ANTAGONISM OF AMINO ACIDS IN THE GROWTH OF LACTIC ACID BACTERIA*

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Inhibition of microbial growth by compounds closely related to essential metabolites has been demonstrated by many investigators (1-3). Early workers (4-7) showed that inhibitory effects of single amino acids could be reversed by altering the concentration of structurally related amino acids. Gladstone (8), using Bacillus anthracis, showed such a relationship among leucine, valine, and isoleucine and between threonine and serine. Threonine exerts an antagonistic action on the utilization of serine by Lactobacillus arabinosus (9). Using Lactobacillus casei, Feeney and Strong (10) demonstrated an inhibitory effect of aspartic acid, reversible by glutamine, glutamic acid, or asparagine.

While investigating a uniform medium for the microbiological determination of amino acids (11), certain difficulties were encountered with the glutamic acid and isoleucine assays with Lactobacillus arabinosus. A lag in the growth response curve was observed at the lower concentrations of these amino acids. The tubes containing samples, particularly in the case of isoleucine assays, did not show this lag to the same extent as the standard curves, resulting in marked downward drift of assay values. To alleviate the lag in the glutamic acid curve and to give a valid assay, a heavy inoculum and adjustment of the medium to pH 6 have been used (12). While this provided a satisfactory assay for glutamic acid, further work on the fundamental defect was indicated. As in previous studies (13, 12, 14, 15), when glutamic acid standard was replaced by glutamine (sterilized by filtration) the lag was absent. This indicated, as suggested previously (12, 14), that glutamic acid is utilized through glutamine and that the lag is the result of partial or complete failure of the small amounts of glutamic acid present to be amidated to glutamine.

In this study the mutual antagonism of the members of two groups of

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amino acids in the growth of *Lactobacillus arabinosus* 17-5 and *Leuconostoc mesenteroides* P-60 was investigated.

**EXPERIMENTAL**

The cultures and assay techniques used were the same as described previously (11), except that light inocula were used. To accentuate the lag in growth, the inocula were diluted until no turbidity was perceptible. In later experiments, the inocula were carefully standardized to a somewhat greater dilution than this by suspending the cells in sufficient sterile water to give a reading of 50 in an Evelyn colorimeter, with standard Evelyn tubes, and a 660 mg filter against a water blank. 1 ml. of this suspension was then diluted to 200 ml. with sterile, distilled water and 1 drop was used to inoculate each 2 ml. of culture medium.

In most studies, 2 ml. volumes in 18 X 150 mm. culture tubes were used. In some of the glutamic acid experiments early growth in 10 ml. volumes, in Evelyn colorimeter tubes, was measured turbidimetrically against an uninoculated blank. In other work where a number of solutions had to be sterilized by filtration 0.2 ml. volumes were used. They were titrated electrometrically (16) after 60 to 72 hours of incubation.

**Results**

_Effect of Other Amino Acids on Isoleucine Requirement—Inhibition indices (3) were determined for *Lactobacillus arabinosus* and *Leuconostoc mesenteroides* with isoleucine as the metabolite and leucine and valine as the antagonists. Typical results are shown in Table I. The inhibition indices were not constant, but were of the same general magnitude over wide concentration ranges. In all cases increasing the concentration of the metabolite reversed the inhibition, indicating that it is competitive. DL-Valine and L-leucine were additive in antagonizing the growth of *Lactobacillus arabinosus* when isoleucine was the limiting amino acid. DL-Leucine was approximately half as effective as L-leucine as an antagonist of isoleucine, indicating that only the L isomer is involved; D-leucine was not tested. Decreasing the concentration of DL-valine and L-leucine in the basal medium from the normal level of 400 and 200 γ per 2 ml. tube to 100 and 50 γ, respectively, eliminated the lag.

Excessive amounts of methionine inhibited the growth of *Lactobacillus arabinosus* when isoleucine was the limiting amino acid. 10 mg. per tube of DL-methionine completely suppressed the growth of *Lactobacillus arabinosus* in the presence of 30 γ of DL-isoleucine. Alanine at 30 mg., or DL-serine or DL-threonine at 50 mg. per tube, caused no inhibition, indicating that the antagonism is not general for all amino acids (Table II). That these relationships are not peculiar to the medium being used in these studies was indicated by parallel experiments in which a medium typical of those currently employed by many other workers (17) was used.
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**TABLE I**

Results of Typical Experiment Showing Effect of Valine and Leucine As Antagonists of Isoleucine for Lactobacillus arabinosus

<table>
<thead>
<tr>
<th>DL-Isoleucine</th>
<th>DL-Valine</th>
<th>L-Leucine</th>
<th>Titer</th>
<th>Molar inhibition index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ per tube</td>
<td>γ per tube</td>
<td>γ per tube</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>500</td>
<td>50</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1,000</td>
<td>50</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1,500</td>
<td>50</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2,000</td>
<td>50</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>15,000</td>
<td>50</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>20,000</td>
<td>50</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>25,000</td>
<td>50</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>30,000</td>
<td>50</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>45,000</td>
<td>50</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>15,000</td>
<td>50</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>75,000</td>
<td>50</td>
<td>179</td>
<td>135-200</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>500</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>200</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>400</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>800</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>1000</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

* Based on concentrations of L forms of amino acids.

**TABLE II**

Summary of Results of Studies of Antagonism of Amino Acids*

<table>
<thead>
<tr>
<th>Limiting amino acid</th>
<th>Inhibiting amino acid</th>
<th>Molar inhibition index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>L. arabinosus</strong></td>
</tr>
</tbody>
</table>

| Isoleucine          | DL-Valine             | 45-60                  | 500-1000                |
|                     | L-Leucine             | 30-40                  | 75-250                  |
|                     | DL-Leucine            | 40-50                  |                         |
|                     | DL-Methionine         | 100-200                | Not determined          |
|                     | DL-Threonine          | " " 3500              | " "                    |
|                     | DL-Alanine            | " " 1400              | " "                    |
| Leucine             | DL-Isoleucine         | 450                    | None at 3000            |
|                     | DL-Valine             | 2000                   |                         |
|                     | DL-Methionine         | None at 1500           | Not determined          |
|                     | DL-Threonine          | " " 5500              | " "                    |
|                     | DL-Serine             | " " 6200              | " "                    |
| Valine              | L-Leucine             | 360                    | None at 1800            |
|                     | DL-Isoleucine         | 20-40                  | 700                     |
|                     | DL-Methionine         | <300                   | Not determined          |

* All based on concentrations of the L isomers.
When five organisms were used to determine isoleucine in acid-hydrolyzed casein, all except Lactobacillus arabinosus gave nearly identical values, with no drift. With L. arabinosus high values at the lower levels and low values in the upper part of the standard curve, i.e. a drift downward, were encountered. Thus, for example, values of 6.1 per cent with Streptococcus faecalis R, 6.0 per cent with Leuconostoc mesenteroides, 6.0 per cent with L. delbrueckii-3, and 6.1 per cent with L. casei were obtained. Values with L. arabinosus drifted from 10 to 5.6 per cent, indicating that the standard curve was below the "sample curve" at low levels and above it at the higher levels.

**Table III**

<table>
<thead>
<tr>
<th>Sample weight (mg)</th>
<th>L. mesenteroides</th>
<th>L. arabinosus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Regular</td>
</tr>
<tr>
<td>0.1</td>
<td>6.4</td>
<td>9.5</td>
</tr>
<tr>
<td>0.2</td>
<td>6.8</td>
<td>7.3</td>
</tr>
<tr>
<td>0.3</td>
<td>6.0</td>
<td>6.4</td>
</tr>
<tr>
<td>0.4</td>
<td>6.0</td>
<td>6.2</td>
</tr>
<tr>
<td>0.5</td>
<td>6.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Average</td>
<td>6.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

* Regular levels were DL-valine 0.4 mg. per 2 ml. tube and L-leucine 0.2 mg. per 2 ml. tube. Low levels were one-fourth of the regular concentrations.

The effect of lowering the level of leucine and valine in the medium for the determination of isoleucine in another protein hydrolysate is shown in Table III. With regular concentrations of leucine and valine in the medium, there was drift in the values, particularly with Lactobacillus arabinosus. Decreasing the level of these amino acids eliminated the slight drift for Leuconostoc mesenteroides, giving assay values which compare well with those obtained with L. delbrueckii-3 (6.6 per cent). When L. arabinosus was the test organism, the drift was not eliminated by lowering the concentrations of leucine and valine in the medium, and much lower values were obtained. With Leuconostoc mesenteroides, inhibition of growth occurred in the lower portion of the curve when regular levels of leucine and valine were present in the medium, but the upper portion of the curve coincided with that obtained with low levels of these two amino acids (Curves 2 and 3, Fig. 1). With L. arabinosus, low levels of leucine and valine gave a standard curve elevated above that obtained with a medium containing
regular concentrations of leucine and valine, at all levels of isoleucine (Curves 1 and 4, Fig. 1). This indicated utilization of the $\delta$ isomer of isoleucine by *L. arabinosus* at low levels of leucine and valine. To verify the variable utilization of $\delta$-isoleucine by this organism, when regular and low levels of leucine and valine were used, standard curves with $L$- and $DL$-isoleucine were prepared. With regular levels of leucine and valine the $L$ and $DL$ standard curves (Curves 1 and 2, Fig. 2) nearly coincided, except in the upper portion. In this portion, the ratio of the concentrations of leucine and valine to isoleucine was less than in the lower portion of the curve, resulting in greater utilization of the $\delta$ isomer. With low levels of leucine and valine present in the medium, the $L$ and $DL$ curves were not superimposable. The $DL$-isoleucine standard curve (Curve 4, Fig. 2) diverged upward from the $L$-isoleucine standard curve (Curve 3, Fig. 2), indicating that the $\delta$ isomer was being utilized at nearly all concentrations of isoleucine. When $\delta$-isoleucine was added in increasing concentrations to a medium containing the $L$ isomer at a level sufficient for half maximum growth (7.5 $\gamma$ per tube), the titration values increased, indicating 10 per
cent activity at 10 γ per tube, and 19 per cent activity at 30 γ per tube. When d-isoleucine was used alone for preparing a standard curve, it showed no growth-promoting activity.

During these studies the contamination of dL-isoleucine (Merck), presumably with alloisoleucine, was noted. The activity for Leuconostoc mesenteroides and L. delbrueckii-3 of this dL-isoleucine, as compared to a pure sample of L-isoleucine, was only 39.5 per cent, instead of the 50 per cent expected for pure dL-isoleucine. This was also observed by Mr. F. A. Wacher of Merck and Company, Inc., and was reported by Smith and Greene (18).

1 Kindly supplied by Dr. D. G. Doherty of this laboratory.
**Effect Of Other Amino Acids on Leucine and Valine Requirements**—With leucine limiting, the concentrations of either DL-valine or DL-isoleucine required to inhibit growth of *Lactobacillus arabinosus* were nearly the same. The inhibition indices (Table II) indicate that this organism is less sensitive to these antagonisms when leucine is limiting than when isoleucine is the limiting nutrient. Methionine, threonine, and serine did not show the antagonism at the high concentrations used.

When valine was the limiting amino acid for the growth of this organism, DL-isoleucine caused inhibition at lower concentrations than did L-leucine. From Table II it is evident that leucine is antagonized less by isoleucine and valine than is valine by isoleucine and leucine. These differences account for the occasional slight lag in the valine standard curves with this medium and this organism and the absence of such lags in the leucine curves with similar assay conditions. The presence or absence of a lag from one valine assay to another is probably a result of slight variations in the weight and age of the inoculum. Methionine antagonized the utilization of limiting quantities of valine.

An antagonizing action by isoleucine for *Leuconostoc mesenteroides* with low concentrations of leucine could not be shown. Growth could be inhibited, however, by large amounts of isoleucine when the valine level was limiting. The relatively lower susceptibility of *Leuconostoc mesenteroides* to these imbalances is probably one reason why this organism is now so widely used for amino acid assays.

**Glutamic Acid-Aspartic Acid Relationship**—The general lag in the growth of *Lactobacillus arabinosus* when glutamic acid was limiting was evidenced in the lower portion of the standard curve, but occasionally extended over one-half of the range. Adjustment of the medium to pH 6 together with the use of a heavy inoculum (12) was a practical solution. Lyman et al. (14) added small amounts of glutamine to prevent this lag. Replacement of aspartic acid by asparagine has been reported by Baumgarten et al. (15) to relieve the lag, but when asparagine was added to our medium already containing aspartic acid, the lag was accentuated. This suggested that aspartic acid or asparagine might be the active substance causing the lag in the growth curve. When the concentration of aspartic acid in the medium was progressively lowered, the lag was diminished until it was completely eliminated at 40 μ per tube (Fig. 3). A 10- to 20-fold greater concentration of asparagine than aspartic acid was necessary to elicit an equivalent lag in growth. Table IV shows the effect of asparagine and aspartic acid on the metabolism of glutamic acid by *L. arabinosus*. These data show an inhibition index of 400 for asparagine and approximately 20 for L-aspartic acid. Asparagine exerts a stimulatory effect on the growth of this organism (Table IV) as shown by slightly higher titrations.
### Table IV

*Effect of Asparagine and Aspartic Acid on Metabolism of Glutamic Acid for Lactobacillus arabinosus*

<table>
<thead>
<tr>
<th>Concentration of glutamic acid</th>
<th>No aspartic acid</th>
<th>L-Asparagine, 2 mg.</th>
<th>DL-Asparagine, 2 mg.</th>
<th>L-Aspartic acid, 2 mg.</th>
<th>DL-Aspartic acid, 2 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>7</td>
<td>4</td>
<td>5</td>
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<tr>
<td>10</td>
<td>30</td>
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</tr>
<tr>
<td>25</td>
<td>43</td>
<td>56</td>
<td>56</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>80</td>
<td>86</td>
<td>102</td>
<td>98</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>100</td>
<td>96</td>
<td>108</td>
<td>101</td>
<td>11</td>
<td>72</td>
</tr>
</tbody>
</table>

* Final pH of media after autoclaving 7.1. Each count represents 0.05 ml. of 0.04 N NaOH.

**Fig. 3.** The effect of L-aspartic acid and L-asparagine concentration on the response of *Lactobacillus arabinosus* to glutamic acid.
for the medium containing added asparagine. Three different samples of asparagine (one of racemate and two of the L form) were used, at the same concentration, to test the possibility that the lag was due to aspartic acid as a contaminant of the asparagine. All samples gave the same degree of lag in growth. It is unlikely that three different samples would contain equal amounts of aspartic acid. From these data, it appears that the antagonistic action is due to the asparagine per se.

With aspartic acid limiting, high glutamic acid concentrations had no effect on the growth of Leuconostoc mesenteroides.

### Table V

<table>
<thead>
<tr>
<th>NaHCO₃</th>
<th>L-Aspartic acid</th>
<th>Glutamic acid*</th>
<th>Molar inhibition index</th>
<th>Glutamine*</th>
<th>Molar inhibition index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>137</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.9 × 10⁻³</td>
<td>0</td>
<td>133</td>
<td>114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.8 × 10⁻³</td>
<td>0</td>
<td>22</td>
<td>56</td>
<td>35</td>
<td>56</td>
</tr>
<tr>
<td>35.6 × 10⁻³</td>
<td>0</td>
<td>2</td>
<td>83</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td>47.6 × 10⁻³</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>59.5 × 10⁻³</td>
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<td>0</td>
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</tr>
<tr>
<td>0</td>
<td>0</td>
<td>147</td>
<td>130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7.5 × 10⁻³</td>
<td>21</td>
<td>10</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15.0 × 10⁻³</td>
<td>8</td>
<td>35</td>
<td>135</td>
<td></td>
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<tr>
<td>0</td>
<td>22.5 × 10⁻³</td>
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<tr>
<td>0</td>
<td>30.0 × 10⁻³</td>
<td>7</td>
<td>36</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37.7 × 10⁻³</td>
<td>7</td>
<td>37</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>

* The values represent titration counts, each count equivalent to 0.05 ml. of 0.04 N NaOH.

During these studies, Waelsch et al. (13) reported inhibition of the growth of *L. arabinosus* by oxalacetate and sodium bicarbonate. To determine whether these antagonisms had a common basis, inhibition indices were determined for L-aspartic acid and sodium bicarbonate. Table V shows the results of one such experiment. Although inhibition by aspartic acid was not observed when glutamine was present, glutamine had no effect on the bicarbonate inhibition. The molar inhibition index for aspartic acid was considerably lower than that for sodium bicarbonate.

## DISCUSSION

The amino acid requirements of many lactic acid organisms have been studied extensively in the search for more specific and reliable assays, but little has been reported on the effect of high concentrations of amino acids.
The policy has been to add an excess of all required nutrients, with the exception of the one being assayed. The results reported here and those of Meinke and Holland (9) indicate that the amino acids should also be present in correct proportions to avoid imbalances. Addition of an inhibiting amino acid with the sample might result in growth suppression in the sample tubes not encountered in the standard tubes, with resulting drift and invalidation of the assay. This might dictate a medium containing little more of such amino acids than the bacteria need for maximum growth. When such a medium is used, however, the percentage difference in concentration in sample tubes and standard tubes is very much greater and might prove quite significant when proteins of other than average composition are assayed. A safer procedure appears to be that of maintaining moderately high concentrations of all constituents, so that the percentage change in composition of the fermentation liquid is affected only slightly by addition of the sample. In cases in which difficulties arise, as evidenced by lag, drift in assay values, poor recovery of added amino acid, or inconsistent values, other organisms less sensitive to such imbalances should be employed. The use of L standards is advisable to eliminate possible activity of the D isomer.

With an isoleucineless strain of Neurospora crassa, it has been demonstrated that β-methyl-α-ketovaleric acid, the keto derivative of isoleucine, inhibits the conversion of the keto acid analogue of valine to valine (19). This may be due to saturation of the surface of the enzyme which reductively aminates this keto acid to valine by the structurally related isoleucine derivative. A similar mechanism may account for the relationships described here. Isoleucine may be utilized as a peptide or a similar derivative and its incorporation into such an active intermediate may be mediated by an enzyme which is effectively blocked by the homologous or isomeric amino acids.

Lyman et al. (20) have recently reported that the D isomer of isoleucine is utilized by Lactobacillus arabinosus when vitamin B_6 is present in the form of pyridoxamine; utilization was greater when leucine was present at 0.4 mg. than at 2.0 mg. per 10 ml. tube. The concentration of leucine used in our studies (11) is one-half the level reported to inhibit utilization of D-isoleucine. The low levels used in these studies are comparable to those found by Lyman et al. (20) to enhance utilization of the D form. The anomalous results obtained in assaying samples, with a medium containing high concentrations of valine and leucine and in the presence of pyridoxal, when DL-isoleucine was used for the standard curve, can largely be explained by a combination of the antagonism described here and the variable availability of D-isoleucine for Lactobacillus arabinosus shown by Lyman et al. (20).
When aspartic acid-glutamic acid ratios were calculated for the data of Hat et al. (12), inhibition indices ranged from 30 to 200, depending on pH, weight of inoculum, length of incubation, and whether ammonium salts were present. The inhibition indices found here essentially confirm their work.

Waelsch et al. (13), using a 20 hour turbidimetric assay, found that at a concentration of $0.82 \times 10^{-3} \text{M}$ glutamic acid, $24 \times 10^{-3} \text{M}$ oxalacetate at pH 5.7 or $9.5 \times 10^{-3} \text{M}$ NaHCO$_3$ at pH 7.4 would inhibit completely the growth of *Lactobacillus arabinosus*. The inhibition due to oxalacetate could be reversed by a 4-fold increase in concentration of glutamic acid or by $7.0 \times 10^{-5} \text{M}$ glutamine while NaHCO$_3$ inhibition was reversed by a 3-fold increase of glutamic acid or by $2.7 \times 10^{-6} \text{M}$ glutamine. They attributed the inhibition to carbon dioxide, which appeared to prevent the amidation of glutamic acid to glutamine. The competitive aspartic acid-glutamic acid growth inhibition obtained with *L. arabinosus* appears to be a function of the enzyme system which converts glutamic acid to glutamine. Aspartic acid or asparagine may inhibit this reaction by competing with glutamic acid for the enzyme catalyzing this conversion. In our studies, glutamine reversed aspartic acid inhibition, but failed to overcome bicarbonate inhibition. From these results, it appears that these inhibitors do not act by a common mechanism. Aspartic acid is a more effective inhibitor than sodium bicarbonate. Interpretation of the data of Waelsch et al. (13) is complicated by the presence of aspartic acid in the medium. Calculation of the inhibition indices showed that asparagine, methionine sulfoxide (21), NaHCO$_3$ (13), oxalacetate (13), and aspartic acid were 200 to 400, 75, 30, 30, and 20, respectively. In these studies, the index for sodium bicarbonate was approximately 50.

By replacing the aspartic acid in the uniform medium (11) for amino acid assays by one-half as much L- or D- asparagine, a dose-response curve to glutamic acid is obtained which is much more nearly linear and whose slope is largely independent of the size of the inoculum. Such a modified medium has proved highly satisfactory for the determination of glutamic acid.

**SUMMARY**

1. The metabolism of *Lactobacillus arabinosus* is affected by the balance of concentrations of leucine, isoleucine, valine, and methionine present in the medium.

2. When isoleucine was the limiting amino acid, high concentrations of leucine, valine, and methionine in decreasing order of effectiveness caused inhibition of growth. Alanine, threonine, and serine did not cause inhibition when added at high levels, indicating reaction specificity.
3. When leucine was limiting, isoleucine inhibited growth more than valine and when valine was limiting, isoleucine inhibited growth more than leucine.

4. The growth of Leuconostoc mesenteroides P-60 was also affected by imbalances of these amino acids. However, the concentrations necessary to inhibit this organism were approximately 5 times greater than those required to inhibit Lactobacillus arabinosus.

5. The growth of Lactobacillus arabinosus, with glutamic acid limiting, was inhibited by aspartic acid or asparagine; the former was the more effective antagonist. It appears that these amino acids inhibit by preventing the small amounts of glutamic acid present from being amidated to glutamine. This organism is much less sensitive to this inhibition below pH 7.

6. The uniform medium of Henderson and Snell (11) should be modified for the glutamic acid assay when Lactobacillus arabinosus is used by replacing the aspartic acid with asparagine at a lower concentration.

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