PART I. CERTAIN AMINO ACID INTERRELATIONSHIPS AFFECTING THE BIOSYNTHESIS OF SERINE IN *LEUCONOSTOC MESENTEROIDES*

PART II. AMINO ACID COMPOSITION AND BIOLOGICAL VALUE OF TEFF PROTEIN AND ITS IMPROVEMENT BY LYSINE SUPPLEMENTATION

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PART I

CHAPTER I

INTRODUCTION

This part of the thesis is mainly devoted to the study of various amino acid interrelationships that are involved in the metabolism of serine and serine precursors in Leuconostoc mesenteroides P-60. Some of the amino acid interrelationships have been observed to be stimulatory in the synthesis of serine from its precursor, glycine, in a medium devoid of serine, while others markedly inhibit the biosynthesis of serine. Because the ultimate growth of the organism is a net result of the combined effects of all the interrelated amino acids, an attempt has been made to study, in greater detail than has been done previously, the complex interactions which occur and which together affect the metabolism of serine.

There are some amino acids that affect the utilization of exogenous serine without any significant effect on the synthesis of serine by the organism. High concentrations of some amino acids improve the growth of the organism in a medium containing some serine, whereas high concentrations of other amino acids depress the growth. Sometimes depression of growth by a relatively high concentration of an amino acid may not be a direct effect on serine utilization. An optimum ratio exists between some of the amino acids and when the optimum ratio is altered by increasing the concentration of one of them, the growth of the organism is inhibited. While such an inhibition of growth has an apparent

effect on the utilization of serine, the effect is not a direct one.

The organism in which these effects were studied is popularly known as Leuconostoc mesenteroides P-60. One of the main criteria for the identification of an authentic culture of Leuconostoc mesenteroides P-60 is its ability to anaerobically ferment sucrose with the production of characteristic slime on sucrose media (1). McCleskey (2) reported that the widely-used Leuc. mesenteroides P-60 originally obtained from the American Type Culture Collection did not form the characteristic gum dextran from sucrose. In addition to the above behavior, the organism produces carbon dioxide from peptone but not from glucose, and it does not produce mannitol from fructose. The above peculiarities of the organism show that it does not properly belong to the genus, Leuconostoc, but instead is a Streptococcus species. Although the organism differs from Streptococcus equinus by an ability to produce ammonia from peptone, McCleskey has suggested the name Streptococcus equinus P-60. However, because this organism has been termed Leuconostoc mesenteroides in most reports of biochemical studies conducted with it and because a species name has not been clearly established, the name Leuconostoc mesenteroides will be used in this thesis.

Leuconostoc and Streptocccus are closely related genera in the family, Lactobacteriaceae. Members of this bacterial family are characterized by the ability to ferment carbohydrates either to lactic acid alone or lactic and acetic acids, ethanol, and carbon dioxide. The family is made up of gram positive cocci and rods. Members of this family reportedly obtain their energy by partial fermentation of sugars without utilization of free oxygen. Consequently the utilization of large amounts of sugar is necessary to obtain relatively small amounts

of energy for growth and maintenance, and a large amount of fermentation end-products, such as lactic acid is formed. Because of this ability to form large amounts of lactic acid as an end product of fermentation, the members of this family are often referred to as lactic acid bacteria.

The lactic acid bacteria are very fastidious in their nutritional requirements. In this respect, Leuc. mesenteroides is by no means an exception. Dunn et al. (3) showed that the microorganism, Leuconostoc mesenteroides P-60 required seventeen of twenty-one amino acids tested for growth. Alanine, hydroxyproline, norleucine, and norvaline were found to be non-essential for the growth of the organism. They demonstrated that this rather absolute requirement for seventeen amino acids by Leuconstoc mesenteroides P-60, might serve as a basis for the quantitative microbiological assay of amino acids.

The amino acid, serine, is one of the seventeen amino acids which was described as indispensable for the growth of Leuconostoc mesenteroides P-60. Therefore, this microorganism has been employed as an assay organism for the quantitative determination of serine in biological materials. It has been shown by several investigators that the requirement for serine can be met by increasing the amount of glycine in the medium. Thus Lascelles and Woods (4) have shown that the requirement for serine by Leuc. mesenteroides can be partially satisfied by glycine in the presence of formate or carbon dioxide, pyridoxal, p-aminobenzoate, and pteroylglutamate. They observed greater activity with formylpteroylglutamate than with pteroylglutamate. Similar biochemical interconversions of glycine and serine have been revealed in animal systems. (5).

Winnick et al. (6) have demonstrated that when rat-liver homogenates were incubated with C^{14}- labeled glycine, 60 % of the activity was found

in the serine liberated by hydrolysis of the protein. In other studies of this biochemical conversion of glycine to serine in the intact rat, the beta-carbon of serine was found to arise from formate or other suitable one-carbon donors and the alpha-carbon of serine arose from the glycine (7). In fasted rats the administration of glycine labeled with carbon^{14} in the methyl position, has indicated that the activities of the beta- and alpha-carbons of serine isolated from the liver were almost the same (8). This work indicates that a glycine molecule can presumably serve as a source of the one-carbon fragment, such as formate, which in turn can condense with another molecule of glycine to yield a serine molecule. It has also been shown that one or more of the choline methyl groups can be utilized as a source of the one-carbon fragment (9). Mitoma et al. (10) have also demonstrated that sarcosine and glycine condense to yield serine in rat liver homogenates. In contrast to earlier work, they report evidence against the incorporation of formate into the second carbon of serine. They speculate that a formaldehyde-like compound is the intermediary one-carbon compound which gives rise to the beta- (or third) carbon of serine.

The above studies were undertaken with animal tissues; nevertheless they have important bearing on the mechanism of serine biosynthesis in microorganisms. Leuconostoc mesenteroides P-60 possesses the ability to synthesize serine from glycine and a one-carbon source. The biosynthesis of serine from glycine in a serine-free medium requires pyridoxal (11). Lascelles and Wood (12) have shown that serine was synthesized by Streptococcus faecalis R in a serine-free medium containing glycine, formate, glucose, pyridoxal and pteroylglutamic acid. Under the above conditions, folic acid and vitamin B_6 were not required. Their

work suggests that pteroylglutamic acid is first converted to a form of folic acid before the compound is active in the biosynthesis of serine. Other workers (13) have found that there is a quantitative relationship between the concentration of glycine and the partial pressure of carbon dioxide in growing cultures of Leuconostoc mesenteroides. In another study (14), Leucovorin, which is a tetrahydro derivative of formyl-pteroylglutamate, was found to replace p-aminobenzoic acid and carbon dioxide for the growth of L. mesenteroides. The formyl group of leucovorin appears to serve directly as the source of the one-carbon unit. Wold and Sirny (15), in their work with L. mesenteroides P-60, have demonstrated that high levels of folinic acid (leucovorin) which are not required under the conditions of the Henderson-Snell Medium (16), can reverse the inhibition of serine synthesis caused by relatively high concentrations of alanine. Their work also shows that low initial pH (5.0 - 6.5), a relatively high glycine, a low cystine concentration, and absence or low amounts of alanine in the medium favor the synthesis of serine. Pyridoxal was more stimulatory to serine synthesis than either pyridoxine or pyridoxamine.

In recent work by Cross (17), carbon dioxide-C^{14} was not incorporated into serine by Leuc. mesenteroides P-60, even though carbon dioxide is essential for the growth of the organism. The work shows that carbon dioxide does not serve as a donor of the beta-carbon of serine. The requirement of the organism for carbon dioxide was not satisfied by the addition of formate and optimum serine synthesis was only achieved in the presence of both carbon dioxide and formate. In view of the above observations, experiments were designed to show the role carbon dioxide plays in serine synthesis by the same author (18). Carbon dioxide was

found to partially inhibit the inactivation of leucovorin by suspensions of Leuc. mesenteroides. If this inactivation is assumed to mean the loss of the coenzyme (a form of folic acid), then carbon dioxide may exert its influence on serine synthesis, by inhibiting the degradation of the coenzyme. It is assumed that the concentration of the coenzyme is sufficiently high, even in the absence of carbon dioxide, for the requirements of the organism growing in the presence of serine. On the other hand, the coenzyme concentration is insufficient, in the absence of carbon dioxide for the synthesis of serine either by growing organisms or by suspensions of organisms previously incubated in phosphate-buffered glucose.

Thusfar, the mechanism of the biochemical transformation of glycine into serine, and the vitamins and other factors that affect it, have been reviewed both in the animal system and the bacterial system. This interconversion is also governed by the relative concentrations of amino acids other than glycine. Of special importance in this respect is the inhibition of serine synthesis caused by a relatively high concentration of alanine in the medium. Thus, Wold and Sirny (15) reported that increasing the level of L-alanine to twice the amount in the Henderson-Snell Medium (16) in a serine-free medium completely inhibits the growth of Leuc. mesenteroides P-60. Growth is presumably retarded by the inhibition of the serine synthesizing system by the L-isomer of alanine. In this regard, the L-isomer is more effective than either the D-isomer or the DL-racemic mixture. (19)

In addition to the amino acids that directly affect the biosynthesis of serine, there are other amino acids that indirectly affect this synthesis. Thus at a given level of serine in the medium, increasing

concentrations of threonine decrease the growth of Leuc. mesenteroides (20). The latter effect, however, is simply that of decreasing the utilization of serine. The growth of the organism is retarded because of its inability to utilize serine, and as a result, any serine synthesis which might occur would not be apparent. Similar antagonisms have been reported recently by O'Barr et al. (21). New antagonisms involving other amino acids have also been found to affect the biosynthetic mechanism and will be discussed later.

There are several amino acids that favorably affect the synthesis and utilization of serine in this organism.

The nutrition of Leuc. mesenteroides with regard to its serine synthesis and serine utilization has been further studied. Amino acids that affect the serine biosynthesis and utilization have been investigated. Attempts have been made to explain the mechanism of action of those amino acid interrelationships that either favorably and unfavorably affect the metabolism and biosynthesis of serine.

CHAPTER II

EXPERIMENTAL

The organism, Leuconostoc mesenteroides P-60, which is listed in the American Type Culture Collection as a Streptococcus species ATCC 8042, is carried in agar stab cultures (appendix I) under refrigeration. Approximately once a month transfers are made. For use, the organism is transferred to previously autoclaved liquid medium (appendix I) and incubated for approximately 12 to 18 hours at 37^{o} C. After the incubation, the bacterial suspension is centrifuged and the supernant discarded. The cells are then washed with sterile 0.9 % KCl and centrifuged again. Finally they are suspended in sterile 0.9 % KCl prior to inoculation. Hereafter the KCl-suspended bacterial cells will be referred to as the inoculum. This inoculum is relatively free of any extracellular amino acids. One drop of the inoculum is dispensed to each tube by means of an ordinary sterile syringe.

Throughout this work a modification of the Henderson and Snell medium (16) was employed for the experimental studies. The composition of this medium is shown in Appendix I. MacLeod and Snell (22) have shown a competitive inhibition between K^+ and Na^+ and NH_4^+. The original Henderson and Snell medium consists of sodium citrate, sodium acetate, ammonium chloride and potassium monohydrogen phosphate in the ratio of 20:1:3:5 by weight, respectively. Sirny et al. (23) have shown that the replacement of the sodium salts by potassium salts

results in certain growth improvements in this medium. In the course of this work, all the sodium salts, with the exception of sodium acetate, have been replaced by potassium salts.

Further appropriate modifications of the medium have been made in the experimental procedures designed to study the many complex amino acid interrelationships that affect serine synthesis and utilization. These experimental modifications will be discussed later in the presentation of the discussions and the results.

The basal medium, devoid of the amino acids under study but otherwise containing all other amino acids, is dispensed with a Cannon Automatic Dispenser (24) into tubes contained in special racks. Likewise, the amino acids under study are dispensed in appropriate concentrations. The final tube volume is adjusted to 2 ml. with distilled water. The racks, containing 60 tubes per rack, are autoclaved at 121^{o} C for 5 minutes in a previously heated autoclave. After autoclaving, one drop of the inoculum is added to each tube by means of a syringe. The racks are then incubated for approximately 72 hours in a water bath maintained at 37^{o} C.

The organism anaerobically ferments one mole of glucose to yield one mole each of lactic acid, ethanol, and carbon dioxide as end products (25). Radioisotope work using C^{14}-labeled glucose strongly suggests that the fermentation takes place through the pentose phosphate pathway. The lactic acid produced in the course of the growth of the organism has been used as a measure of the growth of the organism. Many workers employ turbidimetric methods for the quantitative determination of the growth. In this study, quantitative estimation of growth has been made by titration of the acid produced.

Mention has been made of the need for seventeen of the eighteen of the naturally occurring amino acids for the growth of this organism (3). The amino acid, serine, is one of these amino acids that is indispensable for growth, and is found in the protein of this bacterium; consequently, a serine-free medium cannot support growth of the organism unless serine is synthesized by the organism. Therefore, the growth of the organism in a serine-free medium can be used as an index for the determination of serine being synthesized by the organism. It should also be pointed out that even if there is an exogenous source of serine, other amino acids can interfere with the utilization of serine and thus depress the growth of the organisms. Since lactic acid production is an end product of glucose metabolism, it is in turn used as an index of growth.

The amount of acid produced was determined by titration with 0.05 N KOH. An electrometric method, using a 1 M KCl calomel half-cell as the reference electrode and a quinhydrone electrode as the primary electrode, was employed for titration. The galvanometer was adjusted to zero with a phosphate buffer at pH 7.3 prior to use. Throughout the work titration counts are used as indices for growth and 100 titration counts are equivalent to approximately 4 ml. of 0.05 N KOH.

CHAPTER III

RESULTS AND DISCUSSIONS

A. Serine Synthesis from Glycine

In routinely-used quantitative microbiological assays, it has been observed that varying amounts of growth occur under serine-free conditions; i.e., in the blank tubes. This is primarily due to the fact that the medium contains 200 micrograms of glycine per 2 ml. medium. This concentration is in excess of the amount of glycine actually required for the organism per se.; consequently, the organism utilizes some of the glycine for the synthesis of serine. A typical standard curve, using Leuconostoc mesenteroides as the assay organism, is shown in Figure 1. It should be pointed out that the medium also contained 2000 micrograms of DL-alanine, in addition to the other constituents.

The relatively high growth of the organism in the serine blanks makes such a medium unsuitable for the quantitative determination of serine. However, certain possibilities exist for depressing this high growth of the organism in a serine-free medium, and these involve the study of amino acid interrelationships that affect the biosynthesis of serine from glycine. Mention has been made previously of the work by Wold and Sirny (15) in which they found that increasing the amount of alanine to twice the concentration in the normal Henderson and Snell medium completely inhibits growth of the organism under serine-free conditions presumably by interfering in the synthesis of serine.

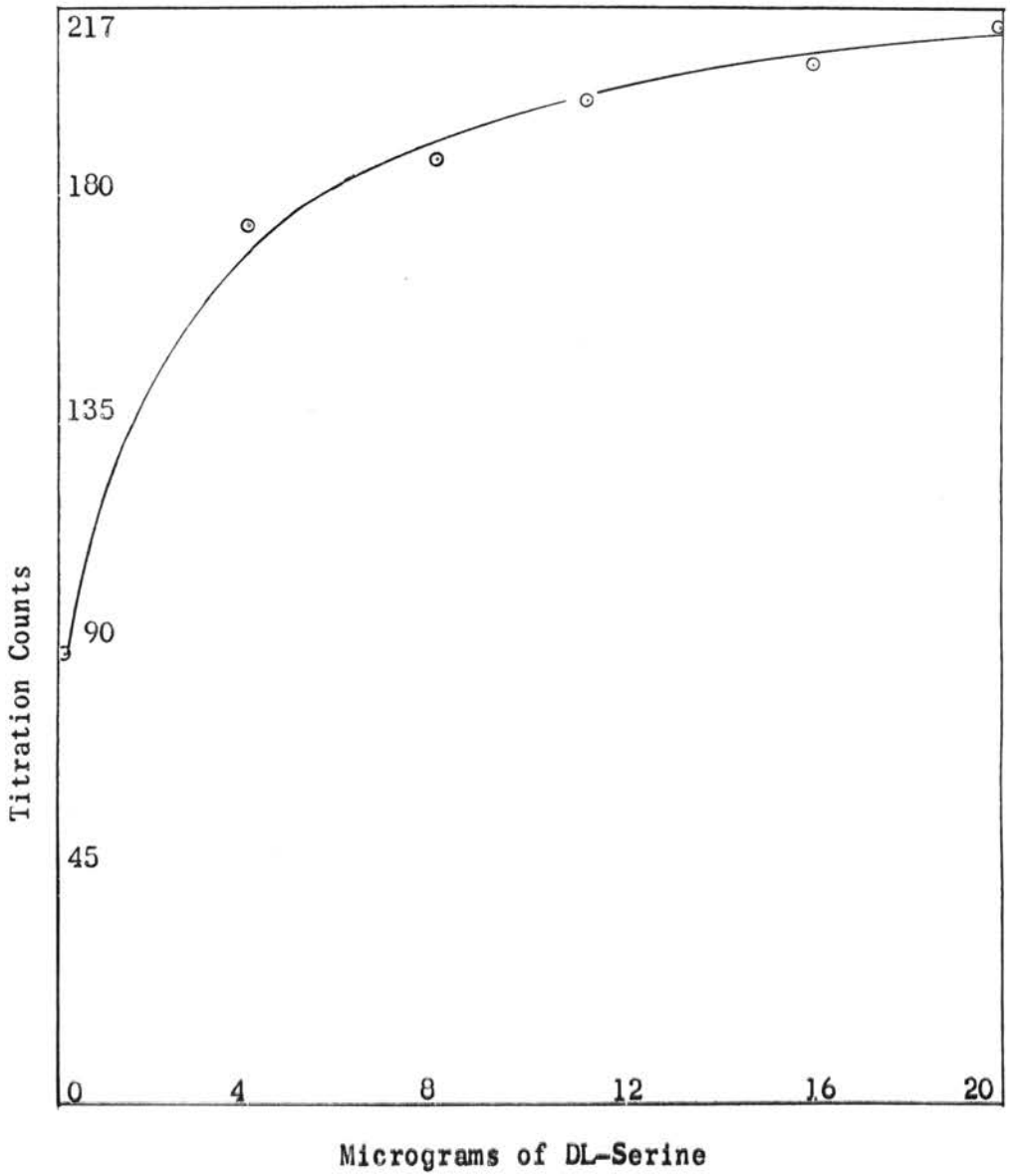

Figure 1. A typical serine standard curve using the modified Henderson-Snell medium. 200 micrograms of glycine and 2000 micrograms of DL-alanine present.

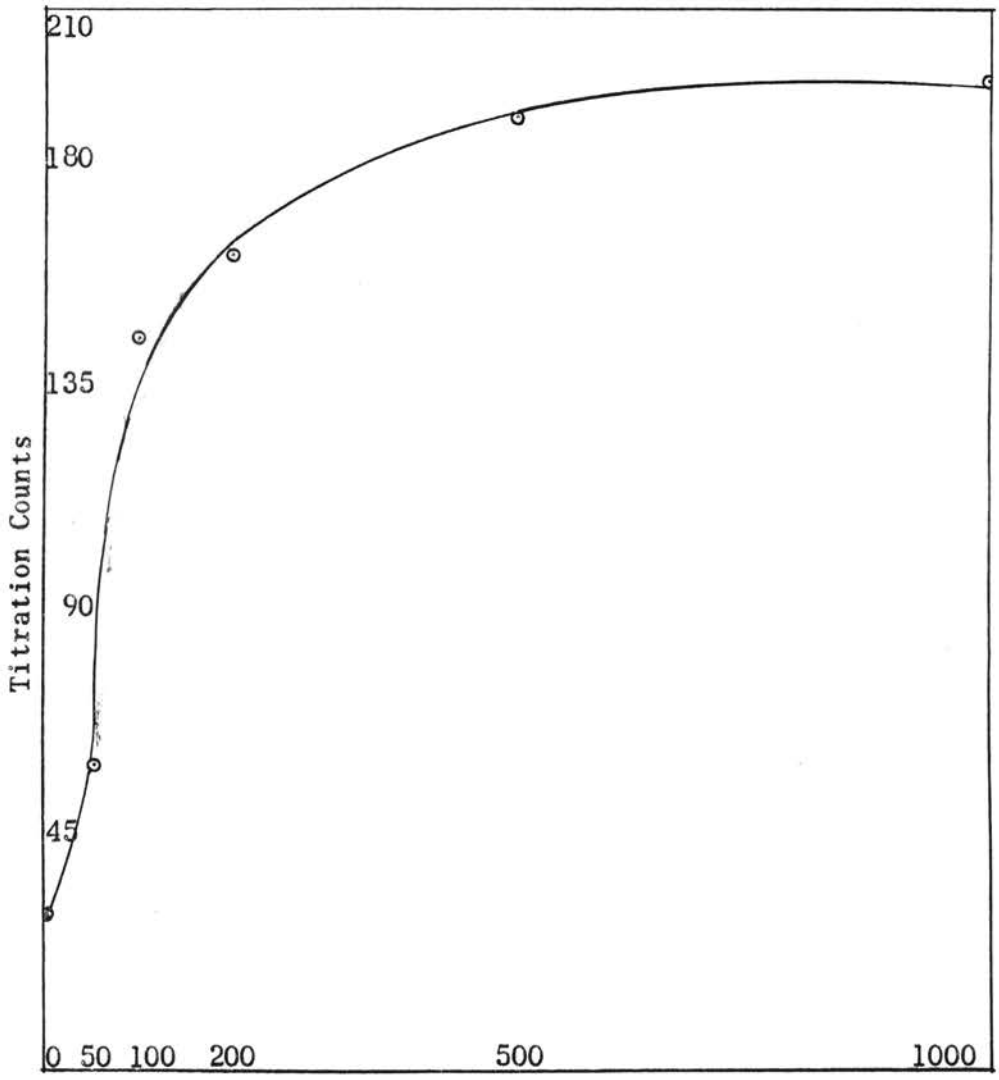

Figure 2. Growth response of Leuc. mesenteroides to increasing levels of glycine in a serine- and alanine-free medium.

Figure 2 illustrates the growth response of the organism to increasing levels of glycine in a serine-free medium. Under the specific conditions employed in this experiment (the Henderson-Snell concentrations of all other amino acids), it is seen that relatively little, or no, growth is obtained in the presence of 20 micrograms of glycine. This level of glycine, if in the presence of adequate serine, would ordinarily support maximum growth. It is further seen that higher concentrations of glycine produce increasing growth and that near-maximum growth is not obtained until levels above 100 micrograms of glycine are used. At these levels it would be presumed that the glycine needs of the organism were being met directly and that the serine needs were also being met by synthesis of serine from the relatively high concentrations of glycine.

B. A Preliminary Survey of the Amino Acids that Affect Serine Synthesis and Utilization

A preliminary study was made to investigate the amino acids that affect the growth of the organism, in a serine-free medium as well as in a medium containing serine. All the media contained 200 micrograms of glycine per 2 ml. volume, and therefore the amino acids that interfere or inhibit the growth of the organism in a serine-free medium would possibly exert their effect by depressing its ability to synthesize serine from glycine. If growth is inhibited in a medium containing serine, then the amino acid involved may play a role in the depression of serine utilization by the organism. On the other hand, if growth is stimulated in a serine-free medium by another amino acid under study, the favorable effect might be directly on the serine synthesizing system. Similarly, a favorable effect of another amino acid in a serine-containing medium might conceivably be improving the utilization of serine. It is recog-

nized that other interpretations of effects such as postulated above must be considered: for example, there are some amino acids that interfere with the utilization of other amino acids without any direct effect on serine synthesis or utilization. In such a situation, growth is primarily inhibited by an antagonism of some essential amino acid.

In the course of the survey, the modifying amino acids were generally added in three different concentrations - low, medium, and high. The medium concentration represents the concentration of the amino acid in the modified Henderson and Snell medium. The low and the high concentrations represent one-tenth and ten times the concentration in the Henderson and Snell medium respectively. A 20 microgram per ml. standard serine solution was added to rows of six tubes in a gradual concentration of 0.0, 4.0, 8.0, 12.0, 16.0, and 20.0 micrograms per 2 ml.

Sixteen of the naturally occurring amino acids were tested for their effect on serine synthesis and utilization. The amino acid, alanine, inhibited the synthesis of serine but had no noticeable effect on the utilization of pre-existing serine. Other amino acids that unfavorably affected serine synthesis were leucine and lysine. The effect of leucine was quite pronounced, whereas the inhibitory effect of lysine, at times, appeared to be insignificent. High amounts of glutamic acid and aspartic acid had a stimulatory effect. The effect of the latter amino acids, however, may be an indirect one, as will be explained later.

There were some amino acids that did not demonstrate any effect on serine synthesis, but had significant effect on the utilization of exogenous serine. High amounts of arginine favorably affected the utilization of serine. On the other hand, threonine appeared to interfere with serine utilization. The probable mechanism of the inhibitory effect of

threonine will be presented later under an appropriate title. Relatively high concentrations of histidine somewhat depress the utilization of serine. Unlike the other amino acids interrelationships, the role of histidine has not been extensively investigated.

C. Effect of Alanine on Serine Utilization

The effect of alanine on serine synthesis has been studied very extensively. Contrary to the findings of Dunn et al. (3), some alanine is required for the maximum growth of Leuconostoc mesenteroides P-60. The amount of alanine so required is very minute relative to the requirements of the organism for the other 16 essential amino acids. Figure 3 shows a substantially less growth in the absence of alanine than in the presence of 20 micrograms of DL-alanine. Since the medium is free of serine and alanine in the case of the alanine-free medium, the organism must be synthesizing both serine and alanine. The fact that high glycine depressed the growth in the absence of alanine could be interpreted as an inhibition of alanine synthesis by high glycine and/or a definite requirement of small amount of alanine for the utilization of high amounts of glycine. Camien and Dunn (26) have shown that high amounts of glycine and serine inhibit the utilization of alanine by Leuconostoc citrovorum. This also appears to be a plausible explanation in the case of L. mesenteroides.

It should be noted that in the presence of relatively high alanine concentrations growth is inhibited significantly. This unfavorable effect of increasing amounts of alanine on serine synthesis is shown in Figure 4. About ten times the concentration of glycine as in the normal Henderson and Snell medium was able to completely reverse the inhibitory effect of alanine. The above considerations suggest that the inhibition

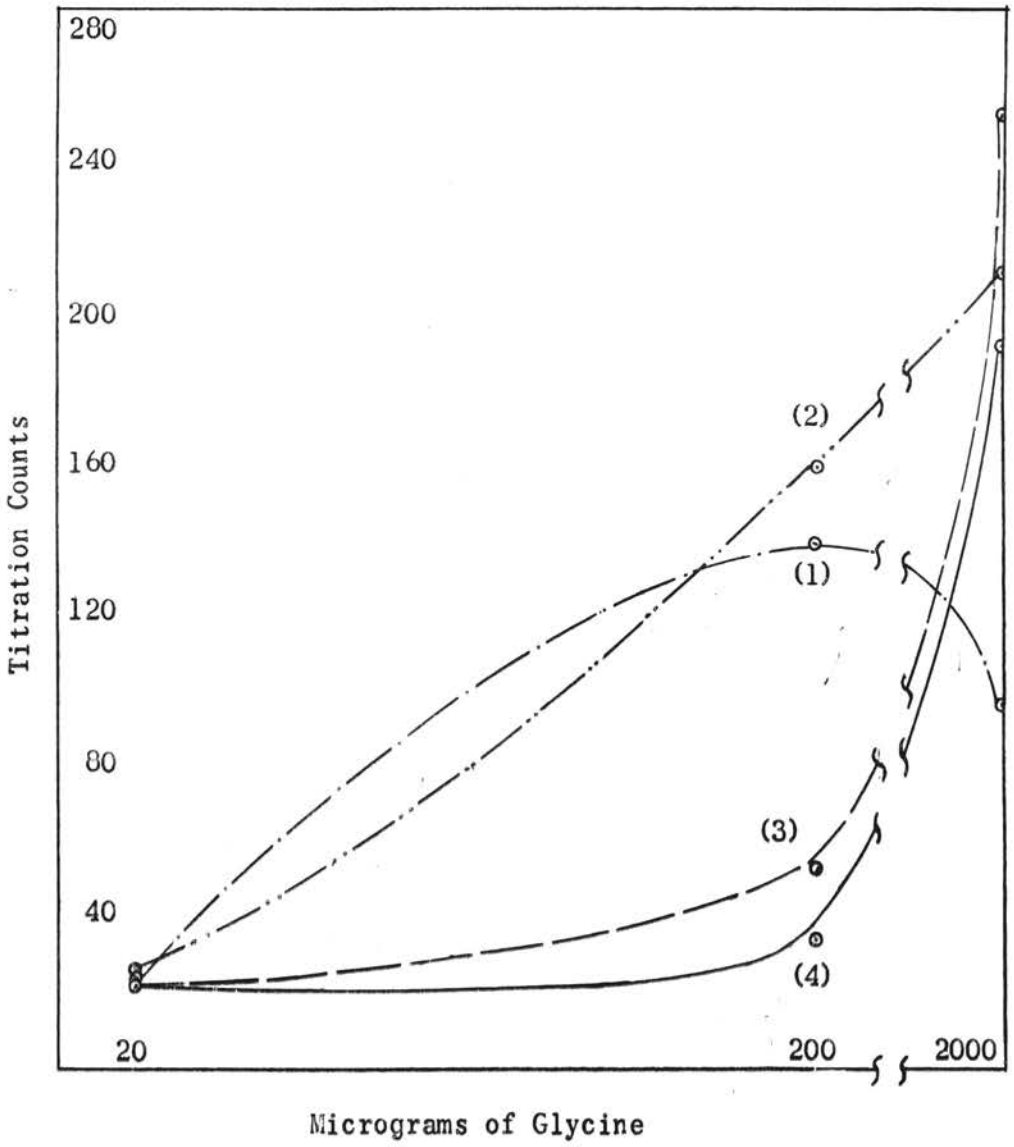

Figure 3. Effect of glycine and alanine on serine synthesis. (1) No DL-alanine, (2) 20, (3) 2000, (4) 4000 micrograms of DL-alanine.

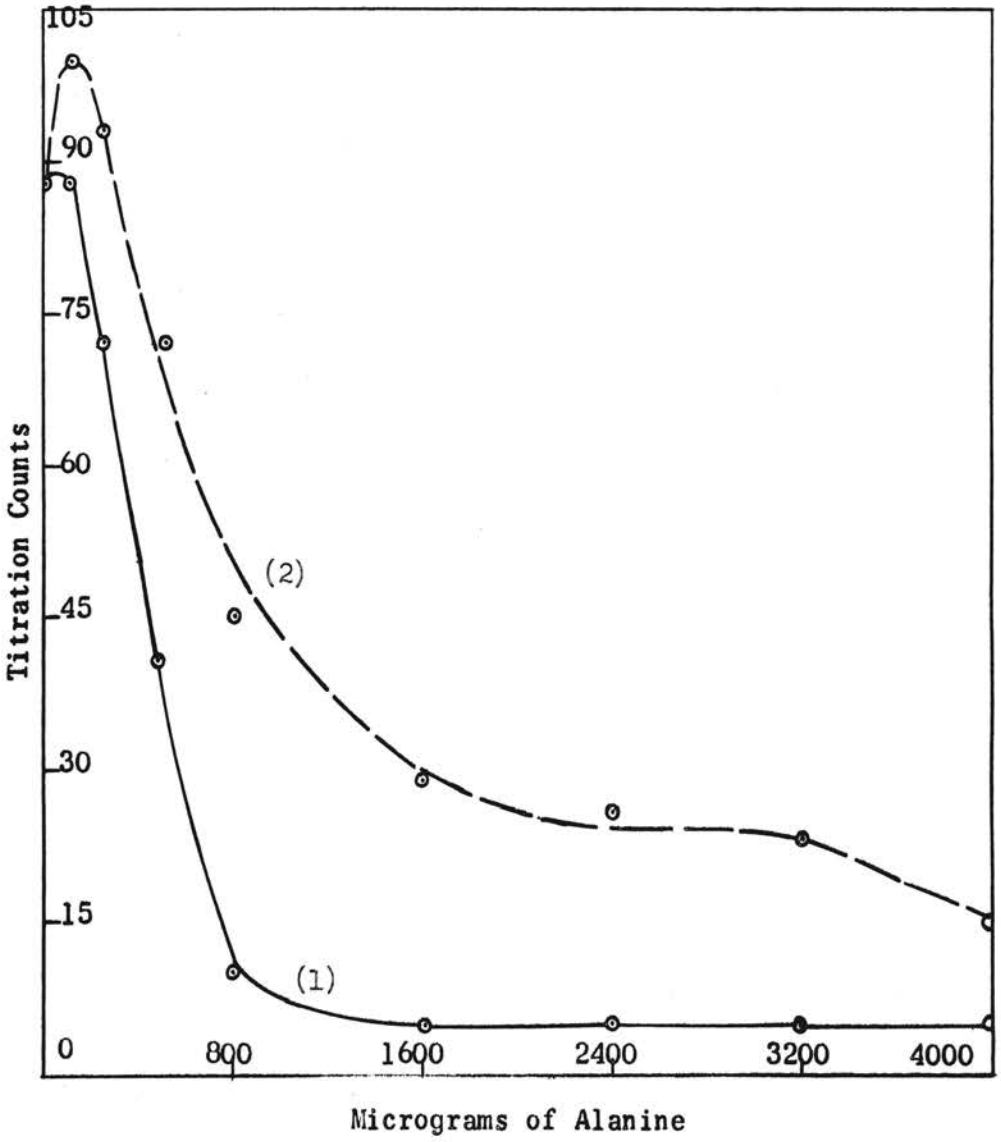

Figure 4. Inhibition of serine synthesis by L- and DL-alanine. (1) L-alanine, (2) DL-alanine.

is competitive and reversible.

The specific mechanism of the alamine inhibition of serine synthesis is yet to be elucidated. Mention has already been made of the experimental fact that high alanine does not inhibit the utilization of exogenous serine. This would probably mean that the site of inhibition by alanine may not be in the incorporation of serine into bacterial protein. With possible elimination of the latter mechanism of inhibition, there are two other possibilities that should be considered as sites of the inhibition. The first involves the competitive interference of alanine with the uptake of glycine from the extracellular medium. The second possibility involves the competition of alanine for the site on the enzyme (3) that is responsible for the transformation of glycine into serine.

D. The Effect of Leucine on Serine Synthesis

High amounts of leucine inhibited the growth of Leuc. mesenteroides in a serine-free medium. Figure 6 shows such an inhibition with increasing amounts of L-leucine. Maximum growth was observed at a concentration of 40 to 100 micrograms L-leucine. There was no significant difference when 20 micrograms or 2000 micrograms of DL-alanine was used, indicating that alanine was not involved in the inhibition. In the presence of 4000 micrograms DL-alanine, growth was completely inhibited, thus masking the effect of leucine.

Brickson et al. (27) have reported that imbalances of valine, methionine, leucine, and isoleucine caused inhibition of growth in Lactobacillus arabinosus. The same inhibition was observed in Leuc. mesenteroides, but to a much smaller degree. There are several other references to the same effect with other organisms (28, 29, 30).

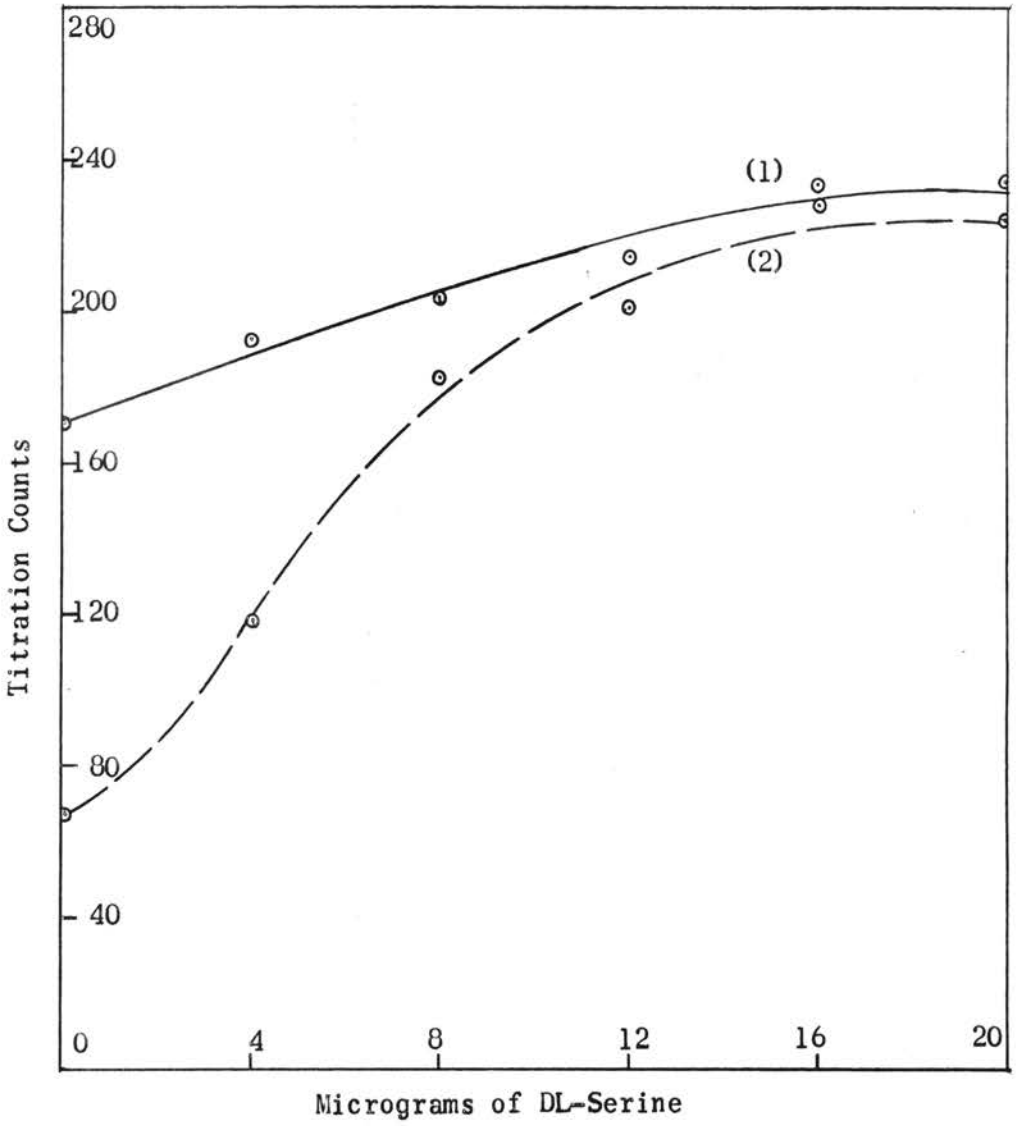

Figure 5. The effect of alanine on serine synthesis and utilization in the modified Henderson-Snell medium. (1) 40 and (2) 4000 micrograms of DL-alanine.

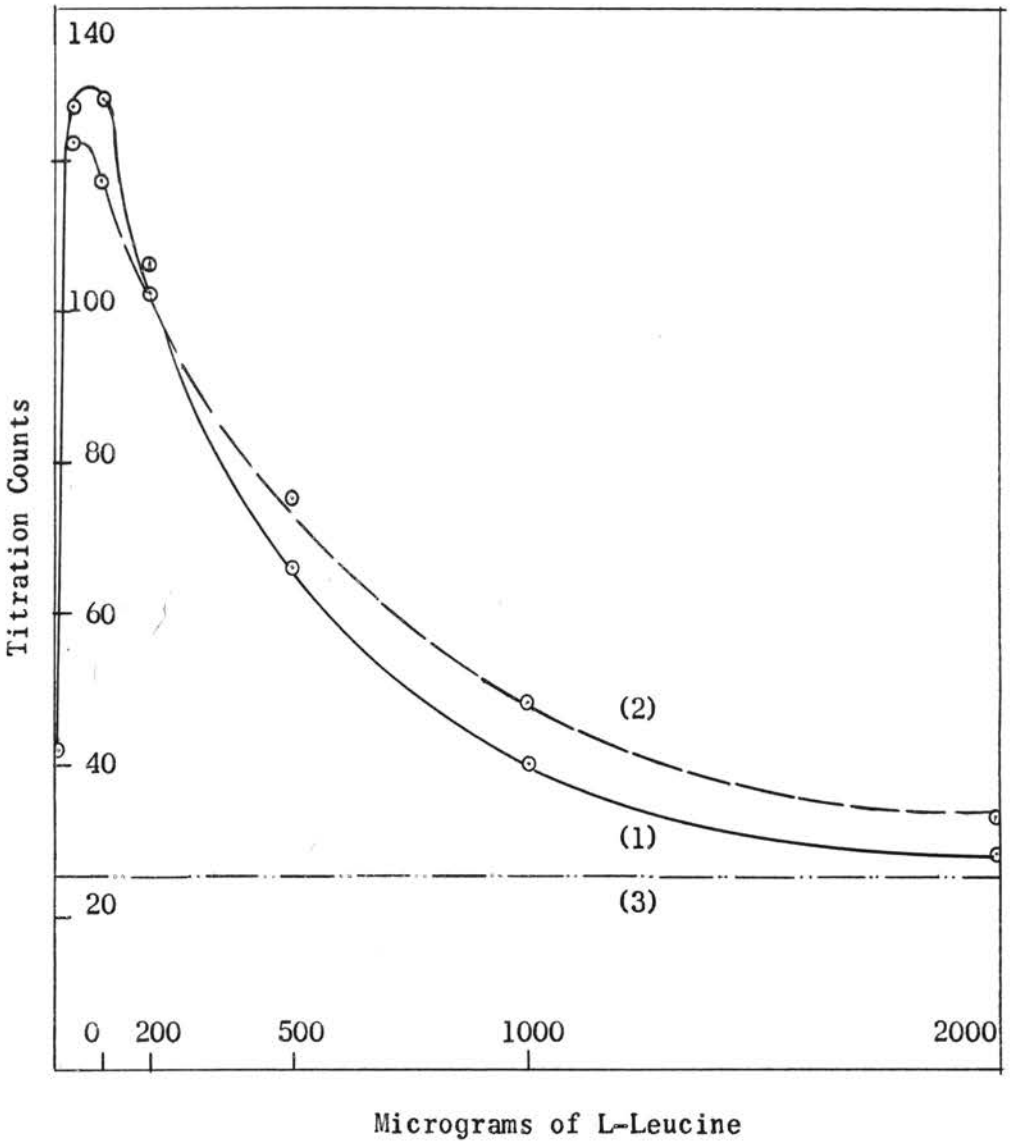

Figure 6. Inhibition of serine synthesis by leucine in a serine-free medium. (1) 20, (2) 2000, (3) 4000 micrograms of DL-alanine.

In view of these reports, experiments were conducted to see if high concentrations of methionine, isoleucine and valine could reverse the inhibition caused by 2000 micrograms of L-leucine. None of these structurally-related amino acids relieved the inhibition. The only way the inhibition could be reversed was by the addition of 2000 micrograms of glycine. The fact that a high glycine concentration was able to repress the antagonism caused by relatively high leucine concentration in the serine-free medium would suggest that leucine was interfering with the utilization of glycine, consequently affecting the serine biosynthesis from glycine. On the other hand, if the antagonism was instigated by an imbalance in the ratio of leucine to valine, isoleucine, and methionine, it would be difficult to explain why high glycine concentrations were able to reverse the antagonism.

Although these observations appear to be contradictory to the previous reports by other workers, it should be pointed out that their investigations were conducted in media containing serine. Speculation regarding the exact mechanism of the leucine inhibition of serine synthesis is not possible with present information. Since leucine and glycine or serine are quite dissimilar structurally, any explanation analogous to that offered for the alanine inhibition appears quite unlikely.

With knowledge of the inhibitory actions of alanine and leucine, attempts have been made to utilize this knowledge in improvement of the serine assay with Leuc. mesenteroides P-60. It should be noted that there are, among others, two important criteria for a good microbiological assay. One is low or no growth in the blanks and the other is high sensitivity in the response of the organism to increasing amounts of the amino acid to be assayed.

Figure 7 shows that while serine synthesis in the blanks was depressed with 200 micrograms of L-leucine and 4000 micrograms of DL-alanine, the sensitivity of the organism to serine was much lower than in the routinely-used medium (200 micrograms of L-leucine and 4000 micrograms of DL-alanine). The figure also shows that the utilization of exogenous serine is depressed by high amounts of L-leucine in the medium. Although high concentrations of leucine inhibit the growth of the organism, the low growth in the absence of leucine merely confirms the well-known fact that a certain amount of leucine is indispensable for growth.

The effect of pH on the growth of the organism has also been studied (Fig. 8). The organism is more sensitive to the effect of leucine and alanine at pH 6.8 or 7.0 than at pH 6.0. There is a general increase in serine synthesis and utilization when the pH of the basal medium was decreased from 6.8 or 7.0 to 6.0.

E. Effect of Lysine

The effect of lysine in antagonizing serine synthesis, while not as pronounced as is the case with either alanine or leucine, is, nevertheless, significant. Table I shows the effects of the interrelationships of alanine, lysine, and glycine on the growth of Leuc. mesenteroides in a serine-free medium. Increasing the lysine from 40 micrograms to 4000 micrograms significantly decreased growth. This was more striking in the medium containing 200 micrograms of glycine than in the medium containing 2000 micrograms. Lysine and alanine appear to have an additive effect in inhibiting the growth. The antagonism exerted by lysine and alanine was partially relieved by increasing the glycine from 200 micrograms to 2000 micrograms.

The effect of lysine on serine synthesis lends itself to little

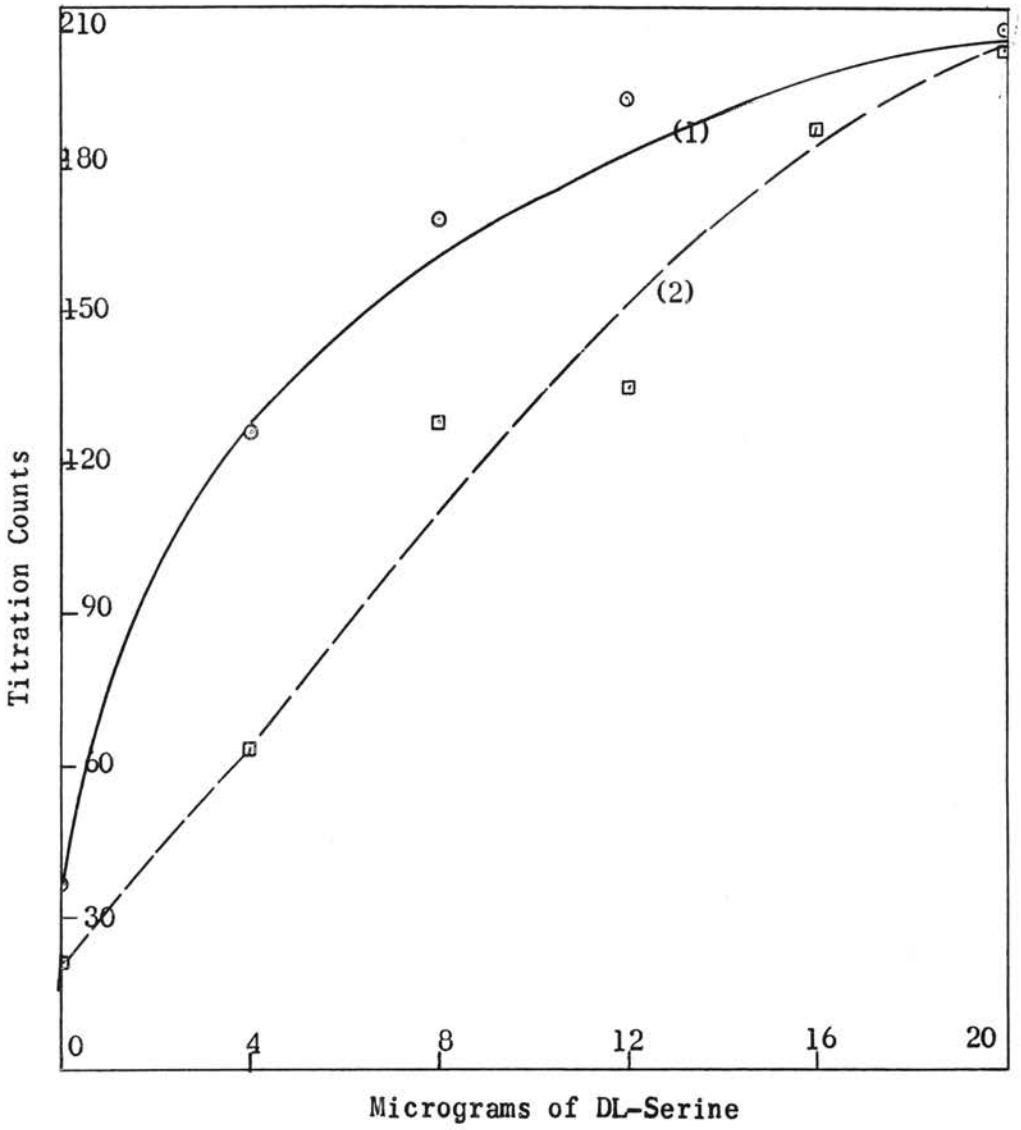

Figure 7. Serine synthesis and utilization as affected by L-leucine and DL-alanine. (1) 200 micrograms of L-leucine and 4000 micrograms of DL-alanine. (2) 2000 micrograms of L-leucine and 4000 micrograms of DL-alanine.

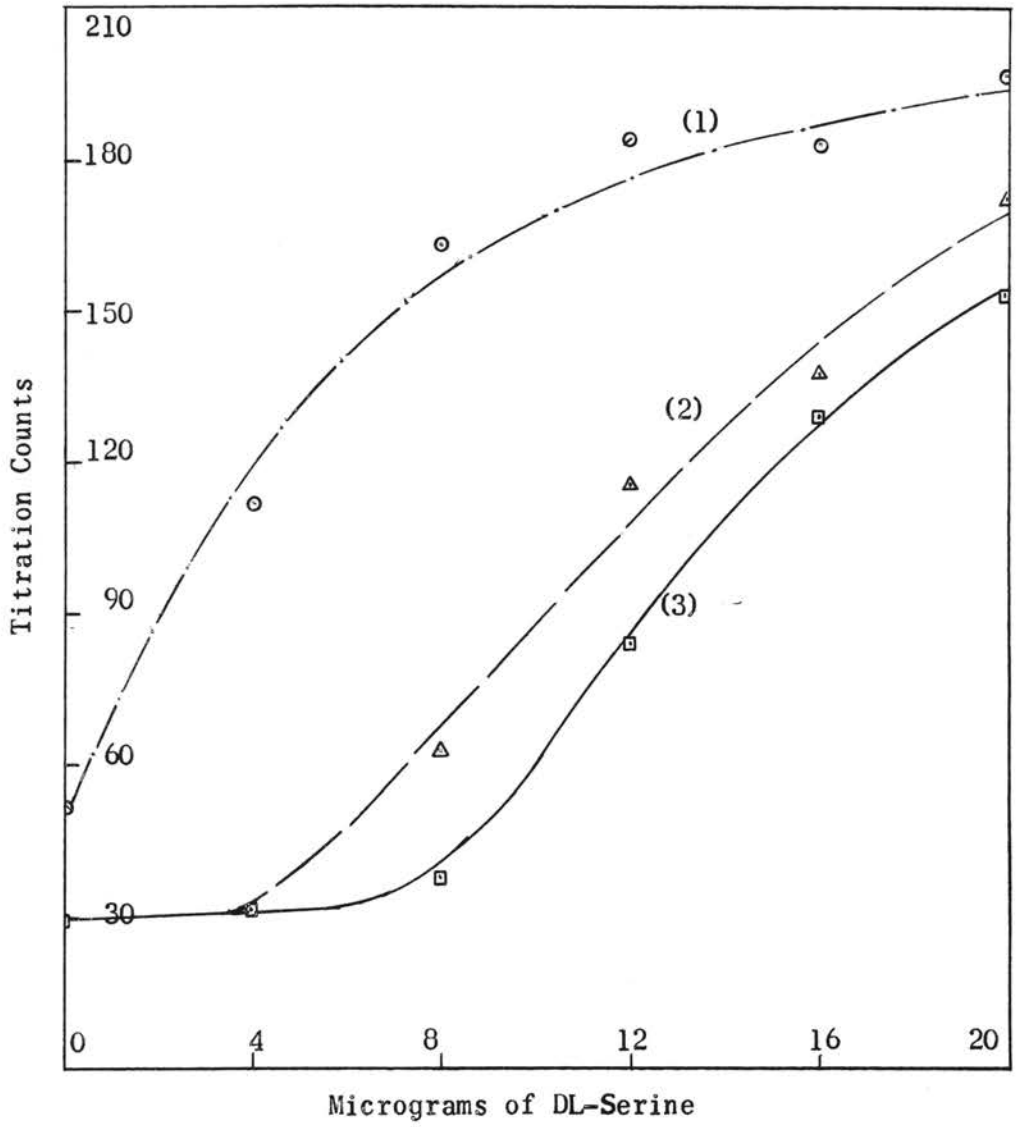

Figure 8. The effect of pH on serine synthesis and utilization in the presence of 2000 micrograms of L-leucine and 4000 micrograms of DL-alanine. (1) pH 6.0 (2) pH 6.8 (3) pH 7.0.

TABLE I

EFFECT OF VARYING CONCENTRATIONS OF LYSINE, ALANINE, AND GLYCINE ON SERINE SYNTHESIS

L-Lysine·HCl Micrograms	DL-Alanine Micrograms	Growth in Titration Counts	
		200 Micrograms Glycine	2000 Micrograms Glycine
40	0	160	172
400	0	149	170
4000	0	139	166
40	20	169	193
400	20	164	193
4000	20	119	140
40	2000	144	217
400	2000	90	216
4000	2000	61	130
40	4000	117	229
400	4000	110	213
4000	4000	46	127

speculation as to the mechanism of action at the present time. It is possible that the lysine is exerting its effect indirectly, by influencing the utilization of some other amino acid in the medium.

F. Effect of Glutamic Acid and Aspartic Acid

Thusfar, the amino acids that unfavorably affect the biosynthesis of serine have been considered. There are certain other amino acids, which, because of antagonism to, or by, amino acids other than serine in the media, indirectly affect the ability of the organism to synthesize serine. Such is the case with glutamic acid. When the level of glutamic acid in the medium was increased from 100 micrograms to 2000 micrograms, growth was stimulated. The experimental results are shown in Table II. These results may, at first glance, appear to indicate a direct stimulation of serine synthesis by an increase in glutamic acid concentration. However, it should be recognized that the Henderson and Snell medium contains 2000 micrograms each of glutamic acid and aspartic acid, a ratio of 1:1 on a weight basis. A reduction in the glutamic acid in the medium to 100 micrograms results in a 1:20 ratio of glutamic acid to aspartic acid. While the effect of increasing the glutamic acid concentration does stimulate the growth of the organism in a serine-free medium, this may not necessarily be a direct effect on serine synthesis. Aspartic acid is known to antagonize the utilization of glutamic acid (31, 32), and this may be the primary effect when the level of glutamic acid is low. Accordingly, experiments were conducted to test the effect of varying concentrations of aspartic acid on the synthesis and utilization of serine in a medium with a low glutamic acid content. The results, which are summarized in Table III, demonstrate that growth of the organism is progressively depressed with

TABLE II

EFFECT OF GLUTAMIC ACID AND ALANINE
ON SERINE SYNTHESIS

L-Glutamic Acid Micrograms	DL-Alanine Micrograms	Average Growth in Titration Counts
100	8	114
2000	8	229
100	40	102
2000	40	230
100	4000	28
2000	4000	168

TABLE III

EFFECT OF ASPARTIC ACID - GLUTAMIC ACID ANTAGONISMS ON SERINE SYNTHESIS AND UTILIZATION

Micrograms DL-Aspartic Acid	Growth in Blanks		Growth with 20 Micrograms DL-Serine	
	30 Micrograms L-Glutamic Acid	50 Micrograms L-Glutamic Acid	30 Micrograms L-Glutamic Acid	50 Micrograms L-Glutamic Acid
0	25	25	25	24
30	69	59	112	118
100	32	46	76	135
200	45	39	82	124
500	34	45	32	123
1000	25	36	25	111

increasing concentrations of aspartic acid. The antagonism results from an imbalance of the concentrations of aspartic acid and glutamic acid. It is significant to note that both the growth in the blanks and the growth in the presence of 20 micrograms of serine are affected by the aspartic acid-glutamic acid antagonism.

G. Stimulation of Serine Utilization by Arginine

The amino acid, arginine, stimulates the utilization of serine in an alanine-inhibited system. Growth of the organism was significantly stimulated when the arginine concentration was increased from 40 micrograms to 2000 micrograms of L-arginine·HCl. The experimental results showing this effect of arginine on the utilization of serine are shown in Figure 9. Sirny et al. (33) have shown an interdependence between arginine and proline in the metabolism of Leuc. mesenteroides P-60 in which an abnormally high arginine is required for growth in the presence of limiting amounts of proline, and vice versa. Their work indicates that the optimum ratio of arginine and proline for most efficient growth is approximately 1:1. Wold (19) has shown a similar relationship between arginine and relatively low concentrations of glycine.

The results of the present experiment may be explained on the basis of a proline-arginine interrelationship. However, since the growth was directly proportional to the amount of serine present in the medium, the difference in growth between the medium with 40 micrograms L-arginine·HCl and the one with 2000 micrograms L-arginine·HCl, might also represent stimulation of growth as a result of more efficient utilization of serine in the presence of high arginine. This is an excellent example of the difficulties involved in accurately interpreting the results of amino acid interrelationships studies.

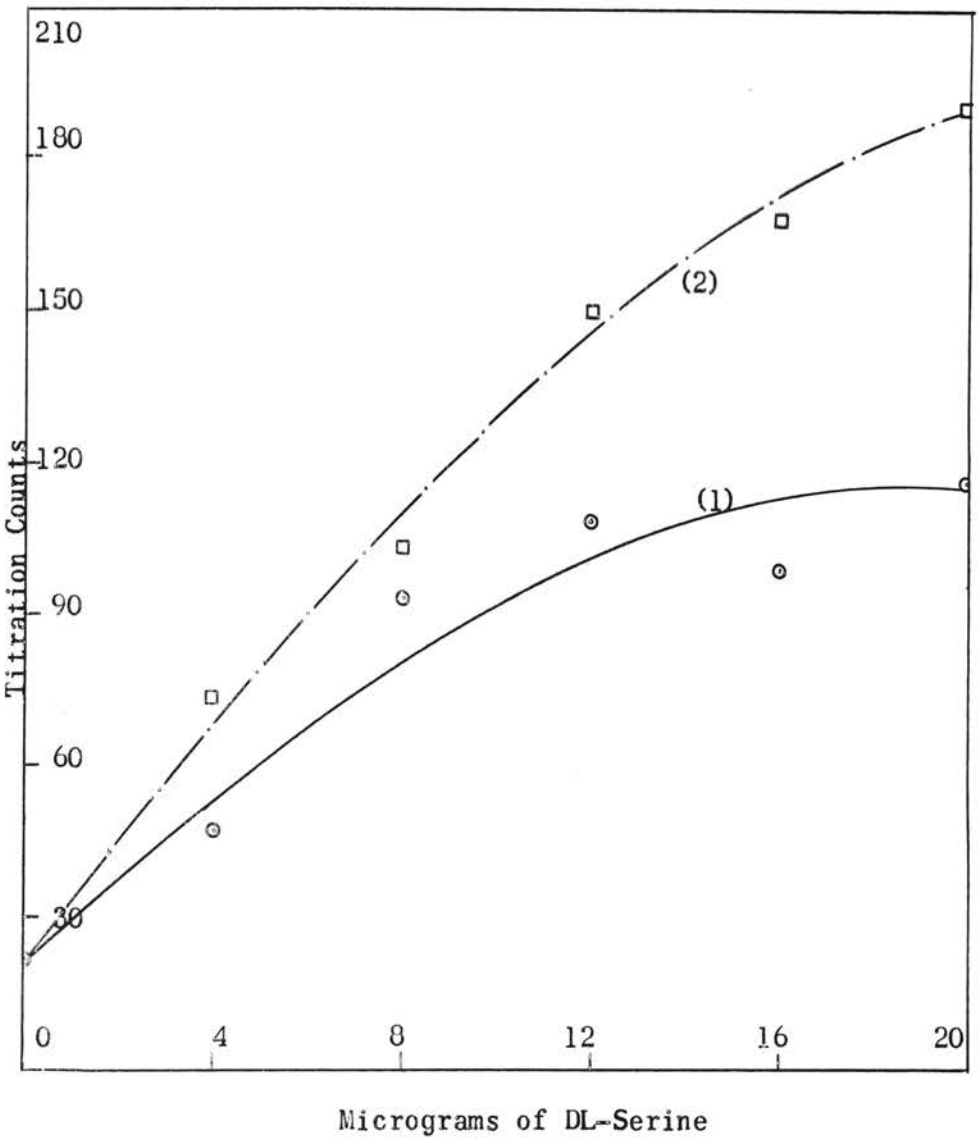

Figure 9. Effect of arginine on the utilization of serine in an alanine inhibited system. (1) 40 micrograms, (2) 2000 micrograms of L-arginine·HCl 200 micrograms of glycine present in the medium.

H. The Effect of Threonine

The antagonism of the utilization of serine by threonine has been reported by several workers (20, 21, 24). Meinke and Holland (20) have shown that at a given level of serine, increasing concentrations of threonine inhibit the growth of Leuc. mesenteroides. Ratios of threonine to serine higher than 75 to 1 completely prevented the growth of the organism. A further study was made to test this interrelationship of threonine and serine.

The organism responds to a 20 microgram standard of DL-serine when the threonine level is 40 micrograms. Increasing the threonine concentration to 4000 micrograms has an adverse effect on the growth of the organism. This unfavorable effect of threonine on the utilization is corrected by an increase in the level of serine. Figures 10 and 11 demonstrate the antagonism of serine utilization by relatively high levels of threonine.

The experimental findings show that the inhibition caused by the imbalance of these amino acids may be competitive and reversible. This appears to be a plausible explanation, since threonine and serine are structurally related.

I. Utilization of L-, D-, and DL-isomers of Serine

The L-stereoisomer of serine is the one normally present in bacterial protein. Since, in this study, of amino acid interrelationships, the DL- form has been exclusively used, experiments were conducted to see if differences existed in growth responses to the two isomers of serine and to their racemic mixture. The DL-serine was added in concentrations two times that of the other two isomers. The growth response of the organism to the three forms of serine is shown in Figures

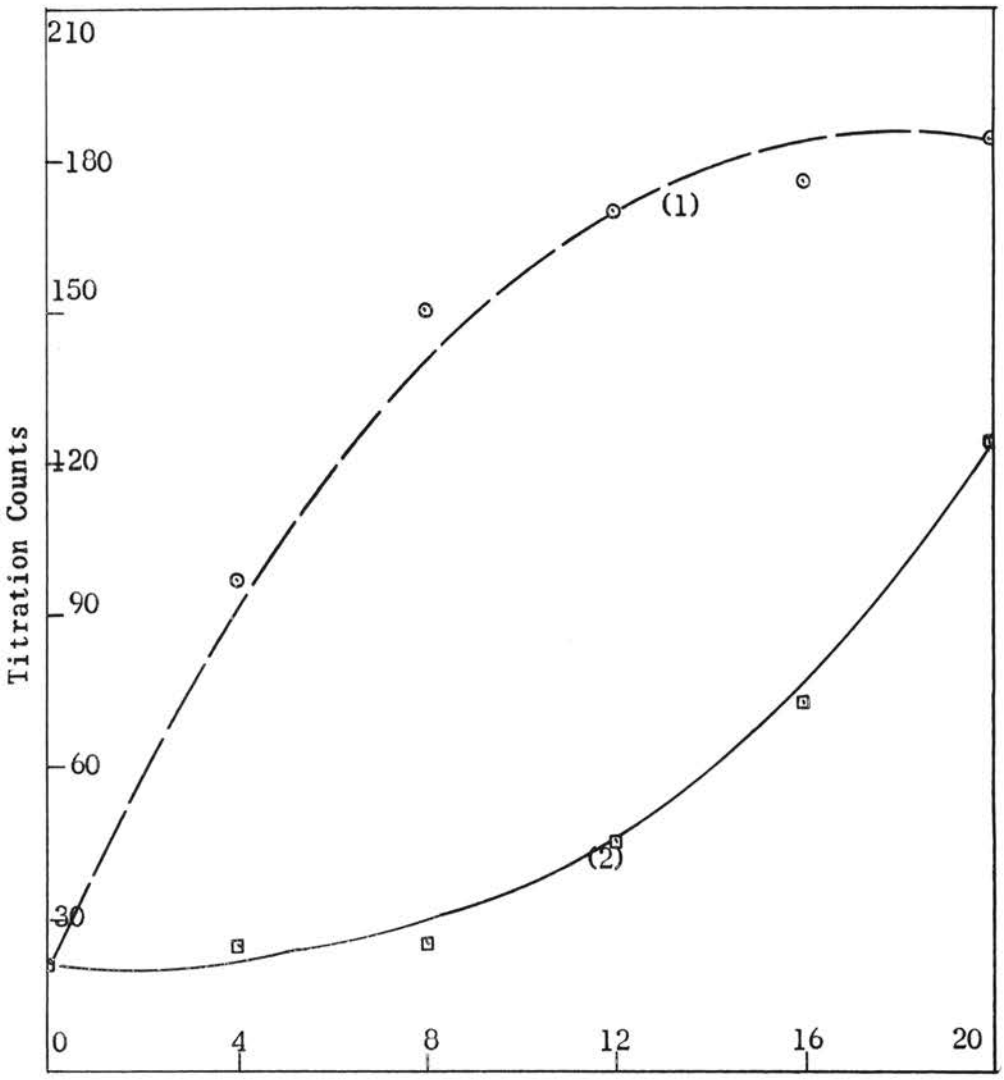

Figure 10. Effect of threonine on serine utilization in a medium containing 80 micrograms of valine. (1) 40 micrograms, and (2) 4000 micrograms of L-arginine·HCl.

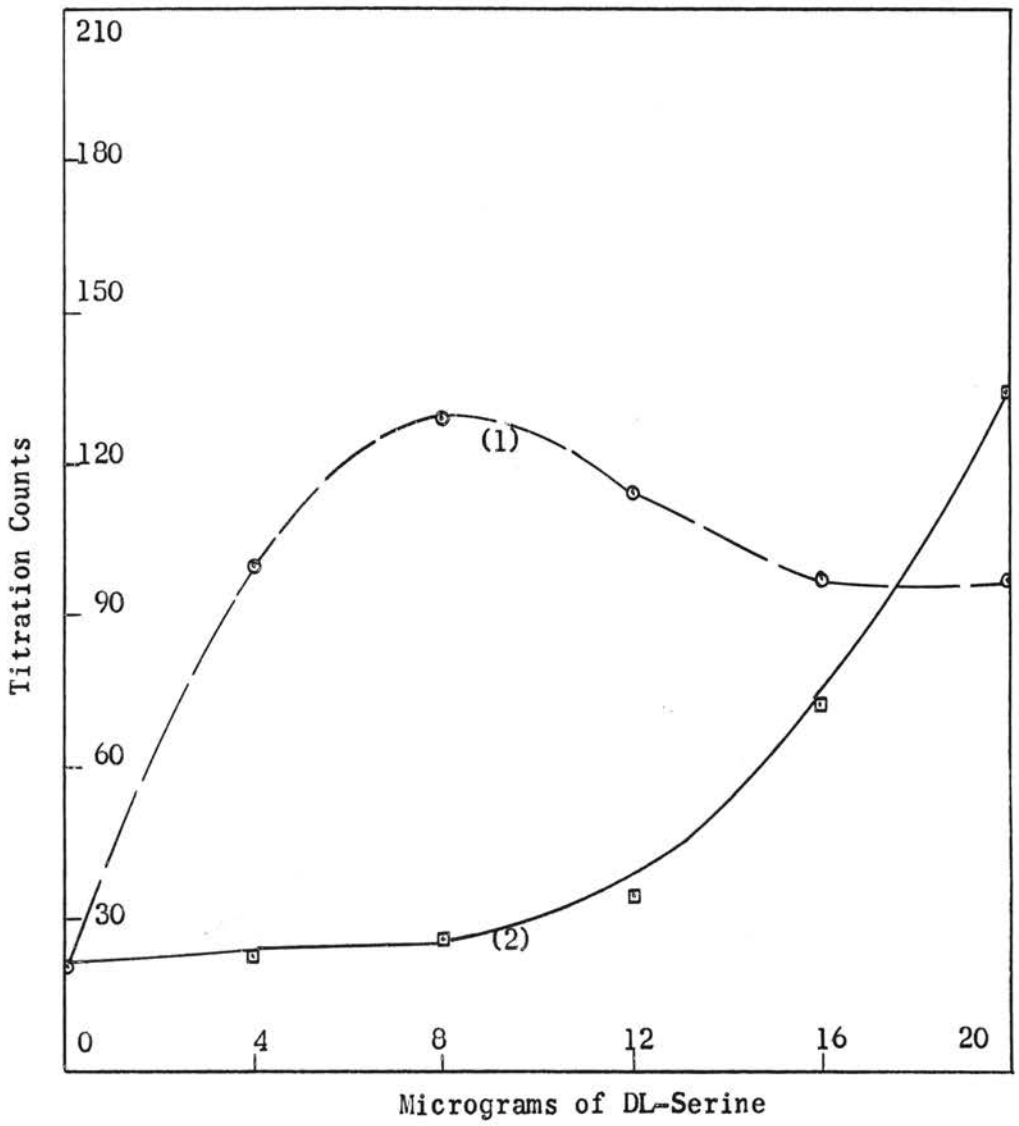

Figure 11. Effect of threonine on serine utilization in a medium containing 320 micrograms of valine. (1) 40 micrograms, and (2) 4000 micrograms of arginine.HCl.

12 and 13.

It seems that the D- form supports very little growth of Leuc. mesenteroides. The activity of two moles of DL-serine was approximately equal to the activity of one mole of the L-serine. This shows that the D-form does not, in any respect, interfere with the utilization of the L-form of serine when the two are present in equimolar amounts as in the racemic DL-form. These studies of the utilization of the different stereoisomers were conducted in a medium relatively low with respect to aspartic acid and glutamic acid.

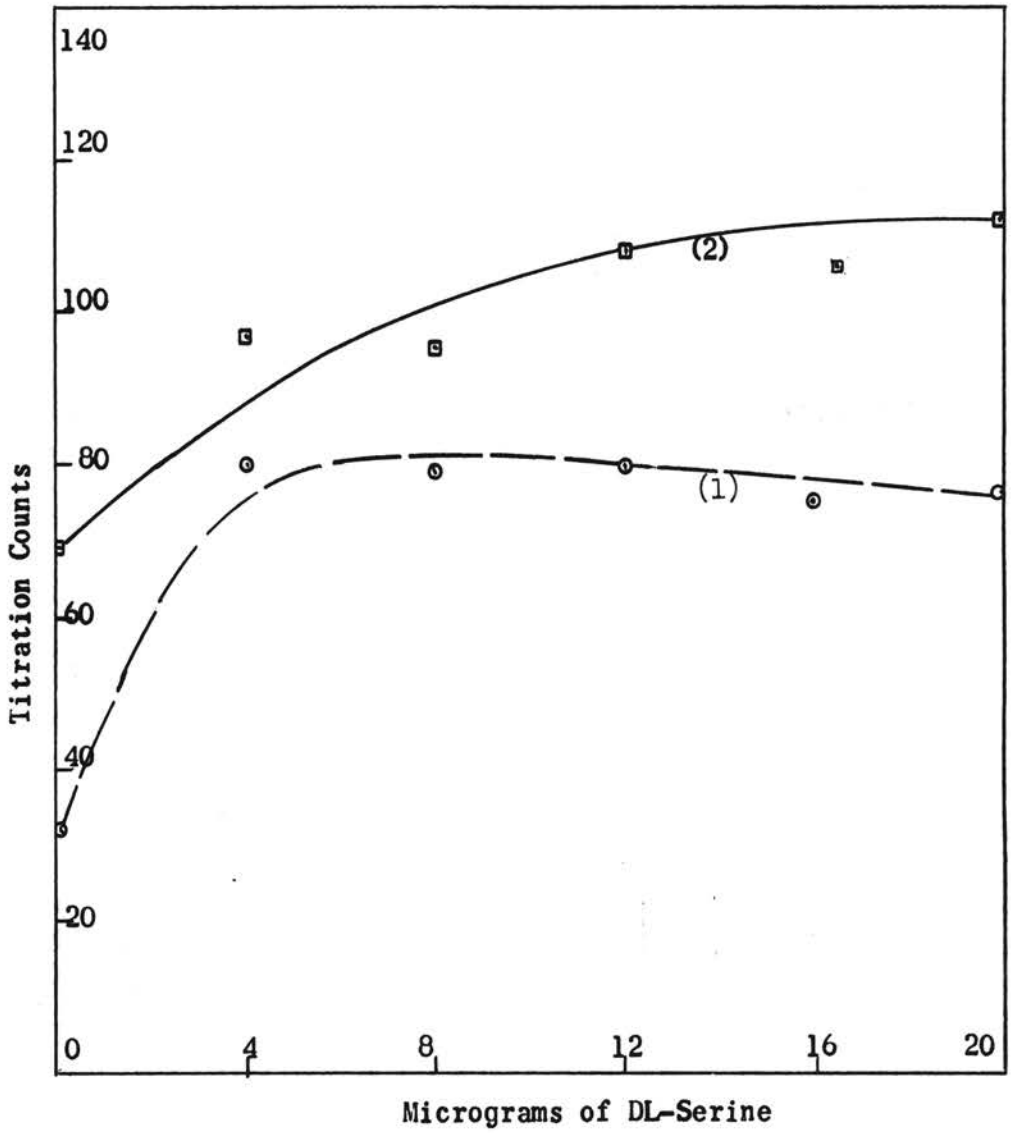

Figure 12. Response of Leuc. mesenteroides to DL-serine in a medium containing low aspartic acid and glutamic acid. (1) 100 micrograms (2) 30 micrograms of DL-aspartic acid.
30 micrograms of L-glutamic acid present in the medium.

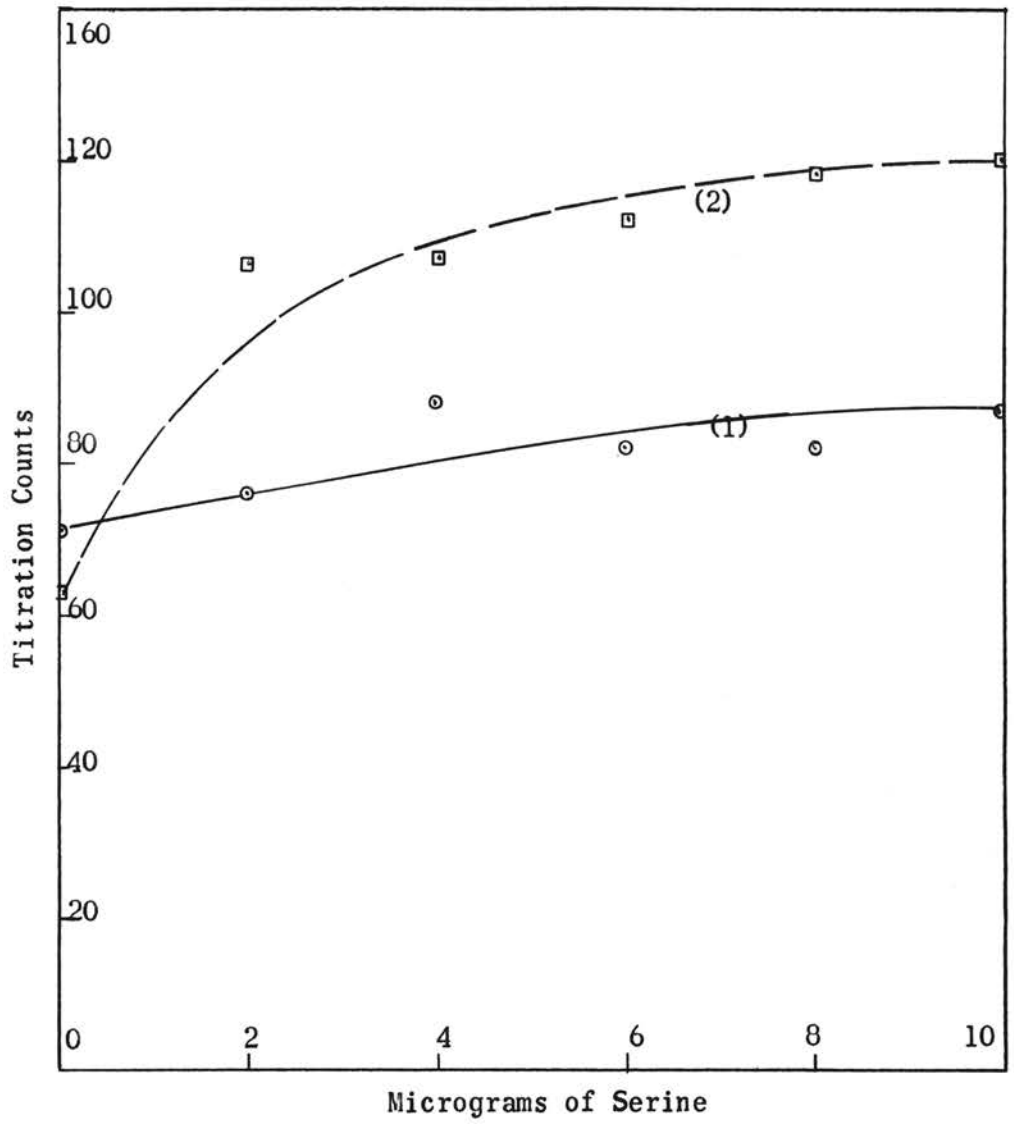

Figure 13. Response of Leuc. mesenteroides to L- and D-isomers of serine. (1) D-serine (2) L-serine. 30 micrograms of DL-aspartic acid and L-glutamic acid each present in the medium.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The bacterium Leuconostoc mesenteroides P-60 has been reported to require seventeen amino acids for normal growth. This was confirmed under routinely-used conditions, but with modifications of amino acid concentrations in the media, the requirement for certain amino acids could be partially or completely eliminated.

A striking example of this phenomenon is the synthesis of serine by this organism when the medium is relatively high in glycine. It has been previously reported and further elucidated in this work that relatively high amounts of alanine inhibit the synthesis of serine. In the present work, high amounts of leucine have been observed to markedly inhibit the biological conversion of glycine to serine. A similar inhibition, though of lesser degree, has been observed with lysine. While the inhibition of serine synthesis by alanine may be explained on the basis of structural similarities between glycine, serine and alanine, no analogous explanation can be offered for the inhibition caused by leucine and lysine.

Certain other amino acids improved the growth-response of the organism in a serine-free medium, but their effects have been found to be indirect with respect to their effect on serine synthesis. In this category is glutamic acid, which was found to increase growth in a serine-free medium, not by directly increasing serine synthesis, but by overcoming

an inhibition attributable to high concentrations of aspartic acid in the medium.

Still other amino acids have been observed to exert their influence by affecting the utilization of serine rather than its synthesis. Thus threonine appeared to inhibit serine synthesis actually by its well-known competitive antagonism of serine utilization. One which appeared to favorably affect serine synthesis was arginine, which was subsequently found to have a marked favorable effect on the utilization of serine in an alanine-inhibited system.

PART II

CHAPTER I

INTRODUCTION

Teff (Eragrostis abyssinica), a millet-like cereal grain, is the most widely used grain in Ethiopia. A nutrition survey made by the Interdepartmental Committee on Nutrition for National Defense showed that teff accounted for over half of the cereal production in Ethiopia (34). According to the survey, a typical daily Ethiopian diet contained 65 gm. of protein, 63 % of which was obtained from teff. Teff is not only the major source of protein and carbohydrates; it is a good source of vitamins and minerals as well. Because of the important role teff plays in the protein nutrition of an average Ethiopian, an investigation of the amino acid composition and biological value of teff protein would serve the purpose of evaluating its quality for the nutrition of animals. The present study is intended to make such an evaluation.

Most cereal grain proteins are deficient in the amino acid, lysine. A preliminary determination of the amino acid composition of teff protein showed that the only limiting essential amino acids for rat growth should be lysine and histidine. Attempts have been made to improve the biological value of teff protein by supplementation with these limiting amino acids. Comparison of teff protein with other well known protein sources has also been studied by rat growth.

Several nutritional studies on the supplementation of wheat protein

with its limiting amino acid, lysine, have been conducted over a period of many years. As early as 1914, Osborne and Mendel (35) showed that wheat protein was deficient in lysine for rat growth and supplementing the wheat protein diet increased the growth of rats. Similar studies by Mitchell et al. (36) using the paired method of feeding rats showed that lysine supplementation caused a large increase in the growth rate of rats receiving wheat protein. Sure (37) showed that supplementing whole wheat with 0.25 % L-lysine·monohydrochloride resulted in an increase of about 45 % in protein efficiency ratio (PER) over the unsupplemented whole wheat. He also obtained additional improvement from supplementation with valine and threonine. The biological value of milled wheat flour, supplemented with lysine, threonine, and valine was approximately the same as for the proteins of dried non-fat milk solids (38). There are several studies concerning the effect of lysine supplementation on the biological value of whole wheat and products based on rat growth (39, 40).

In experiments using 91 % polished, ground rice, supplementation with graded amounts of L-lysine·HCl substantially increased growth rate and efficiency of food utilization in rats (43). Rosenberg et al. (41) have demonstrated that supplementation of a protein with its first limiting amino acid is highly beneficial if it brings the total amount of this amino acid present in the protein and available to the organism into balance with second limiting amino acid. Their work with rats showed that the best biological value was obtained when rice diet was supplemented with .425 % L-lysine·HCl and 0.36 % DL-threonine (42). This work illustrates that it is not enough to add an excess of an essential amino acid which is found to be deficient in a protein, but

that a proper balance or ratio should be effected by the supplementation.

Scrimshaw et al. (44) have studied the effect of amino acid supplementation in man. They fed a simplified basal diet in which corn was the only source of protein to two boys recently recovered from severe protein malnutrition. The protein was supplied at a level of 3.0 grams per kilogram of body weight. The boys continued to gain weight during the progressive supplementation of the basal diet with the amino acids indicated to be deficient by comparison with the amino acid pattern of an FAO "reference protein." When the basal diet was supplemented with tryptophan and lysine to bring the amino acid levels of intake to the reference point, there was a marked increase in nitrogen retention. The work showed that appropriate amino acid supplementation to corn protein resulted in good nitrogen retention and satisfactory gain in weight. Other experiments (45) by the same authors showed that even at an intermediate level of protein intake (less than 2.0 grams of protein per kilogram of body weight per day), supplementation of corn protein with the appropriate amino acids resulted in good nitrogen retention by young children.

In the present studies the amino acid composition and protein value of teff have been studied. The amino acid analysis of this cereal grain showed that it was limiting in lysine and perhaps, in histidine. The biological value of the protein as determined by rat growth was significantly increased by supplementation with lysine but was unaffected by histidine. The teff protein value was also compared with that of whole egg protein, fish protein, and casein.

While this work was in progress, Jansen et al. (46) reported on the amino acid composition and supplementation of teff with lysine at the Fifth International Congress on Nutrition. The results of this work are in essential agreement with the results obtained by Jansen and coworkers.

CHAPTER II

EXPERIMENTAL

A. Amino Acid Analysis

In all the experiments conducted in this study, a mixture of equal weights of white and red teff is used. Hydrolysis was carried by heating the teff samples in 40 volumes 3N HCl for 12 hours at an autoclaving temperature of 121° C. After neutralization and appropriate dilutions, the protein hydrolysate was analyzed for its amino acid composition by microbiological assay by the microbioassay procedure (16). The following microorganisms were used as assay organisms for the different amino acids:

Leuconostoc mesenteroides P-60 (Streptococcus species ATCC 8042)

Aspartic acid, glycine, proline, tyrosine, isoleucine, lysine.

Leuconostoc citrovorum (Pediococcus cerevisiae ATCC 8081)

Alanine.

Lactobacillus arabinosus

Phenylalanine, glutamic acid.

Lactobacillus delbrueckii (Lactobacillus acidophilus ATCC 4913)

Histidine, valine, leucine, arginine.

Streptococcus faecalis ATCC 8043

Threonine.

B. Whole Egg Preparation

Preliminary experiments conducted to compare the biological value

of teff with that of commercial whole egg protein, casein, and fish showed that the biological value of the whole egg preparation was much lower than its reported value in the literature. Consequently, it was desired to prepare whole egg protein in the laboratory. The procedure that was used is described as follows[1].

Eggs are broken into a pail and cooked by running in dry steam to give what looks like a pail full of scrambled eggs. The eggs were air dried at room temperature. After four days of drying, they were extracted at room temperature with 95 % alcohol for three days, a fresh batch being used each day. This was followed by another alcohol extraction for a day, using absolute alcohol this time. They were then extracted at room temperature with ethyl ether for four days using fresh ether each day. Finally they were spread out to let the ether evaporate and were ground to a powder in the Wiley mill.

C. Feeding Procedures and Materials Used

Weanling male rats of the Sprague-Dawley strain were used in all of the experiments. The initial weights of the rats were from 40 to 50 grams. They were kept in individual cages; food and water were supplied ad libitum. Each day they were given a weighed amount of diet, and the amount of feed they did not consume was weighed back the next day.

The compositions of some of the dietary components are tabulated in Appendix II. These were incorporated into the experimental diets which essentially consisted of the following: a source of protein, an inorganic salts mixture (47), Mazola corn oil, a vitamin mixture (48), and sucrose. Two drops of the fat-soluble vitamin mixture (Appendix II)

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[1] B. C. Johnson, private communication.

were orally administered to each rat once a week. The rats were weighed twice each week, and the duration of the experiments was approximately four weeks. Six rats were used for each set of feeding experiments, and the results are expressed as averages of the data obtained from 6 rats on each of the dietary treatments in a four-week period.

CHAPTER III

RESULTS AND DISCUSSION

A. Chemical Composition of Teff and Other Protein Sources

The chemical analysis of mixed, white and red teff is shown in Table IV. The sample used for the analysis represented a mixture of equal portions, by weight, of red and white teff. The protein content of the mixture, as determined by the Kjeldahl method, was found to be approximately 9.0 %. Thus, it is seen that teff would be considered relatively low in protein.

The protein content of this sample of teff, which was used for the experimental studies reported here, is seen to be slightly lower than other values, also shown in Table IV, which were previously obtained for another sample of teff. This difference is to be expected, since the protein content of a grain may vary slightly with climate, soil, and cultural conditions. It is significant to note that there was no appreciable difference in the protein content of the commercial whole-egg protein and the whole-egg protein prepared in this laboratory. The biological values of the two sources of whole egg protein, however, were quite different, as will be shown later.

Analyses for certain other constituents of teff are also shown in Table IV.

B. Amino Acid Composition of Teff

The amino acid composition of teff as determined by microbiological

TABLE IV

CHEMICAL ANALYSIS OF TEFF[2] AND OTHER PROTEINS USED FOR COMPARATIVE PURPOSES[3]

Sample Description	Percent Dry Matter (Air Dried)	Percent Ash	Percent Protein	Percent Fat	Percent Fiber	Percent N.F.E.	Percent Ca	Percent P
			Air Dried Basis					
White Teff		2.42	10.63	2.45	1.80	73.77	.197	.380
Red Teff		2.42	9.69	2.77	2.64	71.99	.200	.360
			Moisture Free Basis					
White Teff	91.07	2.66	11.67	2.69	1.98	81.00	.216	.417
Red Teff	89.51	2.70	10.83	3.09	2.95	80.43	.223	.402
Whole egg protein (commercial)			54.73					
Whole egg protein (laboratory prepared)			55.25					
Fish protein			84.27					
Casein			76.03					

[2]Data made available by G. R. Waller, Jr., Assistant Professor of Biochemistry, Oklahoma State University.

[3]Analyses conducted in the course of this research.

assay is shown in Table V. The values obtained for the amino acids essential for man are in close agreement with those reported by the Interdepartmental Committee on Nutrition for National Defense (34).

The amino acid composition of teff was also compared with the amino acid requirements for rat growth (52); the only two amino acids which appeared limiting in teff were lysine and histidine. However, histidine did not appear as limiting as lysine for rat growth. The lysine content of teff was also considerably lower than that which is indicated in the FAO reference protein. Except for lysine and possibly histidine, the amino acid balance in teff appears to be good.

From these comparisons, it was assumed that the biological value of teff protein, which is well-balanced with respect to all amino acids essential for rat growth except for lysine and possibly histidine, would be improved by supplementation with the limiting amino acids. The experiments which were conducted were designed to test this assumption.

C. The Biological Value of Teff Protein as Compared to Certain Other Proteins

Preliminary experiments were conducted to compare the biological value of teff protein with certain other protein sources that are widely used as standard proteins or as food for human consumption. The composition of the diets used for these comparisons is shown in Appendix II. The biological values are expressed in terms of protein efficiency ratios (P E R), and are shown in Table VI. A summary is given of the protein efficiency ratio of teff compared to those of commercial whole-egg protein, casein, and fish protein. The protein level of all the diets was adjusted to 5.0 percent protein by weight.

TABLE V

AMINO ACID ANALYSIS OF TEFF

Amino Acid	Percent in Sample	Percent in Protein[4]
Glycine	0.35	3.9
Alanine	0.26	2.9
Valine	0.50	5.5
Leucine	0.60	6.7
Isoleucine	0.35	3.9
Cystine[5]	0.24	--
Methionine	0.27	3.0
Threonine	0.30	3.3
Phenylalanine	0.40	4.4
Tyrosine	0.20	2.2
Proline	0.42	4.7
Tryptophan[5]	0.14	--
Aspartic Acid	0.56	6.2
Glutamic acid	1.61	17.9
Lysine·HCl	0.28	3.2
Histidine·HCl	0.15	1.7
Arginine·HCl	0.33	3.7

[4]Sample contained 9.02 percent crude protein (N x 6.25), air-dry basis.

[5]Data for this amino acid obtained from bibliographical reference (34).

TABLE VI

COMPARISON OF TEFF PROTEIN EFFICIENCY RATIO[6] WITH THAT OF CERTAIN OTHER PROTEIN SOURCES
Protein Content of Diets = 5.0 Percent
Based on a four-week Period

Diet	No. Rats Per Group	Av. Gain In Wt. (g)	Av. Prot. Intake (g)	Av. Food Intake (g)	P E R
Teff	6	4.08	6.58	131.10	0.62
Fish	6	7.125	5.70	113.90	1.25
Casein	6	9.50	6.53	130.60	1.45
Commercial whole egg	6	17.37	7.30	145.90	2.38

[6]Protein Efficiency Ratio (P E R) = $\frac{\text{Weight Gained (g)}}{\text{Protein Consumed (g)}}$

The protein efficiency ratios of all the proteins used, as measured by rat growth, were considerably low. This was primarily due to a low protein content of the diets. In rat feeding experiments using a synthetic amino acid diet, RamaRoa and coworkers(49) showed that optimum growth was not obtained until the protein level was 8 %. Harris et al. (50) obtained higher protein efficiency ratios with wheat when the protein level was increased from 8 % to 15 %. Their results were in accord with the work of Barnes and coworkers (51), who reported that efficiencies of animal proteins were highest when fed at low levels in rat diets, whereas efficiencies of cereal proteins improved as the level of protein in the diet approached 20 %.

Although the protein efficiency ratios of the proteins were low, as a whole, the whole-egg protein was superior to any other protein tested. Consequently, commercial whole-egg protein or laboratory-prepared whole-egg protein has been used as the standard protein.

D. Effect of Lysine and Histidine Supplementation on the Protein Efficiency Ratio of Teff

Since Lysine and possibly histidine were found to be limiting in teff protein, experiments were conducted to investigate the effect of these amino acids on the protein value of teff. Commercial whole-egg protein was used for comparative purposes, since it proved to be superior in protein quality to any other protein tested (Table VI). The results obtained by supplementation of teff-containing diets with 0.3 % L-lysine·HCl and 0.2 % L-histidine·HCl are summarized in Table VII.

The protein efficiency ratio of the lysine-supplemented teff was more than twice the value of the unsupplemented teff. Histidine supplementation of teff either alone or combined with lysine did not produce

TABLE VII

EFFECT OF LYSINE AND HISTIDINE SUPPLEMENTATION ON THE PROTEIN EFFICIENCY RATIO OF TEFF AS COMPARED WITH COMMERCIAL WHOLE EGG PROTEIN
Protein Content of Diets = 8.25 Percent
All Data Based on a Four-week Period

Diet	No. Rats Per Group	Av. Gain In Wt. (g)	Av. Prot. Intake (g)	Av. Food Intake (g)	P E R
Teff	6	22.80	15.80	191.00	1.44
Teff + 0.3 % lysine·HCl	3	90.80	27.60	334.80	3.29
Teff + 0.3 % lysine·HCl and 0.2 % histidine·HCl	3	84.50	26.30	318.30	3.21
Teff + 0.2 % histidine·HCl	3	26.67	17.80	216.20	1.50
Commercial whole egg	6	36.80	16.10	194.50	2.29

any significant improvement in the protein efficiency ratio of teff. The amino acid analysis had indicated that histidine might be slightly limiting for optimum rat growth, but apparently this slight limitation of teff protein is not sufficiently significant to affect rat growth.

It is interesting to note that the protein efficiency ratio of teff increased when the protein level of the basal diets was increased from 5 % to 8.25 % (Table VII). On the other hand, the protein efficiency ratio of commercial whole-egg protein slightly decreased when the level of protein was similarly increased. These observations conform, in principle, to the findings of Barnes et al. (51).

E. Comparison of the Protein Efficiency Ratio of Lysine-Supplemented Teff with Laboratory-Prepared Whole-Egg Protein

The protein efficiency ratio of the commercial whole-egg preparation used in the previous experiments was much lower than values in the literature. Since this poor performance might be attributable to the method of preparation of the commercial product, it was decided to undertake the preparation of whole-egg protein in the laboratory. The procedure followed in this preparation has been described in Chapter VI.

When this laboratory preparation of whole-egg protein was used in another set of experiments, the results shown in Table VIII were obtained. It is seen that the protein efficiency ratio of this new whole-egg preparation is approximately twice that of the whole-egg protein prepared commercially. While the efficiency ratio of this laboratory preparation of egg protein is considered to be unusually high, the large difference obtained suggests that the protein of the commercial source must have been damaged from a nutritional standpoint, in some manner which is not obvious at the present time.

TABLE VIII

IMPROVEMENT OF PROTEIN EFFICIENCY OF TEFF PROTEIN BY LYSINE SUPPLEMENTATION AS COMPARED TO LABORATORY PREPARED WHOLE EGG PROTEIN
Protein Content of Diets = 8.25 Percent
All Data Based on a Four-week Period

Diet	No. Rats Per Group	Av. Gain In Wt. (g)	Av. Prot. Intake (g)	Av. Food Intake (g)	P E R
Teff	6	39.00	23.60	286.20	1.63
Teff + 0.3 % lysine·HCl	6	86.80	29.50	357.60	2.90
Laboratory prepared whole egg protein	6	127.60	28.09	340.50	4.52

It is also seen in Table VIII that the lysine supplementation substantially increases the protein efficiency ratio of the teff. The value for the lysine-supplemented teff is slightly lower than that obtained in the earlier experiments, but the small difference is not considered significant. In comparison to the whole-egg protein in this experiment, the lysine-supplemented teff approaches, but does not equal, the performance obtained with the whole-egg protein. However, the improvement obtained with the supplementation is of such magnitude that the supplemented teff should compare vary favorably in quality to many of the major protein sources in human diets.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The chemical and amino acid composition of teff (Eragrostis abyssinica), which is widely used for human consumption in Ethiopia, has been investigated. Comparison of the amino acid pattern of teff with the amino acid requirements of rats indicated that lysine and possibly histidine might be the limiting amino acids in teff.

The protein efficiency ratio of teff was first compared with that of commercial whole-egg protein, casein, and fish protein by rat growth studies. The results of these comparisons showed that the protein efficiency ratio of teff is very low. Supplementation of the teff protein with 0.3 % L-lysine·HCl doubled the protein efficiency ratio. Supplementation with histidine did not have a significant effect in the rat growth studies.

Since the commercial whole-egg protein preparation used as the standard protein in the above studies showed a very poor performance, a laboratory preparation of whole-egg protein was made. In another set of experiments, this preparation gave a protein efficiency ratio which was approximately twice that obtained with the commercial protein. Lysine-supplemented teff showed an improvement over unsupplemented teff similar to that obtained in the earlier experiments.

It appears that lysine is the most limiting amino acid in teff and that supplementation of teff with lysine significantly improves its protein quality.

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A P P E N D I X

APPENDIX I

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	5.0gm.
Water 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 soln.*	1.0 %
Vitamin soln.*	0.5 %

Dissolved in water, and pH adjusted at 6.0

The media were sterilized and stored in the refrigerator.

B. Stock Solutions for Microbiological Assays and Studies

Amino acid solution** (for 100 tubes at 2 ml. final assay volume):

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	40 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 minimal acid and heat	

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*Composition given in Appendix B.

**The amino acid(s) under study to be omitted, and added separately.

B. (continued)

Salts C

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

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

Vitamin soln.

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.

The riboflavin dissolved first with hot water, then other vitamins added, and made up to 250 ml.

AGU-soln.

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

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

X-soln.

Xanthine	250 mg.

Dissolved in dilute KOH and made up to 250 ml.

Biotin soln.

Biotin	0.25 mg.

Made up to 250 ml. with 50 % EtOH

Folic Acid soln.

Folic Acid	0.25 mg.

Made up to 250 ml. with 50 % EtOH made slightly alkaline with dil. KOH

C. Double-Strength Basal Medium

(for 100 tubes at 2 ml. final assay volume):

Glucose	4.0 gm.
K-citrate.H_2O	4.4 gm.
K-acetate (anhydr.)	0.2 gm.
NH_4Cl	0.6 gm.
K_2HPO_4	1.0 gm.
Salts C soln.	4.0 ml.
AGU-soln.	2.0 ml.
X-soln.	2.0 ml.
Vitamin soln.	2.0 ml.
Biotin soln.	2.0 ml.
Folic acid soln.	2.0 ml.
Amino acid soln.	25.0 ml.

The pH adjusted to desired value, usually 6.8-7.0, and volume adjusted to 100 ml.

Appendix II

A. Vitamin Mixture for Rat Growth Studies per 10 Kilograms of Diet

Thiamine·HCl	20.0 mg.
Riboflavin	30.0 mg.
Pyridoxine·HCl (B_6)	25.0 mg.
Calcium pantothenate	200.0 mg.
Choline chloride	10.0 gm.
L-Inositol	1.0 gm.
Biotin	1.0 mg.
Folic acid	2.0 mg.
B_{12}	0.17 mg.
Niacin	250.0 mg.

Made to 200 grams with sucrose

B. Fat-Soluble Vitamin Mixture

PABA	100.0 mg.
Menadione	2.5 mg.
Vitamin A acetate	200.0 I.U.
Calciferol	30.0 I.U.

Made to 5.0 ml. with corn oil

C. Salts IV Mixture per 100 Grams

NaCl	17.0 gm.
K_2HPO_4	32.25 gm.
$CaHPO_4$	6.20 gm.
$MgSO_4 \cdot 7H_2O$	10.20 gm.
$CaCO_3$	30.00 gm.
Ferric citrate	2.75 gm.
KI	0.08 gm.
$MnSO_4 \cdot H_2O$	0.50 gm.
$ZnCl_2$	0.025 gm.
$CuSO_4 \cdot 5H_2O$	0.030 gm.

D. Composition of the Basal Diets

Protein	5.0 and 8.25 gm.
Salts IV mixture	4.0 gm.
Vitamin mixture (A, above)	2.0 gm.
Corn oil*	2.5 gm.

*An additional 2.5 gm. of corn oil was added to all diets except the teff diet to compensate for the fat in teff. All diets were made to 100 gm. with sucrose.

VITA

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Master of Science

Thesis: PART I. CERTAIN AMINO ACID INTERRELATIONSHIPS AFFECTING THE BIOSYNTHESIS OF SERINE IN LEUCONOSTOC MESENTEROIDES

PART II. AMINO ACID COMPOSITION AND BIOLOGICAL VALUE OF TEFF PROTEIN AND ITS IMPROVEMENT BY LYSINE SUPPLEMENTATION

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