Transport and Metabolism of Vitamin B6 in Lactic Acid Bacteria*

JAMES H. MULLIGAN† AND ESMOND E. SKEFF§

From the Department of Biochemistry, University of California, Berkeley, California 94720

Streptococcus faecalis 8043 concentrates extracellular [14C]pyridoxal or [3H]pyridoxamine primarily as the corresponding 5'-phosphates. Accumulation of pyridoxamine requires an exogenous energy source and is inhibited by glycolysis inhibitors. A membrane potential is not required for transport of pyridoxamine, and an artificially generated potential does not drive uptake in this organism. Based on this and other evidence, it is concluded that S. faecalis accumulates pyridoxamine by facilitated diffusion in conjunction with trapping by pyridoxal kinase. Pyridoxamine-P is not concentrated, but equilibrates with that provided externally. Lactobacillus casei 7469 concentrates radioactivity only from pyridoxal, which appears internally as pyridoxal-P, suggesting that it too absorbs the vitamin by facilitated diffusion plus trapping. The specificity of the growth requirement of S. faecalis and L. casei for vitamin B6 parallels the specificity of the transport systems for this vitamin in these organisms. Lactobacillus delbrueckii 7469, however, which specifically requires pyridoxamine-P or pyridoxal-P for growth, accumulates both these compounds and pyridoxine-P from the medium, apparently by active transport, but not pyridoxine, pyridoxal, or pyridoxamine. While pyridoxal-P and pyridoxamine-P are interconvertible in this organism, pyridoxine-P is not further metabolized, thus accounting for the specificity of the growth requirement. These and previous results show (a) that different organisms may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the growth requirement for a given form of vitamin frequently arises from the specificity of transport, but that internal metabolism of the compounds also plays a significant role in some organisms.

The transport of amino acids and sugars by microorganisms is a subject of intensive study (1, 2). However, uptake and utilization of vitamins has received less attention. Transport systems for vitamin B12 (3), biotin (4), folic acid (5, 6), lipoic acid (7), nicotinamide (8), and thiamine (9) have been studied frequently arises from the specificity of transport, but that the specificity of the growth requirement may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the transport systems for this vitamin in these organisms. Lactobacillus delbrueckii 7469, however, which specifically requires pyridoxamine-P or pyridoxal-P for growth, accumulates both these compounds and pyridoxine-P from the medium, apparently by active transport, but not pyridoxine, pyridoxal, or pyridoxamine. While pyridoxal-P and pyridoxamine-P are interconvertible in this organism, pyridoxine-P is not further metabolized, thus accounting for the specificity of the growth requirement. These and previous results show (a) that different organisms may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the growth requirement for a given form of vitamin frequently arises from the specificity of transport, but that internal metabolism of the compounds also plays a significant role in some organisms.

The transport of amino acids and sugars by microorganisms is a subject of intensive study (1, 2). However, uptake and utilization of vitamins has received less attention. Transport systems for vitamin B12 (3), biotin (4), folic acid (5, 6), lipoic acid (7), nicotinamide (8), and thiamine (9) have been studied frequently arises from the specificity of transport, but that the specificity of the growth requirement may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the transport systems for this vitamin in these organisms. Lactobacillus delbrueckii 7469, however, which specifically requires pyridoxamine-P or pyridoxal-P for growth, accumulates both these compounds and pyridoxine-P from the medium, apparently by active transport, but not pyridoxine, pyridoxal, or pyridoxamine. While pyridoxal-P and pyridoxamine-P are interconvertible in this organism, pyridoxine-P is not further metabolized, thus accounting for the specificity of the growth requirement. These and previous results show (a) that different organisms may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the growth requirement for a given form of vitamin frequently arises from the specificity of transport, but that internal metabolism of the compounds also plays a significant role in some organisms.

The transport of amino acids and sugars by microorganisms is a subject of intensive study (1, 2). However, uptake and utilization of vitamins has received less attention. Transport systems for vitamin B12 (3), biotin (4), folic acid (5, 6), lipoic acid (7), nicotinamide (8), and thiamine (9) have been studied frequently arises from the specificity of transport, but that the specificity of the growth requirement may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the transport systems for this vitamin in these organisms. Lactobacillus delbrueckii 7469, however, which specifically requires pyridoxamine-P or pyridoxal-P for growth, accumulates both these compounds and pyridoxine-P from the medium, apparently by active transport, but not pyridoxine, pyridoxal, or pyridoxamine. While pyridoxal-P and pyridoxamine-P are interconvertible in this organism, pyridoxine-P is not further metabolized, thus accounting for the specificity of the growth requirement. These and previous results show (a) that different organisms may employ quite different transport machinery in utilization of a given external nutrient, and (b) that the specificity of the growth requirement for a given form of vitamin frequently arises from the specificity of transport, but that internal metabolism of the compounds also plays a significant role in some organisms.

The transport of amino acids and sugars by microorganisms is a subject of intensive study (1, 2). However, uptake and utilization of vitamins has received less attention. Transport systems for vitamin B12 (3), biotin (4), folic acid (5, 6), lipoic acid (7), nicotinamide (8), and thiamine (9) have been studied briefly. Investigations concerning vitamin B6 transport by microorganisms have been reported by Oya (10), and more recently by this laboratory (11, 12).

Oya found that Escherichia coli accumulated extracellular pyridoxine by a process which is stimulated by exogenous glucose and inhibited by dinitrophenol (10). However, all intracellular vitamin appeared as pyridoxine phosphate and pyridoxal phosphate; thus there was no concentration gradient of free vitamin. We reported a somewhat similar situation for uptake of vitamin B6 by Salmonella typhimurium (11). This organism accumulated extracellular pyridoxine and pyridoxal as the phosphorylated derivatives, but did not accumulate pyridoxine. On the basis of this and other evidence, we concluded that S. typhimurium accumulates vitamin B6 by facilitated diffusion, and that intracellular vitamin was trapped as phosphorylated derivatives by pyridoxal kinase (11). Whether E. coli actively transports vitamin B6 or employs facilitated diffusion and trapping is unknown.

The transport of vitamin B6 by Saccharomyces carlsbergensis has entirely different characteristics than the systems in E. coli and S. typhimurium. This organism concentrated pyridoxine, pyridoxal, and pyridoxamine intracellularly more than 100-fold by a process that is energy-dependent and sensitive to metabolic inhibitors. In this case none of the intracellular vitamin was phosphorylated initially, and the authors concluded that S. carlsbergensis possesses an active transport system for vitamin B6 (12). The lack of parallels between uptake mechanisms in S. typhimurium and S. carlsbergensis led us to inquire into the mechanism of vitamin B6 uptake in other types of microorganisms. Specifically, it would be interesting to know if pyridoxal kinase plays a recurring role as a means of trapping intracellular vitamin. We have chosen pyridoxamine uptake by Streptococcus faecalis for detailed study, and also report suggestive data concerning the mechanism of vitamin B6 uptake by Lactobacillus casei and Lactobacillus delbrueckii. These three lactic acid bacteria are of additional interest because they require specific forms of vitamin B6 for growth (13-17); the present work also correlates the specificity of transport systems for vitamin B6 with the specificity of this growth requirement.

MATERIALS AND METHODS

Isotopically Labeled Compounds - The preparation and purification of [3H]pyridoxine, [3H]pyridoxal, [3H]pyridoxamine, 5'-deoxy[3H]pyridoxine, and 5'-deoxy[3H]pyridoxal have been described

* This work was supported in part by Research Grants AM-1448 and AI-1575 from the National Institutes of Health, United States Public Health Service.
† Recipient of a United States Public Health Service predoctoral traineeship from Grant GM-00031.
§ Present address, Department of Microbiology, The University of Texas at Austin, Austin, Tex. 78712, where reprint requests should be sent.

(Received for publication, July 23, 1976)
(11). ["H]Pyridoxine-P, ["H]pyridoxal-P, and ["H]pyridoxamine-P were prepared enzymatically, using pyridoxal kinase purified from bovine brain by the procedure of Neary and Diven (18). One unit of pyridoxal kinase activity represents 1 nmol of pyridoxal-P formed/min at 37°. The reaction mixtures for preparation of the tritiated vitamin B6 phosphates contained (in a final volume of 1 ml) 10 μmol of potassium phosphate (pH 6.5), 2 μmol of ATP, 50 nmol of ZnCl₂, 90 units of pyridoxal kinase (specific activity 44 units/mg), and either 200 μCi of ["H]pyridoxine, 300 μCi of ["H]pyridoxal, or 1.5 mCi of ["H]pyridoxamine. All solutions were incubated at 37° for 30 min, then those containing pyridoxal-P or pyridoxamine-P were diluted with 4 ml of H₂O and 50 μl of 1 M HAOAc, while that containing pyridoxamine-P was diluted with 3.5 ml of H₂O and 0.5 ml of 1 M NaOAc (pH 4.7). For purification the solutions containing pyridoxal-P or pyridoxamine-P were applied to columns (1.1 x 5 cm) of DE-52 DEAE-cellulose (Whatman) equilibrated with 0.1 M HOAc and eluted sequentially with 48 ml of 0.01 M HOAc, 96 ml of 0.1 M HOAc, and finally 30 ml of 1 M HOAc (19). Both phosphorylated compounds were well separated from starting materials; pyridoxine-P eluted in the 0.1 M HOAc wash, while pyridoxal-P eluted with 1 M HOAc. The solution containing pyridoxamine-P was purified on a column (1.1 x 15 cm) of P11 phosphocellulose (Whatman) equilibrated and eluted with 0.1 M NaOAc (pH 4.7) (19). The yields of tritiated vitamin B6 phosphates were 15% for ["H]pyridoxine-P, 77% for ["H]pyridoxal-P, and 30% for ["H]pyridoxamine-P. The compounds were pure as judged by the radioactivity profile of thin layer chromatography plates developed as described elsewhere (11), and the positions of the radioactive peaks corresponded to those of authentic standards.

"S"-Deoxy ["H]pyridoxamine was prepared by reduction of the Schiff's base formed between "S"-deoxy["H]pyridoxal and ammonia. To a solution containing 0.5 mCi of "S"-deoxy["H]pyridoxal and 200 μl of concentrated NH₄OH in 0.5 ml of ethanol was added 100 μl of 10 N NaOH. After 5 min at 25° solid NαBH₄ (about 20 mg) was added, and after 0.5 h the solution was evaporated to dryness. The residue was extracted with ethanol, and this extract was evaporated. The residue was dissolved in 5 ml of 0.1 N NaOAc (pH 4.7) and purified by chromatography on a column (1.1 x 5 cm) of P11 phosphocellulose eluted with 0.1 M NaOAc (pH 4.7), yielding 30 μCi (% of "S"-deoxy["H]pyridoxamine. This material gave only one radioactive peak when subjected to paper electrophoresis (Whatman No. 1 paper, 1500 V for 2 h in pyridine/acetate/acetone/water (30/190/150), and this peak corresponded exactly to an authentic standard.

"S"-["H]Threonine (300 mCi/mmole) from Amersham/Searle was diluted with L-threonine to a final specific activity of 2.2 mCi/mmol based on the L isomer.

**Cell Growth and Transport Assay Condition**—Bacterial strains were maintained as stabs in nutrient containing 0.2% yeast extract, and 0.25% glucose solidified with 2% agar. Liquid cultures were grown at 37° to stationary phase (about 1 mg/ml, dry weight) in either complete medium (20) or in vitamin B6-free medium (21) (Medium Q, supplemented with 5 to 50 pmol/ml of vitamin B6). The organisms were generally grown in air (not shaken) in test tubes or flasks half-filled with medium. Vitamin B6 uptake was assayed as described earlier (11), except that the uptake and washing medium contained 45 mm K₃PO₄ (pH 6.3) and 5 ml of salts B (20)/liter.

**Preparation and Assay of Spheroplasts from S. faecalis**—Spheroplasts were prepared as described by Mora and Snell (22). Transport assays using spheroplasts were identical with those for whole cells, except that the uptake and washing medium contained 0.4 M sucrose in addition to salts.

**RESULTS**

**Specificity for Vitamin B6 Transport by Streptococcus faecalis, Lactobacillus casei, and Lactobacillus delbrueckii**—Fig. 1 shows the specificity and extent of transport of the six naturally occurring forms of vitamin B6. Assuming that fresh cells of S. faecalis contain an intracellular water space of 2 ml/g dry weight (23), this organism concentrates radioactivity from extracellular ["H]pyridoxal or ["H]pyridoxamine, but not from ["H]pyridoxine. In this organism the level of intracellular pyridoxal-P or PNP, pyridoxine 5'-phosphate; pyridoxal-P or PLP, pyridoxal 5'-phosphate; pyridoxamine-P or PMP, pyridoxamine 5'-phosphate; CCCP, carbonyl cyanide m-chlorophenylhydrazone; DCDCC, N,N'-dicyclohexylcarbodiimide; HOAc, acetic acid; NaOAc, sodium acetate.
Transport and intracellular metabolism of vitamin B6 in lactic acid bacteria

Cells were suspended at 37° in uptake medium containing 0.5% glucose and tritiated vitamin B6 (0.73 μCi PL, 1.17 μCi PM, 0.80 μCi PMP, 0.61 μCi PLP, or 0.44 μCi PNP). After incubation for 40 min (for Lactobacillus casei and Streptococcus faecalis) or 10 min (for Lactobacillus delbrueckii), five 0.5-ml samples from each incubation mixture were filtered, washed twice, and boiled with 5 ml of H₂O for 8 min. These water extracts were then centrifuged, made 0.01 M, and analyzed as described elsewhere (19). Similar results were obtained after 1 min of uptake of [³H]PM by S. faecalis, except that less than 3% of the transported vitamin was unphosphorylated. Compounds whose intracellular forms were not analyzed (e.g. PM or PN in L. casei) are not accumulated by that organism. PIC and PICP are thought to arise by oxidation during hot water extraction and analysis, and not to occur naturally.

Transport and Metabolism of Vitamin B6

**TABLE I**

| Organism and form of vitamin transported | Per cent of transported vitamin* found intracellularly as | PL | PM | PN | PLP | PMP | PNP | PIC | PICP | Total |
|----------------------------------------|------------------------------------------------------|----|----|----|-----|-----|-----|-----|-----|-------|
| S. faecalis                            |                                                      | 14.2 | 9.2 | 26.0 | 3.4 | 26.2 | 2.1 | 0.5 | 4.7 | 79.8 |
| S. faecalis                            |                                                      | 25.9 | 9.2 | 0.4 | 33.1 | 23.4 | 3.7 | 9.6 | 57.8 |
| L. casei                               |                                                      | 28.1 | 2.8 | 0.3 | 20.4 | 70.3 | 1.9 | 1.9 | 10.2 |
| L. delbrueckii                         |                                                      | 0.1 | 0.0 | 0.1 | 4.5 | 19.5 | 3.2 | 0.4 | 7.4 | 8.8 |

* Pyridoxal, pyridoxamine, and pyridoxine are abbreviated as PL, PM, and PN, respectively, and the corresponding 5'-phosphates as PLP, PMP, and PNP; PIC = 4-pyridoxic acid; PICP = 4-pyridoxic acid phosphate.

spheroplasts is greatly stimulated by K⁺; Na⁺ is much less effective (Fig. 4). A similar effect was observed by Mora and Snell during studies of amino acid uptake by S. faecalis (22). They suggested that these effects might be due to differential effects of the two monovalent cations on membrane structure.

**Effects of Metabolic Inhibitors** – A first step in defining the mechanism of pyridoxamine accumulation by S. faecalis involved the use of metabolic inhibitors to determine if uptake occurred under the conditions shown. These results suggest that energy is required for uptake of pyridoxamine, but is not required in the form of a membrane potential.

**Membrane Potential Is Not Required for Pyridoxamine Uptake** – Although neither DCCD nor CCCP inhibited pyridoxamine uptake, it was necessary to show that these compounds did collapse the membrane potential under our conditions. Fortunately, Harold and co-workers have described the use of these reagents in a dissection of factors important for uptake of neutral amino acids (23). They found that either CCCP or DCCD completely inhibited threonine uptake by collapsing the membrane potential. Fig. 5 shows the results obtained on repeating those experiments, and results of similar trials with pyridoxamine under identical conditions. The fact that threonine uptake is abolished while pyridoxamine uptake is unaffected lends us to conclude that a membrane potential is not obligatory for pyridoxamine uptake.

**Membrane Potential Cannot Drive Pyridoxamine Uptake** – Despite the apparent lack of dependence of pyridoxamine transport on a membrane potential, the possibility still existed that under normal conditions pyridoxamine is actively transported at the expense of membrane potential, but is also rapidly trapped by phosphorylation. In the absence of a membrane potential pyridoxamine might enter via facilitated diffusion, and then be trapped. To investigate this possibility, an attempt was made to drive pyridoxamine transport with an artificially generated membrane potential in unenergized...
it lacks a hydroxyl groups at the 5' position and therefore is not accumulated by S. faecalis above the level in the amine-P rapidly reach the concentration of pyridoxamine-P ever, experiments with 5'-deoxy[3H]pyridoxamine showed that S. faecalis involved the use of 5'-deoxypyridoxamine, a nonmetabolizable analogue of pyridoxamine. This analogue is a competitive inhibitor of pyridoxal uptake by yeast, in which active transport of vitamin B6 has been demonstrated (12), but since it lacks a hydroxyl groups at the 5' position and therefore cannot be phosphorylated by pyridoxal kinase, it should not be accumlated by S. faecalis if the accumulation mechanism requires trapping by pyridoxal kinase. Fig. 7 shows that 5'-deoxypyridoxamine does have affinity for the pyridoxamine carrier, since it inhibits pyridoxamine uptake competitively with a $K_v$ value (calculated from changes in the apparent $K_m$ for pyridoxamine at various inhibitor levels) of 0.3 $\mu$M. However, experiments with 5'-deoxypyridoxamine showed that it is not accumulated by S. faecalis above the level in the medium (data not shown). This result argues against active transport, and suggest facilitated diffusion with trapping as the mechanism of pyridoxamine accumulation.

**DISCUSSION**

Pyridoxal and pyridoxamine are accumulated and appear intracellularly in S. faecalis primarily as pyridoxal-P and pyridoxamine-P. In addition, intracellular levels of pyridoxamine-P rapidly reach the concentration of pyridoxamine-P supplied in the medium but do not rise above that level. Other forms of the vitamin (pyridoxine, pyridoxine-P, pyridoxal-P) are apparently unable to cross the cell membrane. These findings are in harmony with the fact that S. faecalis specifically requires either pyridoxal, pyridoxamine, or pyridoxamine-P for growth (13, 14, 24).

The lack of cross-inhibition between pyridoxal and pyridoxamine indicates that they are transported by different systems, although both have similar pH optima for transport. A carrier is involved for each since pyridoxine competitively inhibits pyridoxal uptake and 5'-deoxypyridoxamine competitively inhibits pyridoxamine uptake. The accumulation of pyridoxamine is sensitive to monovalent cations, being stimulated by either K$^+$ or (CH$_3$)$_2$N$^+$ and inhibited by Na$^+$. These effects are similar in protoplasts and whole cells, and may be due to ion effects on membrane structure, as judged by differential binding of 8-anilinonaphthalene sulphonate to protoplast membranes in the presence of sodium or potassium (data not shown).

The following data show that pyridoxamine is not accumulated by an active process in S. faecalis: (a) dissipation of the membrane potential by addition of DCCD or CCCP does occur, as shown by their inhibition of threonine transport. However, such dissipation has no effect on uptake of pyridoxamine. (b) An artificial membrane potential produced by valinomycin-induced potassium efflux does not drive pyridoxamine uptake, but is effective in promoting threonine transport. (c) 5'-Deoxypyridoxamine, a competitive inhibitor of pyridoxamine uptake, is not accumulated by S. faecalis. Since active transport is not involved in pyridoxamine uptake, and yet pyridoxamine is accumulated by S. faecalis in an energy-requiring process, it seems clear that this uptake involves facilitated diffusion followed by trapping of intracellular vitamin as phosphorylated derivatives, as found earlier for S. typhimurium (11).

This explanation is also favored by the finding that less than 5% of the intracellular vitamin is unphosphorylated, even after only 1 min of uptake. The fact that pyridoxine is not accumulated by S. faecalis cannot be attributed to the specificity of the pyridoxal kinase from this organism, since the enzyme has a lower $K_m$ value for pyridoxine than for pyridoxal.
not be freely exchangeable. However, this interpretation is less likely for pyridoxamine-P, which would bind specifically only to the comparatively few aminotransferases (e.g., pyridoxamine-P dehydrogenase) that require it for activity, and the level of pyridoxamine-P employed in these experiments (as judged by the extra-cellular amounts required to support growth) is several orders of magnitude greater than that needed to cover such aminotransferases. Since transport of pyridoxal-P is inhibited by pyridoxamine-P and vice versa, and there is most probably a gradient of diffusible pyridoxamine-P, it appears likely that both components are actively transported by the same system. The situation for pyridoxine-P is more clear-cut. Here intracellular binding (except possibly as an inhibitor of pyridoxal-P-dependent aminotransferases) is not expected, and one may conclude that it also enters L. delbrueckii by active transport.

The finding that L. delbrueckii transports all three forms of phosphorylated vitamin B6 but uses only two of them for growth is unexpected. The disparity is easily explainable, however, in terms of cellular metabolic capabilities: analysis of the intracellular forms of the transported vitamin shows that while pyridoxal-P and pyridoxamine-P are readily interconverted, pyridoxamine-P is not converted to these metabolically useful forms. Thus L. delbrueckii differs from the other organisms studied, in that the specificity of its growth requirement is dictated partially by intracellular metabolic capabilities.

REFERENCES

1. Simoni, R. D., and Pastina, P. W. (1975) Annu. Rev. Biochem. 44, 523-553
2. Boos, W. (1974) Annu. Rev. Biochem. 43, 123-146
3. DiGiralmo, P. M., and Bradbeer, C. (1971) J. Bacteriol. 106, 745-750
4. Waller, J. R., and Lichstein, H. C. (1964) J. Bacteriol. 90, 853-856
5. Shane, B., and Stokstad, E. L. R. (1975) J. Biol. Chem. 250, 2243-2253
6. Henderson, G. R., Zavos, F. M., and Huisman, F. M. (1976) Biochem. Biophys. Res. Commun. 68, 712-717
7. Sanders, D. C., and Leach, F. R. (1982) Biochim. Biophys. Acta 69, 494-508
8. Nejahr, H., and Varga, Z. (1966) Acta Chem. Scand. 20, 1529-1534
9. Griffith, T. W., and Leach, F. R. (1973) Arch. Biochem. Biophys. 154, 388-385
10. Oya, N. (1970) Vitamins (Kyoto) 41, 222-229
11. Mulligan, J. H., and Snell, E. E. (1976) J. Biol. Chem. 251, 1052-1056
12. Shane, B., and Snell, E. E. (1976) J. Biol. Chem. 251, 1042-1051
13. Snell, E. E., and Runnefeld, A. N. (1945) J. Biol. Chem. 157, 475-480
14. Rabinowitz, J. C., and Snell, E. E. (1947) J. Biol. Chem. 169, 643-660
15. Rabinowitz, J. C., and Snell, E. E. (1947) Anal. Chem. 19, 277-280
16. McNutt, W. E., and Snell, E. E. (1969) J. Biol. Chem. 182, 555-557
17. Peters, V. J., and Snell, E. E. (1954) J. Bacterial. 67, 69-76
18. Neary, J. T., and Diven, W. F. (1970) J. Biol. Chem. 245, 5585-5593
19. Contractor, S. F., and Shane, B. (1971) Biochim. Biophys. Acta 230, 127-130
20. Chang, G. W., and Snell, E. E. (1968) Biochemistry 7, 2005-2012
21. Rabinowitz, J. C., and Snell, E. E. (1947) J. Biol. Chem. 169, 631-642
22. Mora, J., and Snell, E. E. (1963) Biochemistry 2, 136-141
23. Asghar, S. S., Levin, E., and Harold, F. M. (1973) J. Biol. Chem. 248, 5235-5238
24. Holden, J. T., Furman, C., and Snell, E. E. (1949) J. Biol. Chem. 178, 789-797
25. McCormick, D. B., Gregory, M. E., and Snell, E. E. (1961) J. Biol. Chem. 236, 2076-2084

(25); rather, it must reflect structural specificity of the membrane carriers.

An additional objective of the present work has been to determine whether growth requirements of various organisms for vitamin B6 are commonly dictated by the discrimination of membrane carriers or by limitations in metabolic machinery. In the case of Salmonella typhimurium mutants (11) and S. faecalis, the specificity of the growth requirement for vitamin B6 stems from discrimination by membrane carriers.

L. casei is even more fastidious than S. faecalis in its vitamin B6 requirement, requiring pyridoxal specifically for growth (13–15). In accordance with this specificity we found that L. casei accumulates only pyridoxal, and most of the intracellular vitamin is phosphorylated. These facts support the idea that specificity in growth requirement may be determined by an organism’s transport system. However, an important caveat is revealed by the knowledge (a) that a 100-fold molar excess of pyridoxine inhibits pyridoxal uptake by about 10%, and (b) that pyridoxal kinase from L. casei discriminates in favor of pyridoxal since its $K_w$ value for this substrate is less than 0.01 that for pyridoxamine or pyridoxine (25). Thus, if accumulation of pyridoxal proceeds by facilitated diffusion with trapping the exclusion of pyridoxine might result from kinase specificity alone, rather than by discrimination at the translocation level. This possibility appears unlikely for two reasons. First, a similar disparity in $K_w$ values of pyridoxal kinase occurs in S. faecalis, where the $K_w$ value for pyridoxamine is more than 100 times that for pyridoxal (25). Nonetheless, pyridoxamine is accumulated by facilitated diffusion and trapping more rapidly than pyridoxal. Therefore, one must conclude that there is excess pyridoxal kinase capacity in S. faecalis. Since the activity of pyridoxal kinase in L. casei is 4 times that in S. faecalis (25), L. casei should trap pyridoxine at least as efficiently as S. faecalis traps pyridoxamine, hence it appears that pyridoxine is not presented intracellularly to the kinase. Second, pyridoxine also inhibits pyridoxal uptake in L. casei; thus the carrier for pyridoxal, although fairly specific, does have some affinity for pyridoxine. It appears likely, therefore, that the basis for the specific growth requirement of L. casei for pyridoxal also lies in the specificity of a membrane carrier, and that the slight inhibition of pyridoxal uptake by pyridoxine stems from less than absolute carrier specificity.

A third pattern in specificity of nutritional requirements for vitamin B6 is presented by L. delbrueckii, which requires pyridoxamine-P or pyridoxal-P for growth, but cannot use pyridoxine-P or any of the unphosphorylated forms of vitamin B6 for this purpose (16, 17). The transport studies have shown that this organism cannot accumulate any of the free forms of the vitamin, but transports pyridoxine-P in addition to pyridoxamine-P and pyridoxal-P. In this case there exists a concentration gradient of unchanged transport substance across the cell membrane. Ordinarily, such a gradient is prima facie evidence for an active transport mechanism, but that interpretation depends on the ability of the intracellular substrate to freely exchange with that in the medium. Certainly in the case of pyridoxal-P, which can bind both specifically to B6-dependent aminotransferases and nonspecifically to many other proteins, one could imagine that the intracellular vitamin might
Transport and metabolism of vitamin B6 in lactic acid bacteria.
J H Mulligan and E E Snell

J. Biol. Chem. 1977, 252:835-839.

Access the most updated version of this article at http://www.jbc.org/content/252/3/835

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/3/835.full.html#ref-list-1