Accumulation of Freely Extractable Glutamic Acid by Lactic Acid Bacteria*

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(Received for publication, August 25, 1958)

Gale (2, 3) initially described the existence in bacteria of a metabolism-dependent process achieving the accumulation of freely extractable amino acids. During the studies described here a number of additional reports have appeared citing examples of amino acid (4, 5), carbohydrate (6), and phosphate (7) accumulations in bacteria. The mechanism of these accumulations is unknown.

Christensen et al. (8) have observed that pyridoxal stimulates amino acid uptake by Ehrlich ascites tumor cells. This and a companion paper describe an investigation intended to determine whether vitamin B₆ deprivation influences the activity of a bacterial amino acid accumulation system. The lactic acid bacteria were selected for study in view of the ease with which the intracellular vitamin B₆ content can be varied in this group of organisms (9). A description of glutamic acid accumulation in several species is presented here. The following paper (10) summarizes experiments designed to test the effect of vitamin B₆ deficiency on the accumulation process.

**EXPERIMENTAL**

** Cultures—Lactobacillus arabinosus 17-5, Streptococcus faecalis R, and Leuconostoc mesenteroides P-60 ** were carried in stabs of glucose-yeast extract-agar (0.25 per cent, 1 per cent, 1.5 per cent), transferred monthly and stored at 4° between transfers. Cells used in accumulation experiments were grown in previously described completely synthetic media (11). For *L. arabinosus* the medium was modified to contain 500 mg. per l. of L-alanine and 100 mg. per l. of n-alanine. Vitamin B₆ was supplied as pyridoxamine (1 µg. per ml.) which was added as a sterile solution to the previously sterilized vitamin B₆-deficient medium. Sterilization was achieved by autoclaving at 15 pounds for 6 to 10 minutes, depending on the volume of medium, which varied between 1 and 8 l.

Inocula were grown at 30° for 16 to 20 hours in the appropriate synthetic medium. Since these experiments were performed during a comparative study designed to determine the effect of vitamin B₆ deficiency on accumulation ability (10), inoculum cultures were routinely grown without vitamin B₆ supplementation. To ensure vitamin depletion in these cells, the inoculum culture was separated from the stab culture by at least one previous transfer in the vitamin B₆-deficient medium. After incubation, the cells were centrifuged, resuspended in cold distilled water and added to the medium with 1.0 µg. dry weight of cells per ml. In the final mass culture, cells used in these experiments were grown at 30° under an atmosphere of carbon dioxide, in medium supplemented with pyridoxamine.

In most instances cells were harvested 12 to 13 hours after inoculation, at which time the cultures were near the end of the exponential phase of growth. They were chilled in ice and collected batch-wise in a refrigerated centrifuge. The cells were washed once in distilled water and then resuspended in cold water to a density of 20 mg. (dry) per ml. The density of the suspension was estimated from its absorbance at 640 mµ with the use of a curve relating dry weight and absorbance. In most experiments this value was checked by measuring the dry weight of an aliquot of the suspension. All descriptions of the weight of cells refer to the dry weight.

**Uptake Conditions**—Initially, experiments were performed with the buffered salt solution described by Gale (2). However, with the use of *L. arabinosus* at a cell density of 1.6 mg. per ml., this solution was found to be inadequately buffered, and, consequently, the amount of phosphate was increased to give a final concentration of 0.12 M. Thereafter experiments were carried out in 0.12 M phosphate, pH 6.5 containing glucose (0.002 M), glutamic acid (0.003 M), NaCl (0.014 M), and MgSO₄ (0.0023 M); 20 mg. of cells were used in a final volume of 12.5 ml. This will be referred to as the standard uptake conditions. The general procedure was to dispense buffer, L-glutamic acid, in most experiments uniformly labeled with C¹⁴, and glucose to plastic centrifuge tubes. These were preincubated in a water bath at 37° to ensure temperature equilibration and then the thick cell suspension (20 mg. per ml.) was added to give a final cell concentration of 1.6 mg. per ml. All experiments included controls lacking either or both glucose and glutamate. In experiments involving variation in the time of incubation, the required number of identically constituted tubes were inoculated and after the requisite period of incubation each of these in turn was transferred to a prechilled rotor and centrifuged in the cold. Cells which had been added to uptake medium at 2° and centrifuged immediately, served as unincubated controls. The tubes were centrifuged at an average of 15,000 x g for 10 minutes. The supernatant buffer was poured off and the inside of the tube was carefully swabbed dry without righting the tube. Both the supernatant solution and the cell pellet were frozen immediately and stored in this condition until analyzed.

**Preparation of Cell Extracts for Assay**—A number of cell extraction procedures have been used, all of them yielding essentially identical extracts as judged by the analyses used in

* A preliminary report of some of this work has appeared (1). This study was supported by a grant (E-1487) from the National Institute of Allergy and Infectious Diseases, United States Public Health Service.
this study. In most experiments the cell pellet was thawed, macerated in the cold, and immediately suspended in 6 ml. of 8% per cent ethanol at 75°. The suspension was transferred to a glass centrifuge tube and heated at 75° for 10 minutes. The tubes were centrifuged and the supernatant removed to a beaker. The residue was resuspended in 6 ml. of ethanol-water and re-extracted for an additional 10 minutes. The combined supernatants were reduced to dryness in a current of air under an infrared lamp. The residues were taken up in water (2 ml of 20 mg. of cells extracted) and centrifuged to remove insoluble material. In experiments in which the intracellular free amino acids were to be examined by paper chromatography, an aliquot of the solution equivalent to 20 mg. of cells was taken to dryness and resuspended in 0.3 ml. of saturated picric acid. The resultant suspension was clarified by centrifugation before application to paper. Extraction with water was carried out similarly of the solution equivalent to 20 mg. of cells was taken to dryness except the temperature of extraction was that obtained in a boiling water bath. When examination of the acid-soluble nucleotide fraction was desired, extraction was achieved with 0.5 N perchloric acid. Ice-cold perchloric acid, 4 ml., was added to the thawed cell pellet. The suspension was transferred to chilled glass centrifuge tubes, allowed to stand for 25 minutes in an ice bath, and centrifuged in the cold for 10 minutes. The residue was re-extracted for 10 minutes in the cold with 4 ml of 0.5 N perchloric acid. The extracts were freed of most of the perchlorate by adjusting to pH 0.5 with concentrated KOH and chilling in an ice bath. The precipitated potassium perchlorate was removed by centrifugation.

Analyses—L-Glutamic acid was measured manometrically by decarboxylation with an acetone powder of Escherichia coli E-26 prepared as described by Ayenger et al. (12). The samples were assayed with the use of 2 mg. of cell powder and 1 ml. of 0.15 M acetate buffer at pH 4.2 containing cetyltrimethylammonium bromide (3.7 mg per ml.), in a final volume of 2.5 ml. L-Glutamine was slowly decarboxylated by the preparation used in all of these experiments. Paper chromatography showed, however, that only small amounts of glutamine occur in experimental cell extracts (at most 5 per cent of the glutamic acid present). Therefore, the error introduced by this lack of specificity is small. In subsequent studies, additional preparations of this powder have been devoid of activity toward glutamine. Recovery of known amounts of L-glutamic acid (measured in the presence and absence of the d-isomer in cell extracts) was 90 to 105 per cent and in most instances 95 to 98 per cent. The supernatant buffer containing 0.15 M phosphate was adjusted to pH 4.5 before assay for L-glutamic acid, but when it contained only 0.024 M phosphate it was assayed directly.

Radioactivity of cell extracts and supernatant buffer was determined with the use of a gas-flow counter (Nuclear-Chicago Corporation). Aliquots of samples were applied to circles of filter paper glued to aluminum planchets. All samples were plated in triplicate. The observed counts were adjusted to the count at infinite thinness by correcting for self-absorption due to paper, cell extract, and supernatant buffer as appropriate for the sample.

Changes in the freely-extractable amino acid content were determined by two-dimensional paper chromatography of cell extracts. The equivalent of 20 mg. of cells as a picric acid filtrate was applied to one corner of an 18 x 22 inch sheet of Whatman No. 1 paper. The paper was developed by the descending technique in chambers with the use of phenol-ammonia-water followed by lutidine-water. After drying at room temperature, the amino acids were visualized by spraying with a ninhydrin solution. A complete description of the pool and its variations will be presented elsewhere. To determine the extent of metabolism of accumulated, uniformly labeled glutamic acid, two-dimensional paper chromatograms of cell extracts and supernatant buffers were exposed to Kodak Industrial Type K x-ray film for 3 weeks.

Chemicals The components of the growth medium were all commercially available substances. Uniformly labeled L-C14glutamic acid was obtained from the Nuclear-Chicago Corporation.

RESULTS AND DISCUSSION

Most of the general characteristics of the glutamic acid accumulation process were investigated in L. arabinosus with cells grown with an excess of pyridoxamine. As shown in Fig. 1A, L-glutamate accumulation depends on the presence of glucose and an elevated temperature of incubation. During incubation with glutamic acid alone, there is only a slight transient increase in the endogenous content of freely extractable L-glutamic acid. Incubation of cells with glucose alone leads to a decline in the endogenous glutamate level. In the presence of both glutamic acid and glucose large amounts of L-glutamate are accumulated, generally on the order of 10 per cent of the dry weight of cells used.

In the succeeding studies, accumulation, measured manometrically, was defined as the increase over control levels of intracellular L-glutamic acid. Since assays were performed on extracts of unwashed packed cells, the amount of glutamic acid adhering to cells and trapped in the intercellular space was corrected for by subtracting the amount of L-glutamate found in cells suspended in buffer and glutamate at 2° and centrifuged immediately. Accumulation of isotope was corrected with the use of the same control. Fig. 1B shows that the intracellular accumulation of isotope corresponds closely to the accumulation of L-glutamate. In keeping with the results of the manometric assay, there was no accumulation of isotope in the cold and only a very small accumulation in the absence of glucose. Radioautograms prepared from two-dimensional paper chromatograms of cell extracts showed that the isotope was confined virtually exclusively to the region of the paper occupied by glutamic acid. However the isotopic method shows the entrance of slightly more glutamate than is measured manometrically as a net increase over control levels. Relative to the amount taken up, the greatest difference is encountered early in the incubation at which time the discrepancy can be partly accounted for as an exchange of intra- and extracellular glutamate (see Table II). There is also at this time a small conversion of L-glutamate to a substance which is resistant to enzymatic decarboxylation and which has the chromatographic properties of glutamic acid. The amount of isotope in this fraction, which is referred to tentatively as d-glutamic acid, increases during the incubation and at 90 minutes generally accounts for 10 per cent of the isotope present in the cell.

The relation between the initial rate of accumulation and the external glutamate concentration is described in Fig. 2. The rate increases sharply as the concentration is raised, but above 3.0 mm further increases in the external concentration produce relatively small increases in the rate. The curve has the form of an adsorption isotherm, and the data yield a straight line.
The total amount of glutamate accumulated during incubation for 60 minutes also increased as the external glutamate concentration was raised from 0.1 mM to 3.0 mM. Further increases above this external concentration did not materially change the amount of glutamate accumulated. The manometric and isotopic assays agreed closely in all cases. From measurements of the volume occupied by a given weight of cells, and estimates of the intercellular volume and intracellular water space, it can be calculated that at 1.0 mM the apparent intracellular glutamic acid concentration exceeded the extracellular concentration 360-fold and at 0.3 mM 390-fold. At these lower concentrations (0.1 to 1.0 mM) the cells accumulated 75 to 60 per cent of the glutamic acid originally present in the external buffer, while at higher concentrations (3.0 to 10.0 mM) this value fell to 30 to 10 per cent.

Therefore, the accumulation of glutamate by this organism occurs in opposition to large, apparent concentration gradients through the operation of a process characterized by a rate-limiting substrate concentration. The relatively insignificant accumulation which is observed in the absence of glucose or when incubation is carried out in the cold, suggests that accumulation is dependent upon cellular metabolism. In all these respects, the process in L. arabinosus is similar to that originally described in bacteria by Gale (2, 3). The nature of this process and its relation to the active transport phenomenon cannot be described with certainty at present, since the intracellular state (free or bound) of the accumulated molecules is unknown.

Effect of pH—Fig. 3 describes the relation between pH and accumulation capacity. Maximal accumulation was achieved with phosphate buffer at an initial pH of 6.5. Maleate at pH 6.0 has been found to be equally effective. The initial accumulation rate was slightly higher in phosphate at pH 6.0, but the amount of amino acid finally accumulated was submaximal as a result of the poor buffering capacity of phosphate in this range. Incubation at pH values below 6 is unfavorable not only to accumulation, but also to the retention of accumulated glutamate. This was observed in early experiments with the use of the buffered salts solution described by Gale (2), and employing cells at 1.6 mg. per ml. Under these conditions glutamate accumulation stopped abruptly after incubation for 30 to 40 minutes. Subsequently L-glutamate (together with D-glutamate) was released to the supernatant buffer, so that at 90 minutes the cells contained only 40 per cent of the isotope and L-glutamate which had been present at 30 minutes. After incubation for 60 minutes, the buffer had a pH of 5.2 and at 90 minutes a pH of
Fig. 3. The effect of pH on l-glutamate accumulation. Standard uptake conditions except for 0.12 M buffers as follows: pH 5.0, acetate; pH 6.0-7.2, phosphate; pH 8.0-8.5 Tris(hydroxymethyl)- aminomethane. The suspensions were incubated for: O—O, 30 minutes; O—O, 60 minutes.

Fig. 4. Effect of cell concentration on the amount of glutamate accumulated by L. arabinosus. Washed cells were incubated for the times shown under the standard uptake conditions. L-Glutamic acid in ethanolic cell extracts was determined by enzymatic decarboxylation. The amounts shown have been corrected for zero time values and refer to the intracellular glutamate per ml. of uptake medium.

4.7. A significant metabolism of glutamic acid occurred under these conditions, evidenced by the intracellular accumulation of smaller amounts of L-glutamate than that predicted from the amount of isotope present, by the intracellular accumulation of radioactive γ-aminobutyric acid and by the return to the buffer of a large amount of isotope without an equivalent rise in L-glutamic acid. Increasing the phosphate concentration to 0.12 M largely prevented the pH variation, and maximal accumulation was achieved and maintained between 60 and 90 minutes of incubation. Use of the more concentrated buffer also decreased the metabolic loss of L-glutamate by eliminating glutamic acid decarboxylation.

Fig. 5. Effect of culture age on the initial rate and total amount of glutamic acid accumulated by L. arabinosus. Standard uptake conditions. Cells were extracted with cold perchloric acid and uptake was calculated from the amount of isotope accumulated.

Effect of Cell Concentration—Under the conditions used in the experiments described here there is a linear relation between l-glutamate accumulated and cell concentration up to 2.4 mg. of cells per ml. (Fig. 4). Hence, the cell concentration normally employed (1.6 mg. per ml.) is a limiting factor in determining the amount of glutamate accumulated.

Effect of Culture Age—Fig. 5 shows the variation in glutamate accumulation activity of cells harvested at different times after inoculation of the growth culture. Maximal activities (rate at 5 minutes and amount at 90 minutes of incubation) were observed with cells from cultures in the exponential growth phase. Cells from cultures entering the phase of maximal growth rate or from cultures no longer increasing in mass showed 70 to 80 per cent of maximal activity. In a few experiments, cells from very young cultures (≤ 0.1 mg. of cells per ml. at harvest), had a decidedly low capacity (50 per cent of maximum). This variability may be related to the degree of damage sustained during harvesting and washing by these cells which are known to be especially sensitive to physical damage at this phase of the growth cycle. With L. arabinosus the initial rate of accumulation was found to be relatively constant and not materially depressed even in those batches of very young cells which exhibited relatively poor accumulation capacity. These results differ from those obtained with S. aureus by Gale and Folkes (14) who observed little change in capacity, but a marked variation in accumulation rate as a function of culture age. Such differences may arise principally in seemingly inconsequential variations in methods of cell prep-
Retention of Accumulated Glutamate—The ability of cells to retain accumulated glutamate was studied as follows. Cells were permitted to accumulate the amino acid for 60 minutes and were then centrifuged and washed once with cold buffer. They were resuspended at 37° in the solutions described in Table I. After additional incubation for 60 minutes the cells were separated from the medium and extracts were prepared in the usual way. Radioactivity was measured in the supernatant solutions and in the cell extracts and l-glutamic acid was measured in the cell extracts. As shown in Table I, incubation at 2° in water, saline, or phosphate led to negligible loss to the external medium of previously accumulated glutamate. A loss of 20 per cent of the isotope during incubation in water at 37° was reduced to 10 per cent or less by incubation in saline or phosphate. In the latter, the presence of glucose tripled the loss as judged by the loss of isotope from the cell. Manometric assays for L-glutamic acid (Table II) showed, however, that in the absence of glucose, despite a superior retention of isotope, there was a greater loss of L-glutamate than in cells incubated with glucose. Thus, in the absence of glucose there was a metabolic loss with a retention in the cell of most of the products. In the presence of glucose, the decline of intracellular L-glutamic acid which was smaller corresponded more closely to the loss of isotope from the cell and to the appearance of isotope in the buffer, and very likely represents a true displacement of the amino acid. Under any of these conditions, the losses were not large and especially in the cold the accumulated glutamate resisted elution from the cell.

The data of Table I show that loss of radioactivity from the cells was greatly increased by the presence in the external buffer of nonradioactive L-glutamic or L-aspartic acids. In the former case, there was a rise rather than a decline in the amount of L-glutamate in the cell indicating a replacement of intracellular (radioactive) by extracellular (nonradioactive) glutamate. Glucose enhanced the glutamate exchange as would be expected if the exchange involved the pathway utilized to accumulate glutamate in the initial incubation. If this interpretation is correct, the large exchange observed with L-glutamate in the absence of glucose might be supported by carbohydrate stores retained from the initial incubation. With aspartic acid the amount of L-glutamate in the cell dropped sharply by an amount closely similar to the glutamate equivalent of the radioactivity appearing in the buffer and lost from the cell. The ability of aspartic acid to elute glutamate from the cell suggests that interaction in the uptake process may account, at least in part, for the lag in the growth response to glutamic acid produced by aspartic acid with this organism (15, 16).

L-Glucose was the least active of the effective displacing compounds. It was almost completely ineffective in displacing isotope in the absence of glucose, and in its presence showed a low level of activity which was not enhanced by varying its concentration between 0.001 and 0.01 M. With the exception of alanine which was slightly active, the other amino acids tested were ineffective in displacing isotope from the cell, testifying to the structural specificity of the displacement phenomenon. However, in the presence of glucose these substances did decrease the amount of L-glutamate (manometric assay) retained in the cell in comparison to the appropriate glucose-incubated control. This enhanced loss of L-glutamate was not observed in the absence of glucose suggesting a requirement for energy, possibly to promote the entry of these amino acids into the cell. Since these amino acids did not stimulate a significant loss of isotope from the cell, the enhanced disappearance of L-glutamic acid probably represents a metabolic conversion of this amino acid to a product which is retained intracellularly. This amino acid-dependent metabolic loss is reminiscent of the observation of Gale and Van Halteren (17) who observed peptide formation.

### Table I

<table>
<thead>
<tr>
<th>Reincubation solution</th>
<th>Temperature</th>
<th>Loss from cell of isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Water</td>
<td>37</td>
<td>21</td>
</tr>
<tr>
<td>NaCl (0.1 M)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>NaCl (0.1 M)</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>Phosphate (0.12 M) + uptake salts</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>Phosphate (0.12 M) + uptake salts</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>Phosphate (0.12 M) + uptake salts + glucose (0.028 M)</td>
<td>37</td>
<td>23</td>
</tr>
</tbody>
</table>

### Table II

Elution of accumulated glutamic acid by amino acids

Conditions of the initial incubation were those described in Table I. Following centrifugation the cells were washed briefly with cold 0.12 M phosphate-salts solution, resuspended at 1.6 mg. per ml. in the solutions described below, and incubated at 37° for 60 minutes. At the time of resuspension 20 mg. of cells contained 1,480 μg. of glutamate, determined by the isotope assay, and 131 μg. of L-glutamate, determined by the manometric assay.

<table>
<thead>
<tr>
<th>Addition to phosphate (0.12 M)-salts</th>
<th>Without glucose</th>
<th>With glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotope assay</td>
<td>Manometric assay</td>
<td>Isotope assay</td>
</tr>
<tr>
<td>L-Glutamic acid (0.003 M)</td>
<td>510</td>
<td>270</td>
</tr>
<tr>
<td>L-Aspartic acid (0.003 M)</td>
<td>410</td>
<td>840</td>
</tr>
<tr>
<td>L-Lysine (0.01 M)</td>
<td>60</td>
<td>220</td>
</tr>
<tr>
<td>L-Alanine (0.01 M)</td>
<td>30</td>
<td>280</td>
</tr>
<tr>
<td>L-Phenylalanine (0.01 M)</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>D-Glutamic acid (0.005 M)</td>
<td>90</td>
<td>440</td>
</tr>
</tbody>
</table>

* The L-glutamate content of these cells increased by 310 μg. per 20 mg.
Glutamate Accumulation by Lactic Acid Bacteria

**Fig. 6.** Comparative removal of isotope and L-glutamate from external buffer during accumulation of glutamate by L. arabinosus. Standard uptake conditions. ○—○, estimation of L-glutamate uptake by assay of supernatant buffer with glutamic acid decarboxylase; •—•, estimation of glutamate uptake by determination of residual isotope in supernatant buffer; X, shows the glutamate equivalent of the isotope found in the respective cell extracts.

**Table III**

Appearance of "n-glutamic acid" in cells during glutamate accumulation by L. arabinosus

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Culture density at harvest</th>
<th>Uptake incubation time</th>
<th>Glutamate in cell extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg./ml.</td>
<td>min.</td>
<td>L-isomer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Manometric assay</td>
</tr>
<tr>
<td>1</td>
<td>0.20 (early log)</td>
<td>5</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>2500</td>
</tr>
<tr>
<td>2</td>
<td>0.70 (late log)</td>
<td>5</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1855</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>2490</td>
</tr>
</tbody>
</table>

When S. aureus was incubated with single amino acids in addition to glutamic acid.

**Metabolism of Glutamate during Accumulation**—As shown in Fig. 1, the amount of L-glutamate accumulated intracellularly corresponded closely to the glutamate equivalent of the isotope accumulated. In addition, the isotope was found exclusively in the region occupied by glutamic acid in two-dimensional chromatograms. Therefore, with the exception of the formation of small amounts of a substance believed to be n-glutamic acid, apparently only a small metabolic loss of L-glutamate occurred during these experiments. Fig. 6 shows, however, that while the loss of isotope from the buffer is accounted for almost entirely by its appearance in the cell extract (there is only a minor incorporation of isotope into the ethanol-insoluble fraction of the cell which accounts for the small difference in these values), the amount of L-glutamate removed from the buffer is considerably higher than that predicted by the amount of isotope removed. This difference is not attributable to the presence in the buffer of glutamic acid decarboxylase inhibitors which would cause an underestimation of L-glutamic acid, and thus indicates the presence of glutamic acid metabolites. Chromatographic studies of the supernatant buffer have demonstrated the absence of isotopically labeled γ-aminobutyric acid or glutamine. n-Glutamic acid is found in the buffer, but in insufficient amounts to account for the major portion of the missing L-glutamic acid.

Therefore, there is a significant metabolic loss of glutamate during these experiments. Since this loss does not occur in the absence of glucose unless the glutamate has already been accumulated (See Table II), it occurs very likely after or during entry into the cell. A metabolic loss of glutamate was shown by Gale (2) and Gale and Mitchell (18) to occur during the accumulation of this amino acid by a strain of S. faecalis. These authors did not report the location of the resultant metabolites, but suggested that their formation did not involve peptide synthesis, deamination, or transamination.

Under the conditions used in most of the accumulation experiments described here the only detectable metabolite which accumulates in the cell is a substance believed to be n-glutamic acid. The formation of this material was studied as described in Table III which shows its appearance in cells during glutamate accumulation. The formation of this material was studied as described in Table III which shows its appearance in cells during glutamate accumulation. The formation of this material was studied as described in Table III which shows its appearance in cells during glutamate accumulation. The amount of n-glutamate found with the use of the calorimetric assay agreed well with the isotopic assay. A variability in the amount of n-glutamate formed during such experiments was traced to the age of the culture used. As shown in Experiment 1 of Table III, the amount of n-glutamate formed is much higher in cells from early exponential phase cultures than in cells harvested at subsequent growth phases. In addition, cells from such low density cultures frequently, although not in every case, accumulated more total n-glutamate than that predicted by the amount of isotope found in this fraction. This suggests that nonradioactive n-glutamic acid, or a substance with closely similar chromatographic properties, is liberated in such cells in a freely excretible form.

1 This material has been isolated from streak chromatograms and treated with dinitrofluorobenzene. Only a single derivative was observed whose chromatographic migration in three solvents was found to be identical to that of authentic dinitrophenyl-glutamate. The authors are indebted to Dr. G. Rouser for details of the chromatographic procedures prior to their publication.
tractable form during some accumulation experiments. These observations may have some relation to the biosynthesis of the bacterial cell wall, which is believed to be incomplete in cells from cultures in the early growth phases (19), and which is known to contain D-glutamic acid (20).

Glutamate Accumulation by Other Organisms—The characteristics of glutamate accumulation in *S. faecalis* R and *L. mesenteroides* P-60 have been studied with the techniques described above. Although these organisms accumulate large amounts of L-glutamate, they achieve apparent gradients somewhat smaller than those observed with *L. arabinosus*. As in the latter organism, the uptake is temperature-dependent and does not occur to a significant extent in the absence of glucose.

**SUMMARY**

Washed cells of *Lactobacillus arabinosus* 17-5 incubated in phosphate buffer containing L-¹⁴C-glutamic acid and glucose accumulated this amino acid in a freely-extractable form. Accumulation was negligible in the absence of glucose and at 2°. Under the conditions used, almost all the intracellular isotope was retained in L-glutamic acid. The amount of glutamate accumulated generally equaled, and in some cases exceeded, 10 per cent of the dry weight of cells used. Accumulation occurred in opposition to large apparent concentration gradients, and was characterized by a rate-limiting extracellular amino acid concentration.

Maximal accumulation capacity was observed at pH values between 6.0 and 6.5. Capacity varied with culture age, and was maximal in cells from midexponential phase cultures. Little elution of accumulated glutamate occurred during incubation in water or buffer. However, aspartic and glutamic acids eluted previously accumulated glutamate from the cell.

The metabolic loss of L-glutamate was enhanced when cells were incubated with glucose and alanine, phenylalanine, or lysine. More L-glutamate, but not isotope, was removed from the external buffer than could be accounted for by intracellular accumulation. Approximately 10 per cent of the intracellular isotope was found in a fraction tentatively identified as n-glutamic acid. The amount of this substance detected in cell extracts varied markedly with the growth phase at harvest.

The accumulation of L-glutamate by *Streptococcus faecalis* R and *Leuconostoc mesenteroides* P-60 also has been demonstrated.

**Acknowledgment**—The authors are deeply indebted to Dr. Eugene Roberts for many stimulating and constructive suggestions.

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