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UPTAKE OF VALINE AND GLYCYLVALINE BY *LEUCONOSTOC MESENTEROIDES*[1]

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ABSTRACT

SHELTON, DAMON C. (West Virginia University Medical Center, Morgantown), AND WILLIAM E. NUTTER. Uptake of valine and glycylvaline by *Leuconostoc mesenteroides*. J. Bacteriol. **88**:1175–1184. 1964.—Uptake systems for valine and glycylvaline were studied in growing cultures and nongrowing cellular suspensions of *Leuconostoc mesenteroides*. Glycylvaline promoted growth more rapidly than did valine. Other dipeptides were shown to be antagonistic to the utilization of glycylvaline by the organism. The experiments with cellular suspensions were done with the use of combinations of labeled and unlabeled amino acids and peptides. Results showed that different uptake systems were present in *L. mesenteroides* for the uptake of valine and glycylvaline. Both systems were energy-dependent and readily saturated. The synthesis, purification, and chromatographic identification of glycyl-DL-valine-*1*-C^{14} is described.

Certain dipeptides promote the growth rate of nutritionally fastidious bacteria, such as *Leuconostoc mesenteroides*, more rapidly and to a greater extent than do comparable concentrations of their component essential amino acids. The effect is most prominent when the organism is in the exponential growth phase and when the concentration of the amino acid and peptide being compared is low enough to be growth-rate limiting. Indeed, the lag phase normally observed when the medium contains amino acids is much greater than when the growth-limiting amino acid is provided as a peptide. To account for this effect, O'Barr, Levin, and Reynolds (1958) suggested that amino acids and peptides enter into the metabolism of *L. mesenteroides* strain P-60 by divergent pathways. By growth data, they showed that a number of amino acid antagonisms exist in the organism. Where this antagonism occurred, peptides containing one of the antagonistic amino acids were more effective than was the free amino acid in restoring the growth response.

Earlier reports by Simmonds and Fruton (1949); Klungsoyr, Sirny, and Elvehjem (1951); Kodicek and Mistry (1952); Kihara, McCullough, and Snell (1952*b*); Kihara and Snell (1952, 1955); Kihara, Klatt, and Snell (1952*a*); Miller and Waelsch (1952); Hirsch and Cohen (1953); Peters, Prescott, and Snell (1953); Prescott, Peters, and Snell (1953); Peters and Snell (1954); Meinhart and Simmonds (1955); Miller, Neidle, and Waelsch (1955); Nutter and Shelton (1956); and the subsequent reports by Levine and Simmonds (1960) and Leach and Snell (1960) showed the effect of peptides on the amino acid nutrition of many different organisms. The conditions whereby peptides appear to exhibit their growth-promoting properties were summarized by Leach and Snell (1960). Furthermore, these authors presented additional results showing that *Lactobacillus casei* used independent mechanisms for the accumulation of glycine, alanine, and their peptides. Kessel and Lubin (1963) used mutant strains of *Escherichia coli* to show that active transport of peptides and peptidase activity are separate functions of that bacterial cell. Summation of results clearly illustrates that some bacterial cells possess independent transport systems for amino acids and their peptides. The present paper concerns the uptake systems for valine and glycylvaline as found in growing and nongrowing (but actively accumulating) cultures of *L. mesenteroides*.

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MATERIALS AND METHODS

Preparation of cells and medium for growth inhibition studies. L. mesenteroides P-60 (ATCC 8042) was maintained on a medium composed of 1% glucose, 1% yeast extract, 1% hydrated sodium acetate, and 1.5% agar. Twice monthly, new cultures were established by transferring organisms from refrigerated stab cultures to nutrient broth (above medium minus the agar). The inoculated broth was incubated for 24 hr at 37 C to obtain actively growing cells. New stab cultures were made, and were incubated for 24 hr and stored at 4 C. These actively growing cells from the nutrient broth culture were centrifuged, washed with 0.9% saline, and resuspended in the saline to an absorbancy of 0.15 (620 mμ). This cellular suspension served as the inoculum for the growth studies. One drop of this suspension dispensed from a syringe fitted with a blunt, 20-gauge needle provided a uniform quantity of cells for each tube used in these studies.

The growth studies were set up to show the comparative effects of DL-valine and glycyl-DL-valine in providing a source of valine for growth of *L. mesenteroides* on an otherwise complete medium. Secondly, these studies were to show the effect of certain other peptides and amino acids on the utilization of the above compounds for support of growth of this organism. The basal medium was that developed by Henderson and Snell (1948), except pyridoxine was used instead of pyridoxal and valine was omitted. Valine, an essential amino acid for *L. mesenteroides*, was added as DL-valine or as the peptide, glycyl-DL-valine, at suboptimal concentration (3.4×10^{-5} M) or at an appropriate concentration as noted later. Pyrex tubes (15 by 150 mm), previously selected for optical uniformity by the method of Moore and Stein (1948), served as culture tubes. The supplemental substrates were added to 5 ml of double-strength medium, and the final volume was adjusted to 10 ml with distilled water. To avoid excessive browning, the medium was autoclaved (120 C) for 10 min, allowed to stand at room temperature for 12 hr, and autoclaved again for 5 min.

Growth of the organism as afforded by the medium as supplemented was measured turbidimetrically with a Bausch & Lomb Spectronic-20 colorimeter at 620 mμ. Uninoculated control tubes served to adjust the instrument to zero absorbance. Three replications were used for each concentration of substrate.

Preparation of resting cells for cellular accumulation studies. Nine liters of medium [1% glucose, 0.5% hydrated sodium acetate, 0.5% yeast extract, 0.25% peptone (Difco), and 0.25% enzymatically hydrolyzed casein] were inoculated with 200 ml of a 10-hr culture grown on the above medium. The 200-ml inoculum had previously been inoculated with 10 ml of a broth culture (24 hr) prepared similarly to those used for the growth studies. The cells in these 9 liters of medium were grown for 10 hr at 37 C and were harvested with a Sharples centrifuge. After harvest, the cells were washed by resuspension in 0.067 M phosphate buffer (pH 6.8), and were passed through a fritted-glass crucible with suction to insure homogenous suspension. This washing procedure was repeated; 8 to 9 g of wet cells were obtained in this manner. The cells were used in the studies immediately after harvesting. Cell weights are expressed as dry weights.

Measurement of substrate uptake was made on the cellular fluid obtained according to the following procedure. Incubation tubes were 50-ml, standard polypropylene centrifuge tubes. Each tube was prepared to contain 30 ml of 0.067 M phosphate buffer (pH 6.8), 1% glucose, the amino acid and peptide substrates, and approximately 100 mg of cells (exact amount determined at end of incubation). The contents of each tube were gently stirred every 4 min. After incubation at 37 C for 20 min, the tubes were centrifuged at 8,170 $\times$ *g* for 10 min. The supernatant fluid was removed and the cells were resuspended in 3 ml of distilled water. These cells were ruptured by boiling for 10 min according to the method of Gale (1947). While still hot, the tubes were centrifuged as above. The supernatant fluid (2 or 3 ml) was removed and was designated the "cellular fluid." The wet residue, termed "cellular debris," was dried by vacuum desiccation to constant weight.

The amount of bacterial cells in each incubation tube was calculated from the dried cellular debris. Previously, the relationship between whole cells and the cellular debris had to be determined. Equal quantities of the homogenous cellular suspension were dispensed into eight tubes of the incubation medium minus the substrates. Except for the absence of amino acid and peptide sub-

strates, the incubation conditions were identical to those described above for measurement of substrate uptake. After the 20-min incubation, the cells were spun down, and those from four tubes were dried to constant weight. The cells in the other four tubes were ruptured by boiling, and the dried cellular debris was obtained as above. Relationship based on dry weights and nitrogen determinations was established on the dried whole cells and the dried cellular debris.

The amount of valine accumulated by the cells was determined by chromatographing the cellular fluids, eluting the valine, and counting the radioactivity. Descending paper chromatography with the use of Whatman no. 1 filter paper and propanol-water (7:3, w/w) at 30 C for 24 hr was the system used. The elution of the valine from the chromatogram was done by the procedure described by Roberts et al. (1955).

Radioactivity measurements were made with the use of a thin-window, gas-flow counter. The efficiency of counting was approximately 10%. Material to be analyzed was pipetted carefully into stainless-steel planchets so as to prevent the sample from flowing upon the edge of the planchets. Samples were prepared in triplicate and were counted for 5 min. Control sources of DL-valine-*1*-C^{14} and glycyl-DL-valine-*1*-C^{14} were counted each time a series of samples was being analyzed. Under conditions employed, no correction for self-absorption was found necessary.

Amino acids and peptides. All amino acids (except DL-valine-*1*-C^{14}) and the nonradioactive peptides were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. In each case, paper chromatographic analysis showed the presence of a single ninhydrin-positive spot. DL-Valine-*1*-C^{14} (specific activity, 1.76 mc/mmole) was obtained from New England Nuclear Corp., Boston, Mass., and was found to give a single radioactive and ninhydrin-positive spot after chromatography on paper.

Synthesis of glycyl-DL-valine-1-C^{14}. The haloacetylhalide method of Fischer and Scheibler (1908) was used as a guide for the synthesis; 100 μc of DL-valine-*1*-C^{14} (6 7 mg) were mixed with 10 g of DL-valine, dissolved in 54 ml of boiling distilled water, and then recrystallized from 1 liter of absolute alcohol. Total recovery was 9.1 g. α-Chloroacetyl-DL-valine-*1*-C^{14} was prepared as follows. A 7-g portion of the recrystallized DL-valine-*1*-C^{14} (as prepared above) was dissolved in 30 ml of 2 N NaOH and placed in an ice bath. With constant magnetic stirring, 9 ml of chloroacetylchloride and 66.6 ml of 2 N NaOH were added alternately, each in five equal portions over a period of 30 min; 15.4 ml of 5 N HCl were added, and the reaction mixture was retained in the ice bath for crystallization. After recrystallization from water, 7 g of the derivative, which had a melting point of 128 to 129 C, were recovered.

By magnetic stirring, the α-chloroacetyl-DL-valine-*1*-C^{14} (6.5 g) was reacted with 130 ml of 27% aqueous ammonia at 28 C for 24 hr. Under reduced pressure, the product was brought to dryness, thus removing any excess ammonia. The residue was dissolved in a minimum of hot water (10 ml), and the glycyl-DL-valine-*1*-C^{14} was precipitated overnight with 500 ml of absolute alcohol at 20 C. Again, the peptide was dissolved (9 ml of hot water); activated charcoal was added and the mixture was filtered while warm; 300 ml of absolute alcohol were added to the filtrate, and recrystallization occurred at refrigerator temperature during the next 6 hr. After extensive desiccation over sulfuric acid, a yield of 2.9 g or 28% of theory, based on the amount of valine, was obtained.

The product was found to be chromatographically pure in two solvent systems, and was not distinguishable from chromatographically pure glycyl-DL-valine. The solvent systems used were *n*-propanol–water (7:3, w/w) and *n*-butanol–acetic acid–water (450:50:125, v/v) in a descending, unidimensional assembly with Whatman no. 1 filter paper.

RESULTS

Growth experiments. Glycyl-DL-valine was very effective in supplying the valine nutritional needs of *L. mesenteroides*. At suboptimal concentrations of glycylvaline in an otherwise complete growth medium, the effect of other peptides or related compounds on the availability of the valine for growth of the organism could be determined. The addition of glycine, glycylglycylglycine, acetyl-DL-valine, or ammonium chloride in a range of concentrations from 2×10^{-3} to 10×10^{-3} M did not affect the growth (Fig. 1, curve A). The presence of glycylglycine, glycyl-DL-serine, and glycyl-L-leucine or DL-alanyl-DL-phenylalanine in

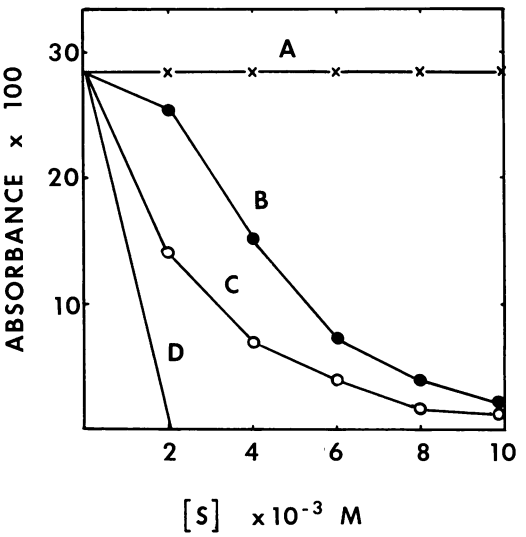

FIG. 1. *Effect of glycine, special peptides, and related compounds on the growth of Leuconostoc mesenteroides as supported by the basal medium plus 3.4 × 10⁻⁵ M glycyl-DL-valine. Curve A shows the growth response afforded by the growth medium. This curve did not change with the subsequent addition of glycine, glycylglycylglycine, acetyl-DL-valine, or ammonium chloride. Curves B and C show the growth inhibition that occurred when glycylglycine and glycyl-DL-serine, respectively, were provided in the growth medium. Curve D shows the growth inhibition caused by the addition of either DL-alanyl-DL-phenylalanine or glycyl-L-leucine.*

the growth medium inhibited growth in each instance; inhibition became increasingly greater as the concentration of the dipeptides was increased (Fig. 1, curves B, C, and D).

The difference in growth of *L. mesenteroides* appeared to be a result of the ability or inability to derive its valine needs from the medium. None of the compounds—glycine, glycylglycylglycine, acetyl-DL-valine, or ammonium chloride—interfered with valine utilization from the medium. However, the dipeptides were effective in inhibiting growth, and this effect appeared to be related to the inability of the organism to gain adequate valine to meet its nutritional requirements.

The basal medium contained all the amino acids as required by the organism except for valine. The amount of growth supported by the medium containing 3.4 × 10^{-5} M glycyl-DL-valine was substantially higher than that supported by 3.4 × 10^{-5} M DL-valine (Table 1). This effect demonstrated that under the experimental conditions the organism was able to obtain valine at a more rapid rate from the peptide than from the free valine.

Additional supporting data were obtained to show that peptides such as DL-alanyl-DL-phenylalanine and glycyl-L-leucine were actually impeding cellular growth by interfering with the amount of valine which the cells could obtain from the medium (Tables 1 and 2). The results

TABLE 1. *Effect of DL-alanyl-DL-phenylalanine, glycyl-L-leucine, and their component amino acids on growth of Leuconostoc mesenteroides as supported by DL-valine and glycyl-DL-valine*

Source and concn of valine	Concn of substrate (10^{-3} M)	DL-Alanyl-DL-phenylalanine		DL-Alanine and DL-phenylalanine		Glycyl-L-leucine		Glycine and L-leucine	
		20*	40	20	40	20	40	20	40
Glycyl-DL-valine	0.0	17†	29						
(3.4 × 10^{-5} M)	0.4	13	26	17	28	3	28	18	28
	0.8	6	23	17	28	0	14	17	29
	1.2	5	23	18	29	0	0	17	28
	1.6	2	20	16	27	0	0	17	28
	2.0	2	19	18	28	0	0	17	28
DL-Valine	0.0	2	15						
(3.4 × 10^{-5} M)	0.4	0	7	0	11	0	0	0	11
	0.8	0	3	0	10	0	0	0	9
	1.2	0	2	0	11	0	0	0	7
	1.6	0	1	0	12	0	0	0	7
	2.0	0	0	0	11	0	0	0	5

* Figures indicate growth period in hours.

† Results expressed as rate of absorbancy × 100.

TABLE 2. *Reversal of* DL-*alanyl-*DL-*phenylalanine-inhibited growth of Leuconostoc mesenteroides by increasing concentrations of* DL-*valine and glycyl-*DL-*valine**

DL-Valine (10^{-4} M)	Glycyl-DL-valine (10^{-4} M)	Growth period	
		20 hr	40 hr
0	0	0†	0†
0	0.34	3	20
0	0.68	6	42
0	1.03	6	54
0	1.37	7	65
0	1.71	8	74
0	0	0	0
0.34	0	0	2
0.68	0	0	17
1.03	0	1	49
1.37	0	2	65
1.71	0	3	74

* DL-Alanyl-DL-phenylalanine was added to the basal medium at a concentration of 2×10^{-3} M.

† Absorbance × 100.

shown (Table 1) were obtained by adding increasing concentrations (4×10^{-4} to 2×10^{-3} M) of DL-alanyl-DL-phenylalanine, glycyl-L-leucine, or comparable concentrations of their component amino acids to media containing 3.4×10^{-5} M valine and supplied as glycyl-DL-valine or as DL-valine. With glycyl-DL-valine as the source of valine, both peptides, DL-alanyl-DL-phenylalanine and glycyl-L-leucine, but not their component amino acids inhibited the utilization of glycyl-DL-valine in supplying valine for the organism. When free DL-valine was the valine source in the medium, both the peptides and their component amino acids caused growth inhibition. By increasing the valine concentration from 3.4×10^{-5} M to 1.7×10^{-4} M, the growth inhibition caused by 2×10^{-3} M DL-alanyl-DL-phenylalanine was reversed (Table 2). Higher valine concentrations may have produced a greater reversal of growth inhibition, but the results demonstrated, nevertheless, that the DL-alanyl-DL-phenylalanine was interfering with valine utilization by the cells.

Accumulation studies with nongrowing cells. The *L. mesenteroides* cells needed an energy source for accumulation of valine. The effect of glucose upon the uptake of valine-*1-*C^{14} was very dramatic (Table 3). In the absence of added glucose, a low level of uptake occurred which probably resulted from some endogenous sources of oxidizable substances in the cells. Dinitrophenol (5.4×10^{-3} M) inhibited uptake also (Table 3). These results emphasized that the uptake mechanism for either source of valine is an energy-dependent process for this organism.

To assure that the accumulating systems were saturated and that the measured uptake was independent of substrate concentration, the concentration of substrate to achieve this goal was determined (Fig. 2). In subsequent experiments, the concentration of either valine substrate used was 1.43×10^{-2} M, which was higher than the saturating concentration.

Uptake was measured at time intervals up to 60 min (Fig. 3). Under conditions used in these experiments, accumulation from each substrate reached the maximum within approximately 20 min, and subsequent observations were made at the 20-min incubation period.

Influence of the simultaneous presence of other substrates on the uptake of valine-1-C^{14}. A sensitive method to study the relationship of the various accumulating systems in *L. mesenteroides* was to follow the effect of unlabeled substrates on the uptake of valine-*1-*C^{14}. Unlabeled glycyl-DL-valine did not inhibit the uptake of free valine (Table 4). This result showed that the uptake system for free valine is different and independent of that system for the valine peptide.

TABLE 3. *Effect of glucose on the uptake of valine-1-*C^{14} *from* DL-*valine-1-*C^{14} *and glycyl-*DL-*valine-1-*C^{14} *by suspensions of Leuconostoc mesenteroides cells*

Substrate*	Concn of glucose	Intracellular valine-*1-*C^{14}†
	%	
DL-Valine-*1-*C^{14}	None	30
DL-Valine-*1-*C^{14}	1	172
Glycyl-DL-valine-*1-*C^{14}	None	13
Glycyl-DL-valine-*1-*C^{14}	1	373
DL-Valine-*1-*C^{14} + dinitrophenol	1	25
Glycyl-DL-valine-*1-*C^{14} + dinitrophenol	1	55

* Each incubation tube contained 30 ml of 0.067 M phosphate buffer (pH 6.8). Incubation was for 20 min at 37 C. Each substrate concentration was 1.43×10^{-2} M. Dinitrophenol was used at a concentration of 5.4×10^{-3} M.

† Expressed as millimicromoles per milligram of cells.

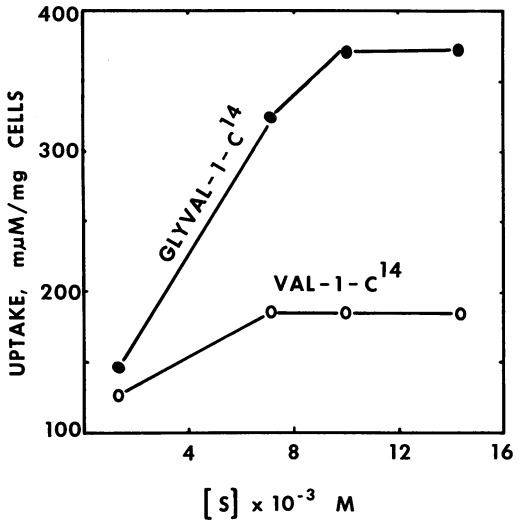

FIG. 2. *Effect of substrate concentration on the valine-1-C^{14} uptake by Leuconostoc mesenteroides. Endogenous valine was very low (see Fig. 5). In addition to the substrate, each incubation tube contained 30 ml of 0.067 M phosphate buffer (pH 6.8) and 1% glucose. Incubation time was 20 min at 37 C.*

The rate of uptake as well as the total uptake were much greater for valine supplied as glycyl-DL-valine-*1*-C^{14} than as free DL-valine-*1*-C^{14} (Fig. 3). These observations explain, in part if not completely, the superior growth response afforded by the valine peptide for the organism maintained under growing conditions. When the organism was incubated with labeled DL-valine and labeled glycyl-DL-valine, the total uptake of valine-*1*-C^{14} was the same as that found when only glycyl-DL-valine-*1*-C^{14} was present. If unlabeled rather than labeled DL-valine was present with the glycyl-DL-valine-*1*-C^{14}, the uptake of valine-*1*-C^{14} was reduced substantially and by an amount equivalent to the uptake of free valine-*1*-C^{14}. Just why the uptake supported by the peptide, glycyl-DL-valine-*1*-C^{14}, was the same as that observed from DL-valine-*1*-C^{14} plus glycyl-DL-valine-*1*-C^{14} was puzzling when first observed. Then, it was recognized that, after the uptake of glycyl-DL-valine-*1*-C^{14} by the cells, free glycine and valine-*1*-C^{14} were being released within the cell. Since these cells were nongrowing and unable to utilize these amino acids in the absence of other essential amino acids and apparently could not retain such high concentrations, both glycine and valine-*1*-C^{14} were released into the extracellular fluid or medium (Fig. 4). The valine-*1*-C^{14} so released would be available for accumulation by the valine-dependent system. Precipitation of the cellular proteins with 10% trichloroacetic acid caused precipitation of less than 1% of the radioactivity, which indicated no binding of the intracellular, labeled valine. The data available do not permit a definite conclusion. The data are suggestive, though, that both systems, valine-dependent and glycylvaline-dependent, were operating independently, and that the only source of valine-*1*-C^{14} was from the intracellular hydrolysis of the peptide. This suggestion comes from the observation that, when unlabeled valine and glycyl-DL-valine-*1*-C^{14} were present simultaneously, the apparent uptake of radioactive valine from the peptide was not as great as when the unlabeled valine was omitted. Yet, when both sources of valine were radioactive, the uptake equaled that attained with the radioactive peptide alone. Another explanation, as observed by Leach and Snell (1963) with glycyl-L-alanine, may account for the effects observed. They found that

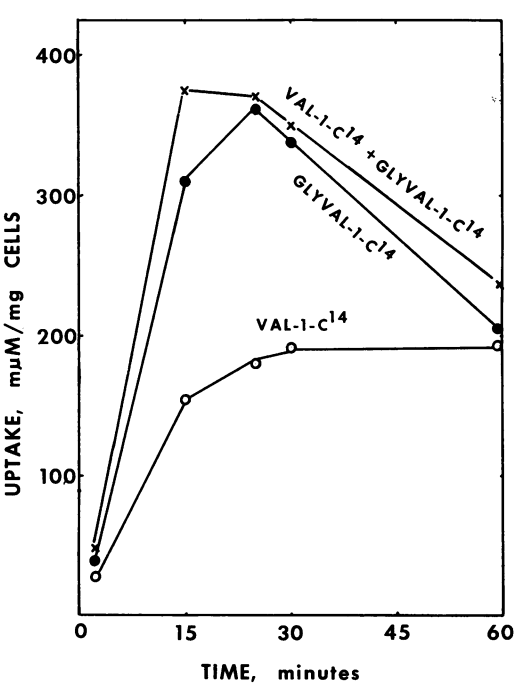

FIG. 3. *Rate of uptake of valine-1-C^{14} by cellular suspensions of Leuconostoc mesenteroides cells. Each substrate, DL-valine-1-C^{14} or glycyl-DL-valine-1-C^{14}, was present at a concentration of 1.43 × 10^{-2} M. In addition to the substrate, each incubation tube contained 30 ml of 0.067 M phosphate buffer (pH 6.8) and 1% glucose.*

TABLE 4. *Effect of the simultaneous addition of labeled and unlabeled valine substrates upon the uptake of valine-1-C^{14} by Leuconostoc mesenteroides*

Incubation tube*	Substrate	Intracellular valine-1-C^{14}†
1	DL-Valine-1-C^{14}	172 ± 14‡
2	DL-Valine-1-C^{14} + glycyl-DL-valine	171 ± 11
3	DL-Valine + glycyl-DL-valine-1-C^{14}	205 ± 16
4	DL-Valine-1-C^{14} + glycyl-DL-valine-1-C^{14}	374 ± 14
5	Glycyl-DL-valine-1-C^{14}	373 ± 11

* Each incubation tube contained 30 ml of 0.067 M phosphate buffer (pH 6.8) and 1% glucose. The substrates, labeled or unlabeled DL-valine and glycyl-DL-valine, were each provided at a concentration of 1.43×10^{-2} M when included in the incubation tube. Incubation was for 20 min at 37 C.

† Expressed as millimicromoles per milligram of cells.

‡ Calculated standard deviations of the mean values.

an exchange occurred between the intracellular alanine and the extracellular alanine. Perhaps in this study, the radioactive intracellular valine-1-C^{14} available after intracellular hydrolysis could exchange with the unlabeled extracellular valine, and the apparent uptake of labeled valine would be less than that observed in the absence of the unlabeled valine. In any case, the valine accumulation from the peptide proceeded to a higher level than was possible with an equivalent concentration of free valine.

Uptake versus time was studied over a 2-hr period. The results differed only during the first 60 min (Fig. 3). The treatments containing the glycyl-DL-valine-1-C^{14} reached maximal uptake slightly earlier than did the one containing valine-1-C^{14} alone. With the valine-1-C^{14}, the cells attained their maximal uptake, and maintained this level during equilibrium over the period of study. On the other hand, the glycyl-DL-valine-1-C^{14} supported a much higher maximal uptake, but apparently the cells were unable to maintain such high intracellular concentrations of valine-1-C^{14} from this source. Thus, at equilibrium, the concentration of intracellular valine-1-C^{14} was no different than that supported by the uptake of valine-1-C^{14}. Either the uptake of the peptide declined or the cells were losing labeled valine more rapidly than they could accumulate it. If, however, the cells continued to absorb the peptide, hydrolyze it, and counter-transport the free amino acids to the medium, equilibrium of uptake no different than that for valine-1-C^{14} would be achieved. The pH of the medium was followed, and no measurable change was observed for the first 30 min. Thereafter, a slight reduction in pH from 6.8 to 6.6 was observed by the end of the 2-hr period. Results similar to these would be expected if the organism secreted extracellular peptidases, but Nutter (1956) showed that dipeptidases were not secreted under the conditions employed. Care was exercised to insure against cell lysis and liberation of intracellular peptidases.

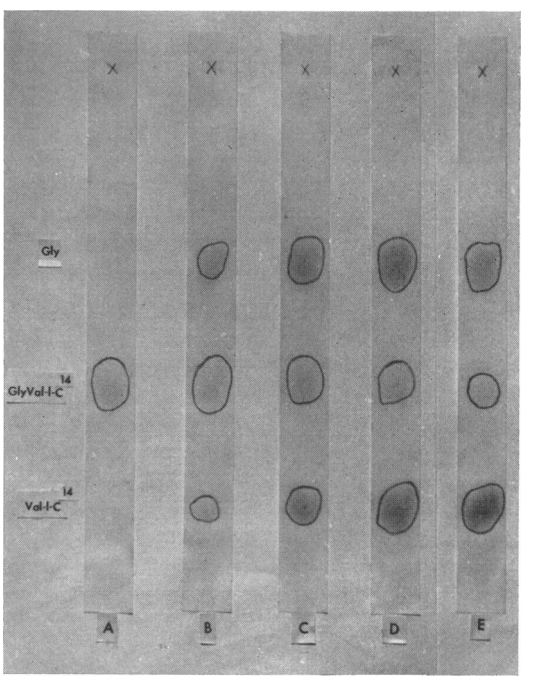

FIG. 4. *Chromatograms of the extracellular fluid showing the presence of glycine and valine-1-C^{14} after incubation of Leuconostoc mesenteroides cells with glycyl-DL-valine-1-C^{14} for increasing time intervals. The x denotes the point of sample application. A, B, C, D, and E refer to 0-, 15-, 30-, 60-, and 120-min incubation periods, respectively. A 0.1% ninhydrin solution in ethanol-collidine (95:5) sprayed over the chromatograms and developed by heating was used to locate the compounds.*

Since leucine is known to antagonize the utilization of valine (Brickson et al., 1948), some insight into the mechanisms involved might be obtained through its use as a substrate in the presence of the free and peptide form of valine (Table 5). L-Leucine was an effective inhibitor of the uptake system for DL-valine-*1*-C^{14} (Fig. 5). When L-leucine was added simultaneously with glycyl-DL-valine-*1*-C^{14}, the same amount (207 mμmoles) of valine-*1*-C^{14} was absorbed as when DL-valine was incubated with the labeled valine peptide (Table 4). Thus, leucine was effective in blocking the uptake of approximately 170 mμmoles of valine-*1*-C^{14}, which is the same amount that unlabeled DL-valine blocked when it was present simultaneously with glycyl-DL-valine-*1*-C^{14}. This finding suggested that perhaps these free, structurally similar amino acids (valine and leucine) were absorbed by the same uptake system, which was different from the one for valine when supplied in peptide form.

Glycyl-L-leucine inhibited the uptake of valine-*1*-C^{14}, but to a greater degree if the valine was supplied as glycyl-DL-valine than as free DL-valine (Table 5). This suggests two explanations: (i) that some of the glycyl-L-leucine was being hydrolyzed and the free leucine then was competing with the valine for uptake; and (ii) that the glycyl-L-leucine competed strongly for the uptake system which accumulated glycyl-DL-valine.

The above results show that independent uptake systems are present in *L. mesenteroides* for the uptake of valine, one specific for valine and another one specific for valine dipeptides. With the independent systems, the amount of radioactive valine accumulated when both substrates

TABLE 5. *Effect of the simultaneous addition of L-leucine and glycyl-L-leucine on the uptake of valine-1-C^{14} by Leuconostoc mesenteroides*

Incubation tube*	Substrate	Intracellular valine-*1*-C^{14}†
1	DL-Valine-*1*-C^{14}	171
2	Glycyl-DL-valine-*1*-C^{14}	372
3	DL-Valine-*1*-C^{14} + L-leucine	21
4	Glycyl-DL-valine-*1*-C^{14} + L-leucine	207
5	DL-Valine-*1*-C^{14} + glycyl-L-leucine	127
6	Glycyl-DL-valine-*1*-C^{14} + glycyl-L-leucine	116

* Each incubation tube contained 30 ml of 0.067 M phosphate buffer (pH 6.8) and 1% glucose. Each substrate was provided at a 1.43×10^{-2} M concentration when included in the incubation tube. Incubation was 20 min at 37 C.

† Expressed as millimicromoles per milligram of cells.

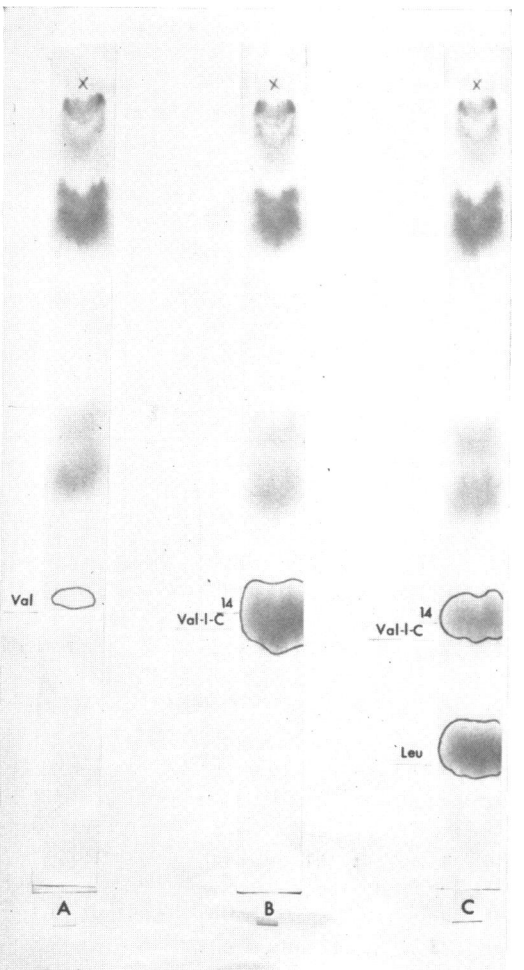

FIG. 5. *Chromatograms of the cellular fluid of Leuconostoc mesenteroides cells showing the low endogenous valine and the inhibitory effect which L-leucine has on the uptake of DL-valine-1-C^{14}. The x denotes the point of sample application. Chromatogram A represents the endogenous amino acids in the cellular fluid. Chromatogram B shows the cellular fluid from cells incubated with DL-valine-1-C^{14}. Chromatogram C is the cellular fluid from cells incubated with both DL-valine-1-C^{14} and L-leucine.*

were present should equal the sum of the amounts when only the individual substrates were present. If a common uptake system was involved, then the simultaneous presence of both substrates should have substantially reduced the uptake possible from either substrate. A common system of uptake was shown for valine and leucine.

DISCUSSION

The finding that glycyl-DL-valine promoted the growth of *L. mesenteroides* more rapidly and to a greater extent than did comparable concentrations of its component amino acids can be explained by the difference in the relative uptake and intracellular utilization of the peptide vs. the amino acids. Where the medium is otherwise complete except for valine, growth of the organism was obtained by the addition of glycyl-DL-valine, which enabled the cells to accumulate valine without the competition from other free amino acids for the same uptake system. Certain other dipeptides added to the medium simultaneously with glycyl-DL-valine inhibited growth and appeared to inhibit competitively the uptake of glycyl-DL-valine, illustrating that a common transport system was employed for the uptake of these peptides.

Under conditions of this study, the growth response of the organism was affected quite differently by glycine, glycylglycine, and glycylglycylglycine. It was found that neither glycine nor glycylglycylglycine inhibited growth in the presence of glycyl-DL-valine but that glycylglycine did. Cole (1954) showed that glycylglycine was an excellent source but that glycylglycylglycine was a poor source of glycine for this organism. The logical explanation was that the organism could not meet its glycine needs with glycylglycylglycine but could with glycylglycine. Berger, Johnson, and Peterson (1938) found that ruptured cells of this organism showed high tripeptidase activity; apparently glycylglycylglycine was unable to gain entry into the cell to furnish a source of glycine. On this assumption, glycylglycylglycine would not have competed with glycyl-DL-valine for a common uptake system, and no antagonism would have occurred. On the other hand, glycylglycine was inhibitory to glycylvaline utilization and was competing for a common transport system for uptake. In a similar manner, the other peptides, glycyl-DL-serine, glycyl-L-leucine, and DL-alanyl-DL-phenylalanine, inhibited growth of the organism. Acetyl-DL-valine caused no antagonism because it did not compete for a common uptake system. Apparently, the free amino group of the glycyl residue is necessary, and is concerned with the specificity of the transport system.

All attempts to detect the accumulated peptide in the cellular fluid of the organism have been unsuccessful (Nutter, 1956; Leach and Snell, 1960). At this time, it is not clear whether (i) the peptide, *per se*, is transported through the cell membrane and rapidly hydrolyzed by the intracellular dipeptidase; or (ii) concomitant peptide hydrolysis occurred during the transport event by the cell. Kessel and Lubin (1963) showed with mutant strains of *E. coli* that active transport of peptides and petidase activity are separate functions of that bacterial cell.

By studying the uptake systems through the use of radioactive valine and glycyl-DL-valine with their unlabeled counterpart in cellular suspensions of *L. mesenteroides*, different uptake systems, one for valine and another for glycylvaline, were shown. Both systems were energy-dependent. Additional, but unpublished, data show that both L- and D-valine are accumulated by *L. mesenteroides*. Leach and Snell (1960) showed that *Lactobacillus casei* has three different systems, one specific for glycine, another one with equal affinity for D- or L-alanine, and a third for the peptide form such as glycyl-L-alanine or L-alanylglycine.

LITERATURE CITED

BERGER, J., M. J. JOHNSON, AND W. H. PETERSON. 1938. The proteolytic enzymes of bacteria. I. The peptidases of *Leuconostoc mesenteroides*. J. Biol. Chem. **124**:395–408.

BRICKSON, W. L., L. M. HENDERSON, I. SOLHJELL, AND C. A. ELVEHJEM. 1948. Antagonism of amino acids in the growth of lactic acid bacteria. J. Biol. Chem. **176**:517–528.

COLE, E. R. 1954. Peptide growth factors for *Leuconostoc mesenteroides*. M.S. Thesis, West Virginia University, Morgantown.

FISCHER, E., AND H. SCHEIBLER. 1908. III. Derivate der activen Valine. Ann. Chem. **363**:36.

GALE, E. F. 1947. The assimilation of amino acids by bacteria. I. The passage of certain amino acids across the cell wall and their concentration in the internal environment of *Streptococcus faecalis*. J. Gen. Microbiol. **1**:53–76.

HENDERSON, L. M., AND E. E. SNELL. 1948. A uniform medium for the determination of

amino acids with various microorganisms. J. Biol. Chem. **172**:15–29.

HIRSCH, M. L., AND G. N. COHEN. 1953. Amino acid utilization in bacterial growth. I. Peptide utilization by a leucine-requiring mutant of Escherichia coli. Biochem. J. **53**:25–30.

KESSEL, D., AND M. LUBIN. 1963. On the distinction between peptidase activity and peptide transport. Biochim. Biophys. Acta **71**:656–663.

KIHARA, H., O. A. KLATT, AND E. E. SNELL. 1952*a*. Peptides and bacterial growth. III. Utilization of tyrosine and tyrosine peptide by *Streptococcus faecalis*. J. Biol. Chem. **197**:801–807.

KIHARA, H., W. G. MCCULLOUGH, AND E. E. SNELL. 1952*b*. Peptides and bacterial growth. I. Purification of a peptide factor required for growth of *Lactobacillus casei*. J. Biol. Chem. **197**:783–789.

KIHARA, H., AND E. E. SNELL. 1952. Peptides and bacterial growth. II. L-Alanine peptides and growth of *Lactobacillus casei*. J. Biol. Chem. **197**:791–800.

KIHARA, H., AND E. E. SNELL. 1955. Peptides and bacterial growth. VII. Relation to inhibitions by thienylalanine, ethionine, and canavanine. J. Biol. Chem. **212**:83–94.

KLUNGSOYR, M., R. J. SIRNY, AND C. A. ELVEHJEM. 1951. Effect of incomplete hydrolysis on microbiological determination of amino acids. J. Biol. Chem. **189**:557–569.

KODICEK, E., AND S. P. MISTRY. 1952. The microbiological assay of strepogenin with *Lactobacillus casei*. Biochem. J. **51**:108–112.

LEACH, F. R., AND E. E. SNELL. 1960. The absorption of glycine and alanine and their peptides by *Lactobacillus casei*. J. Biol. Chem. **235**:3523–3531.

LEACH, R. F., AND E. E. SNELL. 1963. The effects of prior incubation with glycyl-L-alanine on uptake of peptides by *Lactobacillus casei*. Biochim. Biophys. Acta **71**:454–456.

LEVINE, E. M., AND S. SIMMONDS. 1960. Metabolite uptake by serine-glycine auxotrophs of *Escherichia coli*. J. Biol. Chem. **235**:2902–2909.

MEINHART, J. O., AND S. SIMMONDS. 1955. Metabolism of serine and glycine peptides by mutants of *Escherichia coli* strain K-12. J. Biol. Chem. **216**:51–65.

MILLER, A., A. NEIDLE, AND H. WAELSCH. 1955. Chemical stability and metabolic utilization of asparagine peptides. Arch. Biochem. Biophys. **56**:11–21.

MILLER, H. K., AND H. WAELSCH. 1952. The utilization of glutamine and asparagine peptides by microorganisms. Arch. Biochem. Biophys. **35**:184–194.

MOORE, S., AND W. H. STEIN. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. J. Biol. Chem. **176**:367–388.

NUTTER, W. E. 1956. Bacterial utilization of peptides. Ph.D. Thesis, West Virginia University, Morgantown.

NUTTER, W. E., AND D. C. SHELTON. 1956. Bacterial utilization of peptides. Federation Proc. **16**:227.

O'BARR, T. P., H. LEVIN, AND H. REYNOLDS. 1958. Some interrelationships of amino acids in the nutrition of *Leuconostoc mesenteroides*. J. Bacteriol. **75**:429–435.

PETERS, V. J., J. M. PRESCOTT, AND E. E. SNELL. 1953. Peptides and bacterial growth. IV. Histidine peptides as growth factors for *Lactobacillus delbrueckii* 9649. J. Biol. Chem. **202**:521–532.

PETERS, V. J., AND E. E. SNELL. 1954. Peptides and bacterial growth. VI. The nutritional requirements of Lactobacillus delbrueckii. J. Bacteriol. **67**:69–76.

PRESCOTT, J. M., V. J. PETERS, AND E. E. SNELL. 1953. Peptides and bacterial growth. V. Serine peptides and growth of *Lactobacillus delbrueckii* 9649. J. Biol. Chem. **202**:533–540.

ROBERTS, R. B., P. H. ABELSON, D. B. COWIE, T. B. ELLIS, AND R. J. BRITTEN. 1955. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Wash. Publ. 607.

SIMMONDS, S., AND J. S. FRUTON. 1949. A microorganism exhibiting a growth requirement for peptides. Science **109**:561–562.