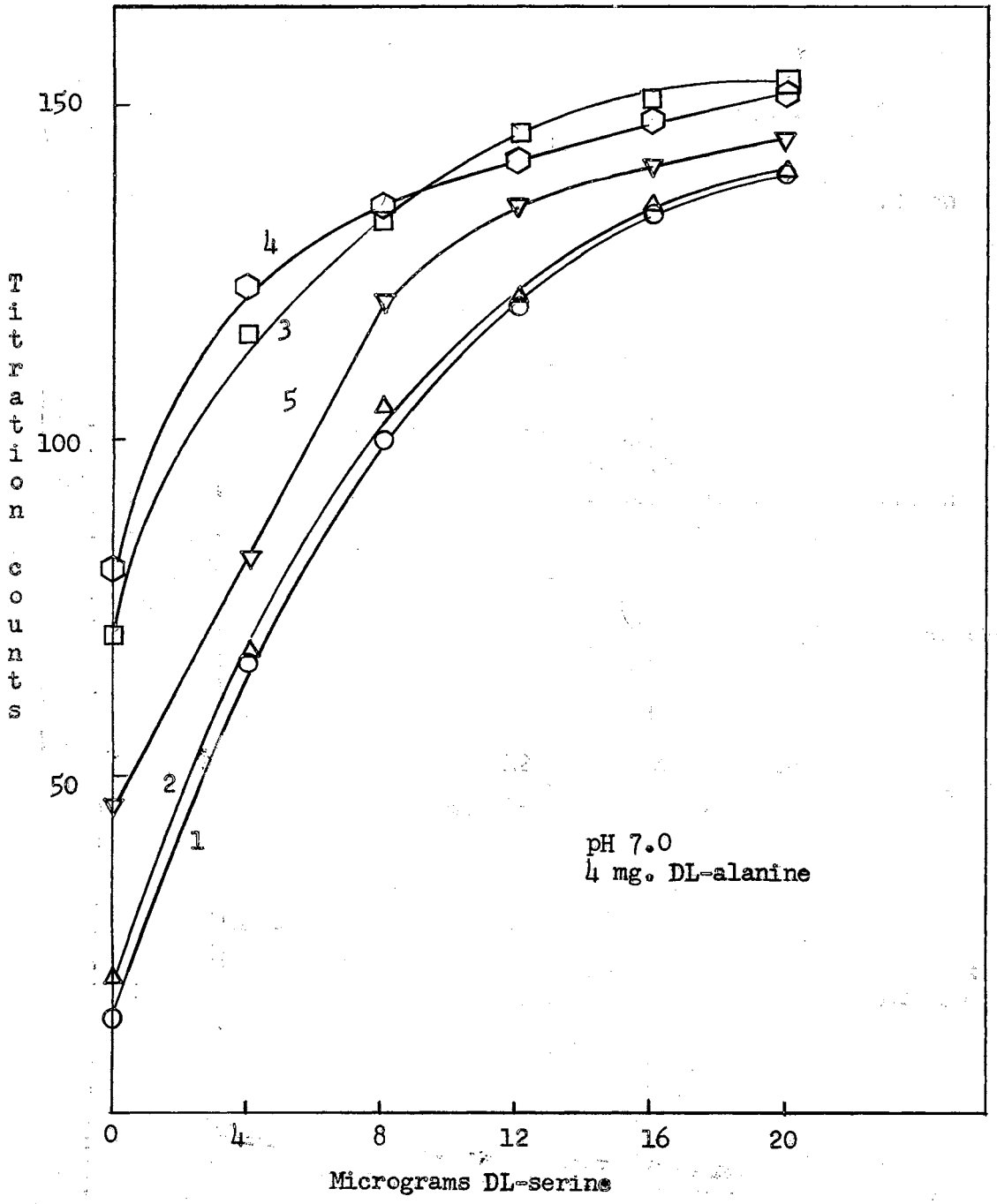


Response to DL-serine in the presence of: (1) 20 µg., (2) 800 µg. L-leucine, (3) 46.5 µg. D-arabonyl-L-leucine ethyl ester.

Response to DL-serine in the presence of: (1) 20 µg., (2) 800 µg. L-leucine, (3) 28.6 µg., (4) 57.2 µg. glycyl-L-leucine.

Figure 4

The Comparison of Activity of Hydrolyzed Peptides with free leucine



Response to DL-serine in the presence of: (1) 20 µg. L-leucine, (2) 28.6 µg. hydrolyzed glycyl-L-leucine, (3) 57.2 µg. hydrolyzed glycyl-DL-leucine, (4) 57.2 µg. hydrolyzed DL-leucyl-glycine, (5) 40 µg. DL-leucine.

APPENDIX

A. Media for Storage and Transfer of Organisms.

Agar medium:

Yeast extract	10.0 gm.
Glucose	2.5 gm.
Agar	15.0 gm.
K-acetate	5.0 gm.

Water to make up to 1000 ml.

Liquid transfer medium:

Glucose	1.0 %
K-citrate	1.0 %
K-acetate	0.1 %
K_2HPO_4	0.5 %
NH_4Cl	0.3 %
Tryptone	0.5 %
Yeast extract	0.5 %
Salts C solution*	1.0 %
Vitamin solution*	0.5 %

Dissolved in water, and pH adjusted at 6.0

The media were sterilized and stored in a refrigerator.

B. Basal Media for Microbiological Assays.

Amino acid mix (for 100 tubes)

DL-alanine	200 mg.	DL-threonine	40 mg.
DL-aspartic acid	200 mg.	DL-tryptophan	40 mg.
L-glutamic acid	200 mg.	DL-valine	40 mg.
L-arginine·HCl	40 mg.	Glycine	20 mg.
DL-isoleucine	40 mg.	L-cystine	20 mg.
L-lysine·HCl	40 mg.	L-histidine·HCl	20 mg.
DL-methionine	40 mg.	L-leucine	20 mg.
DL-phenylalanine	40 mg.	L-proline	20 mg.
DL-serine	40 mg.	L-tyrosine	20 mg.

Made up to 25 ml. with acid and heat.

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*Composition given in Appendix B.

B. (Continued)

Sugar mix (for 100 tubes):

Glucose	4.0 gm.
K-citrate·H_2O	4.4 gm.
K-acetate (anhydrous)	0.2 gm.
NH_4Cl	0.6 gm.
K_2HPO_4	1.0 gm.
Salt C solution	4.0 ml.
AGU-solution	2.0 ml.
X-solution	2.0 ml.
Vitamin solution	2.0 ml.
Amino acid mix	25.0 ml.

The total made up to 100 ml. pH adjusted to 7.0

Solutions for the above sugar mix:

Salts C

$FeSO_4 \cdot 7H_2O$	0.5 gm.
$MnSO_4 \cdot 7H_2O$	2.0 gm.
$MgSO_4 \cdot 7H_2O$	10.0 gm.

Dissolved with the aid of HCl, and made up to 250 ml.

AGU-solution

Adenine-sulphate	250 mg.
Guanine·HCl	250 mg.
Uracil	250 mg.

Dissolved with the aid of HCl, and made up to 250 ml.

X-solution

Xanthine	250 mg.

Dissolved in dilute KOH, and made up to 250 ml.

Vitamin solution

Thiamin	25.0 mg.
Niacin	25.0 mg.
Ca-pantothenate	25.0 mg.
Pyridoxal	5.0 mg.
Riboflavin	25.0 mg.
PABA	5.0 mg.
Biotin*	0.25 mg.
Folic acid**	0.25 mg.

Riboflavin dissolved first with hot water and acid, then the rest of the vitamins added, and the volume made up to 250 ml.

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*Biotin stored in solution in 50 % ethanol.
**Folic acid stored in solution in dilute KOH in 50 % ethanol.

VITA

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