present experiments is not due to intact cell contamination.

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The Uptake of Amino Acids by Cells and Protoplasts of *S. faecalis**

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Both cells and protoplasts of *S. faecalis* contain a transport system for glycine, L-alanine, and D-alanine that requires an energy source, possesses a high temperature coefficient, is saturated at relatively low external concentrations of amino acid, and leads to high internal concentration of these amino acids in a form that is extractable as free amino acid by hot water. The affinities of the transport system for these amino acids are the same in cells and in protoplasts. Uptake of all three amino acids in protoplasts is stimulated by K^+ and inhibited by Na^+; these ions have no detectable effect in the intact cells. High concentrations of pyridoxal greatly increase accumulation of glycine and L-alanine by protoplasts; uptake of D-alanine is not changed. The effect is reasonably specific for pyridoxal, and is not observed in intact cells. 5-Deoxypyridoxal inhibits uptake in both intact cells and protoplasts. D-Cycloserine inhibits uptake of both D- and L-alanine by the transport system. No clear explanation of the differential effects of K^+ and of pyridoxal in cells and protoplasts is yet possible. In each case, the bacterial protoplast resembles mammalian cells more than it does the intact bacterial cell. Both effects may result from conformational changes in the non-rigid protoplast membrane that are not possible when this is in contact with a rigid cell wall.

Extracellular pyridoxal or pyridoxal-5-phosphate greatly increases the rate and extent of concentration of several amino acids by Ehrlich ascites tumor cells (Christensen *et al.*, 1954; Christensen, 1960). Pyridoxal phosphate also increases intestinal absorption of amino acids in animals made B_6-deficient by penicillamine treatment (Akedo *et al.*, 1960), and in isolated intestinal segments of rats previously treated with 4-deoxypyridoxine (Jacobs and Hillman, 1959).

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In contrast to these results in mammalian cells and tissues, Holden (1959a) found that vitamin B_6–deficient cells of *Lactobacillus arabinosus* absorb glutamic acid as rapidly as those grown with an excess of vitamin B_6, though not to the same extent, and Leach and Snell (1960) found that the uptake of L-alanine or of glycyl-L-alanine by cells of *Lactobacillus casei* severely deficient in vitamin B_6 was not increased by the addition of pyridoxal either to the resting cells or to growing cultures.

To determine whether the presence of a rigid cell wall in bacterial cells was responsible for these differences, and to learn more about the possible role of this structure in cell permeability, a comparative study has

been made of the uptake of amino acids by cells and protoplasts of *Streptococcus faecalis*.

Experimental Procedures

Radioactive Amino Acids.—Uniformly labeled glycine-C^{14} (Nuclear Chicago) and L-alanine-1-C^{14} and D-alanine-1-C^{14} (California Corporation for Biochemical Research) were used. Purity of these compounds was checked by paper chromatography in pyridine–acetic acid–water (50:35:15). Only a single radioactive and ninhydrin-positive spot was found; this corresponded exactly to the standard amino acid in position; there was no trace of other radioactive materials.

Preparation of Cells and Protoplasts.—Sterile medium (300 ml) containing 1.0% glucose, 1.0% tryptone (Difco), and 1.0% K_2HPO_4 was inoculated with *S. faecalis* ATCC 9790 and incubated at 38° for 18 hours. The cells were harvested by centrifugation and washed three times with cold distilled water. Freshly harvested cells were used for each experiment.

Protoplasts were prepared by a slight modification of the procedure of Abrams (1959). For this purpose, the cells were suspended at *p*H 6.2 and a cell density of 2 to 3 mg (dry weight) per ml in 0.075 M potassium phosphate (buffer A) or 0.15 M Tris-phosphate buffer (buffer B), each made 0.4 M in sucrose. Crystalline lysozyme (Worthington, 360 μg per ml) was added and the mixture was incubated at 37° for 1 hour. The protoplasts were then centrifuged at 5000 × *g* and resuspended in the same volume of buffer A–0.5 M sucrose, *p*H 7.2. Formation of protoplasts by the lysozyme treatment was virtually complete, as shown by (1) the decrease in optical density during this procedure, (2) the complete lysis that occurred on dilution with water, and (3) the previously described metabolic swelling in the presence of glucose (Abrams, 1959). In comparative experiments intact cells were carried through this same procedure but with lysozyme omitted.

Separate controls for cells and protoplasts were run with each experiment; when comparisons are made between protoplasts and cells, these represent differences between the experimental values and the respective control values. Quantities of protoplasts are expressed in terms of the weight of cells from which they were prepared.

Measurement of Accumulation of C^{14}-Labeled Compounds.—Unless otherwise stated, freshly prepared cells or protoplasts were incubated at 37° for 10 minutes; then glucose was added. After an additional 5 minutes the radioactive amino acid was added. At desired times, further uptake of label was stopped by rapidly mixing 1-ml samples of the cell or protoplast suspension with 1.6 ml of ice-cold buffer A–0.5 M sucrose, *p*H 7.2. The suspension was centrifuged for 10 minutes at 7000 × *g* and 0–4°. The supernatant solution was poured off and the inside of the tube was carefully swabbed dry; the pellet of cells or protoplasts was again centrifuged and the remaining liquid was removed with a filter paper swab. The pellet was resuspended in 25% ethanol, heated in a water bath at 85° for 10 minutes, then centrifuged 10 minutes at 7000 × *g*. The supernatant solution was plated and the radioactivity determined to give the value for *accumulation* or "*pool*" content. The precipitate was resuspended and plated and the radioactivity determined to give the *incorporation* into protein, cell wall, and other insoluble material. Radioactivity of all samples was determined with a Nuclear-Chicago D-47 Micromil counter. In most cases, 5120 counts were measured for each sample (standard error, 2.0%) and corrected for background and nonmetabolic absorption of radioactivity by the pellet of cells or protoplasts. For the latter purpose a non-incubated control was used, to which radioactive amino acid was added at 0° and immediately centrifuged. This correction was usually less than 5% and never more than 10% of the total count. Since only 2 mg of cells were present in each sample, no correction for self-absorption was necessary. Replicate samples prepared and counted in this fashion agreed within 5%.

Other Methods.—Lactic acid was determined by the method of Barker and Summerson (1941).

Table I

Requirement for Glucose for Accumulation of Glycine-C^{14}, L-Alanine-1-C^{14} and D-Alanine-1-C^{14} by Cells and Protoplasts

After 3 hours at 37° without added amino acids or glucose, glucose was added where indicated and incubation was continued for 5 minutes. The radioactive substrate was then added and samples were taken for analysis at 40 minutes. The complete system contained per ml: For cells: K_2HPO_4 (*p*H 7.2), 75 μmoles; glucose, 50 μmoles; and 400 mμmoles of glycine-C^{14}, or 100 mμmoles of L-alanine-1-C^{14}, or 2000 mμmoles of D-alanine-1-C^{14}. For protoplasts: as above plus *500* μmoles of sucrose. Two mg of cells or the equivalent of protoplasts were used.

Components	Glycine-C^{14}	L-Alanine-1-C^{14}	D-Alanine-1-C^{14}
	mμmoles of C^{14} amino acid (in "pool")		
Cells, complete	8.65	15.8	18.7
Cells, complete minus glucose	1.0	2.15	1.0
Protoplasts, complete	12.7	23.7	47.7
Protoplasts, complete minus glucose	1.0	2.3	5.5

Results

Requirement for Energy Source.—When cells and protoplasts were starved before addition of radioactive substrates, a complete dependence on an energy source (glucose) for uptake of glycine, L-alanine, and D-alanine by cells and by protoplasts was shown (Table I). As expected for an energy-requiring reaction, uptake of amino acids did not occur in the complete system at 0°. The results are similar to those reported for these same amino acids in cells of *Lactobacillus casei* by Leach and Snell (1960).

Effect of Concentration of Sucrose on the Rate of Accumulation of Glycine-C^{14} by Protoplasts.—The extent of accumulation of glycine-C^{14} increases with the molarity of sucrose, tending toward but not reaching a plateau when the external concentration of sucrose is 0.5 M (Fig. 1). However, except at the lowest sucrose concentration, which may not be sufficient to stabilize the protoplasts, the initial rate of glycine uptake is the same.[1] Protoplasts were resuspended in 0.5 M sucrose in all subsequent experiments.

Effect of Concentration of Amino Acids.—At low external concentrations, accumulation of label by cells and protoplasts is proportional to concentration of the labeled amino acid, but at higher concentrations the

[1] These results are reminiscent of those of Holden (1959a,b), who found that the extent of accumulation of glutamate by vitamin B_6 deficient cells of *L. arabinosus* was increased toward that of the normal cells by sucrose, and interpreted this effect in terms of formation of a weakened cell wall in the deficient organisms and protection of the weakened cells from lysis by high sucrose concentrations.

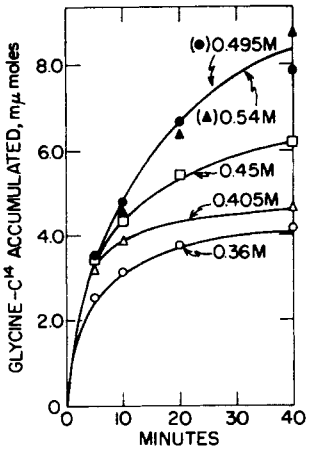

FIG. 1.—Effect of concentration of sucrose on the rate and extent of accumulation of glycine-C^{14} by protoplasts of *S. faecalis*. Protoplasts were resuspended in buffer A, *p*H 7.2, with different concentrations of sucrose as indicated. The suspension was incubated 10 minutes at 37°, and 5 minutes more with glucose, then glycine-C^{14} was added and samples were taken as indicated. The complete system contained per ml: 75 μmoles of K_2HPO_4, 50 μmoles of glucose, 100 mμmoles of glycine-C^{14}, protoplasts equivalent to 1.86 mg of original cells and sucrose as indicated.

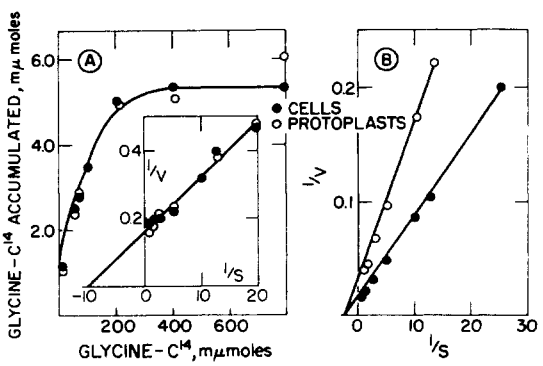

FIG. 2.—*A*, effect of concentration of glycine-C^{14} on the rate of its accumulation by cells (●) and protoplasts (○). Cells and protoplasts were held for 10 minutes in buffer A, *p*H 7.2, and 5 minutes more with glucose at 37°. Glycine-C^{14} as indicated was then added. Samples were analyzed after 5 minutes. The complete system is that described in Table I. *B*, effect of concentration of D-alanine-1-C^{14} on the rate of its accumulation by cells (●) and protoplasts (○). Conditions as in *A* except that the cells were not incubated 1 hour in buffer A–sucrose (without lysozyme) during the period the protoplasts were being prepared. In the reciprocal plots, S = glycine, M × 10^3; V = mμmoles of glycine or D-alanine accumulated per 5 minutes.

accumulating systems become saturated and accumulation is independent of the external concentration (Fig. 2*A*). The accumulating system for D-alanine requires substantially higher concentrations for saturation than do those for glycine or L-alanine (Fig. 2*B*). Saturating concentrations of the labeled amino acids for protoplasts and cells are essentially the same and approximately as follows (mμmoles per ml): glycine, 400; D-alanine, 1,000; L-alanine (data not shown), 100. The removal of the cell wall does not affect the affinities of the accumulating systems for any of these amino acids; the K_m values found for both intact cells and protoplasts are as follows: glycine, 0.8–1.0 × 10^{-4} M; L-alanine, 5 × 10^{-5} M; D-alanine, 4–6 × 10^{-4} M.

Rates of Accumulation and Incorporation.—The rates of accumulation and incorporation of radioactivity

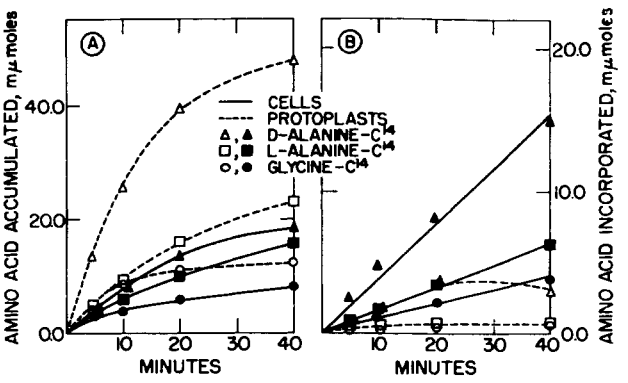

FIG. 3.—Rates of accumulation (*A*) and incorporation (*B*) of radioactive amino acids by cells and protoplasts. After incubation for 5 minutes with glucose at 37° the labeled amino acids were added. One ml samples were taken for analysis as indicated. Complete system as indicated in Table I.

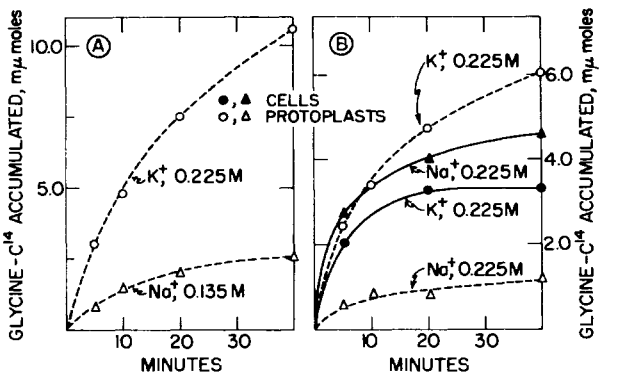

FIG. 4.—*A*, effect of potassium and sodium on the rate of accumulation of glycine-C^{14} by protoplasts. Protoplasts were prepared in buffer A, *p*H 6.2, plus 0.4 M sucrose. They were then suspended either in the same buffer plus 0.5 M sucrose, *p*H 7.2, or in sodium phosphate-sucrose buffer of the same *p*H and concentration. After 30 minutes at 37° glucose was added. Five minutes later glycine-C^{14} was added and aliquots were taken as indicated for analysis. Complete system as in Table I. *B*, effect of K^+ and Na^+ on the rate of accumulation of glycine-C^{14} by cells and protoplasts. Protoplasts were prepared in Tris buffer B, *p*H 6.2, plus 0.4 M sucrose, then resuspended in K^+ or Na^+ phosphate buffers as in *A*. Cells were treated in the same way, but without the addition of lysozyme. Other conditions as in *A*.

from labeled glycine, L-alanine, and D-alanine by cells and protoplasts is shown in Figure 3. As found previously for intact cells of *L. casei* (Leach and Snell, 1960), accumulation reaches near maximum in each case within approximately 20 minutes; incorporation continues for longer periods. Incorporation of amino acids—and especially of D-alanine, which occurs primarily in the cell wall (Snell *et al.*, 1955)—is markedly decreased in protoplasts as compared to the intact cells, and accumulation is correspondingly larger.

Nature of Accumulated Isotopic Materials.—Cells or protoplasts were incubated for 5 minutes at 37° with glucose and labeled glycine, L-alanine, or D-alanine. After centrifugation in the cold, each batch of cells or protoplasts was extracted with boiling water for 10 minutes and the extracted material was concentrated at reduced pressure, applied to paper, and chromatographed with pyridine–acetic acid–water (50:35:15) as solvent. All of the radioactivity of the extracts migrated as free glycine or alanine; no trace of other radioactive material was found.

Effect of K^+ and Na^+ on the Uptake of Glycine.—Potassium is required for optimal uptake of amino acids by Ehrlich ascites cells, and also for the stimulation of amino acid accumulation of these cells by pyridoxal (Riggs *et al.*, 1958). The energy-dependent penetration of sucrose in protoplasts of *S. faecalis* is also K^+-dependent, and is competitively inhibited by Na^+ (Abrams, 1960). Similarly K^+ increases both the extent (Table II) and the rate (Fig. 4*A*, *B*) of accumulation of glycine-C^{14} by protoplasts of *S. faecalis*; Na^+ inhibits. These effects of K^+ and Na^+ are not secondary consequences of changes in the rate of glycolysis as measured by lactic acid production (Table II), and do not occur in intact cells treated in the same way as the protoplasts in the above experiments (Fig. 4*B*).

TABLE II

EFFECT OF K^+ AND Na^+ ON THE ACCUMULATION OF GLYCINE-C^{14} BY PROTOPLASTS OF *S. faecalis*

Protoplasts were prepared in Tris buffer B, *p*H 6.2–0.4 M sucrose, and were resuspended in 0.075 M Na^+ or K^+ phosphate buffer, *p*H 7.2–0.5 M sucrose, with added KCl or NaCl to give the ion concentrations indicated. After 40 minutes at 37°, glucose and labeled glycine were added as in Table I; after 30 minutes 1-ml samples were taken for analysis of pool glycine-C^{14} and for lactic acid.

Addition to Protoplast Suspension	"Pool" Glycine-C^{14} (mμmoles)	Glucose Utilized (%)
0.235 M K^+	4.73	60.0
0.135 M K^+	4.43	72.5
0.135 M Tris-PO_4	3.84	56.0
0.135 M Na^+	2.55	66.0
0.235 M Na^+	0.69	25.0

Effect of Vitamin B_6.—Both the rate and extent of accumulation of glycine-C^{14} by protoplasts of *S. faecalis* were greatly enhanced by 1 mM pyridoxal; no such effect could be found with intact cells (Fig. 5). This stimulation was observable at the earliest time (2 minutes) tested, and occurred even though glycine was present at saturating concentrations (0.4 mM). When the external concentration of pyridoxal was raised to 4 mM a 100% stimulation of accumulation by protoplasts was observed; a small stimulation (approximately 20%) occurred under the same conditions in intact cells (Fig. 5*C*). Pyridoxine and pyridoxamine were very much less active in protoplasts than was pyridoxal (Fig. 5). Pyridoxal also increased greatly the rate of accumulation of L-alanine-1-C^{14} by protoplasts; in marked contrast, however, it was without effect on the rate of accumulation of D-alanine-1-C^{14} (Fig. 6).

These effects of pyridoxal occurred to an equal extent in protoplasts prepared from cells grown with an excess of vitamin B_6 and in protoplasts from vitamin B_6-low cells. As concluded from previous studies (Leach and Snell, 1960), the effect does not appear to be a physiological one; certainly it does not occur at physiological levels of the vitamin.

Protoplasts that have been suspended in sucrose swell on addition of glucose (Abrams, 1960). Pyridoxal did not increase the rate or extent of this metabolic swelling; occasionally some inhibition of it was observed.

Limited further tests of the specificity of the effect of pyridoxal were made. 5-Deoxypyridoxal, which has no vitamin activity but closely resembles pyridoxal phosphate in many properties (Metzler and Snell, 1955), stimulated glycine accumulation at low concentrations but inhibited accumulation strongly at higher

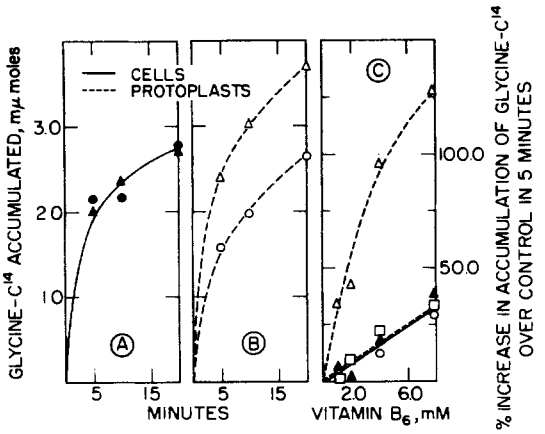

FIG. 5.—*A* and *B*, effect of pyridoxal on the rate of accumulation of glycine-C^{14} by cells (*A*) and protoplasts (*B*); ●, ○ no addition; ▲, △ pyridoxal (1.0 mM) added. The control curve for protoplasts (- -O- -) also represents protoplasts with pyridoxine (1 mM) or pyridoxamine (1 mM). Cells and protoplasts in buffer A, *p*H 7.2, were incubated 30 minutes at 37°, then pyridoxal (1 mM) was added. After 5 minutes glucose was added and 5 minutes later the radioactive glycine. The complete system contained per ml: 100 μmoles of glycine-C^{14}; 1.89 mg of cells (*A*) or protoplasts (*B*). Other conditions were as described in Table I. *C*, effect of concentration of pyridoxal (▲,△), pyridoxamine (□), and pyridoxine (○) on the rate of accumulation of glycine-C^{14} in cells (——) and protoplasts (– – –). Samples were taken 5 minutes after the addition of the radioactive glycine. The system contained 1.61 mg of cells or protoplasts. Other conditions as in *A* and *B*.

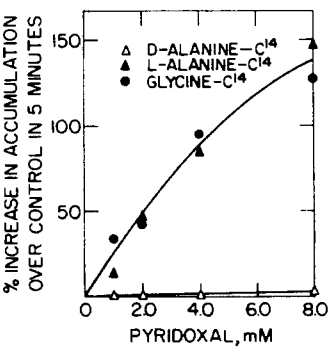

FIG. 6.—Comparative effects of pyridoxal on the rate of accumulation of glycine and D- or L-alanine by protoplasts. The concentration of radioactive amino acids was 0.1 mM. Protoplasts in a quantity equivalent to 2 mg of original cells per ml. Other conditions as in Figure 5.

concentrations. The inhibitory effect also was pronounced in intact cells (Fig. 7). 5-Deoxypyridoxine was without effect. At relatively high external concentrations, inhibition of accumulation of glycine-C^{14} by 5-deoxypyridoxal is competitive in nature (Fig. 7*B*).

4-Nitrosalicylaldehyde, which resembles pyridoxal in certain of its nonenzymatic catalytic effects, decreased the rate of accumulation of glycine-C^{14} by almost 50% at the lowest concentration tested (0.5 mM).

Effect of Unlabeled Amino Acids on the Accumulation of Glycine-C^{14}, L-Alanine-1-C^{14}, and D-Alanine-1-C^{14} by Cells and Protoplasts.—Although the accumulation rates differ for each of these labeled amino acids, a single transport system appears to be mainly involved in their accumulation. This conclusion is based on competition experiments, which show that unlabeled L-alanine decreases the rate of accumulation of both

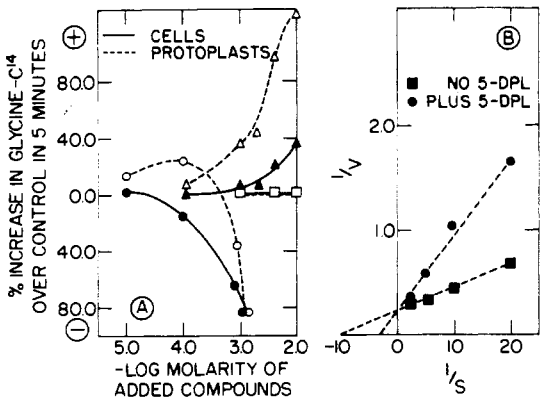

FIG. 7.—*A*, comparative effects of 5-deoxypyridoxal (●,○), 5-deoxypyridoxine (□), and pyridoxal (▲,△) on the rate of accumulation of glycine-C^{14} by cells and protoplasts of *S. faecalis*. Other conditions as in Figure 5. *B*, effect of 5-deoxypyridoxal (DPL) on the rate of accumulation of glycine-C^{14} by protoplasts of *S. faecalis*. Protoplasts were present in a quantity equivalent to 2 mg of original cells per ml. Aliquots were taken 6 minutes after the addition of glycine-C^{14}. Other conditions as in Figure 5.

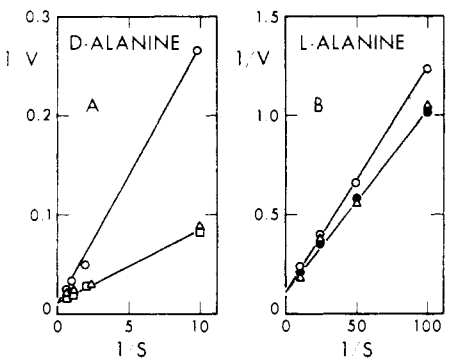

FIG. 8.—Effect of D- and L-cycloserine on the rate of accumulation of labeled D- or L-alanine by cells of *S. faecalis*. D- or L-cycloserine were added at the same time as the radioactive alanine. Aliquots (1 ml) were taken for analysis 6 minutes after the addition of the radioactive alanine. Cells 1.9 mg per ml. Other conditions as in Table I. A, D-alanine (□); D-alanine + 0.1 mM L-cycloserine (△); D-alanine + 0.1 mM D-cycloserine (○). *B*, L-alanine (●); L-alanine + 0.01 mM L-cycloserine (△); L-alanine + 0.01 mM D-cycloserine (○). S = D- or L-alanine, M × 10^3; V = mμmoles of D- or L-alanine accumulated in 6 minutes.

glycine-C^{14} and D-alanine-1-C^{14} by intact cells, and conversely, that unlabeled D-alanine decreases the rate of accumulation of L-alanine-1-C^{14} (Table III). Glycyl-L-alanine and L-alanylglycine both interfered strongly with the uptake of glycine-C^{14}. Entirely similar results were obtained with protoplasts.

These findings emphasize the extent to which transport systems differ in different organisms. In *L. casei*, the same transport system also accumulates D- and L-alanine; however, this system differs from that responsible for accumulation of glycine, and also from that which accumulates amino acids from glycyl-L-alanine and L-alanylglycine (Leach and Snell, 1960).

D-Cycloserine,[2] which causes protoplast formation in *E. coli* and accumulation of *N*-acylamino sugar in *S. aureus* (Ciak and Hahn, 1959), also prevents the normal incorporation of D-alanine into the cell wall of *S. faecalis* 9790 (Shockman, 1959). L-Cycloserine has

[2] We are indebted to Dr. H. Boyd Woodruff, Merck, Sharpe and Dohme, for samples of D- and L-cycloserine.

TABLE III

EFFECT OF UNLABELED AMINO ACIDS ON RATE OF ACCUMULATION OF RADIOACTIVE AMINO ACIDS BY CELLS

Complete system as in Table I. Rates were determined from the linear portion of the curves relating accumulation to time, usually during the first 5 minutes.

Addition	Accumulation (cpm per 5 min.)
Glycine-C^{14} (0.1 mM)	991
Glycine-C^{14} (0.1 mM) + L-alanine (0.1 mM)	373
D-Alanine-1-C^{14} (2 mM)	1250
D-Alanine-1-C^{14} (2 mM) + L-alanine (2 mM)	338
L-Alanine-1-C^{14} (0.1 mM)	450
L-Alanine-1-C^{14} (0.1 mM) + D-alanine (0.1 mM)	380
L-Alanine-1-C^{14} (0.1 mM) + D-alanine (0.5 mM)	356
L-Alanine-1-C^{14} (0.1 mM) + D-alanine (1.0 mM)	278

none of these effects. It was therefore of interest to compare the action of these compounds on an uptake system which acts on D-alanine, L-alanine, and glycine. As shown in Figure 8, D-cycloserine inhibits competitively accumulation of both D-alanine-1-C^{14} and L-alanine-1-C^{14}. The K_i values for D-cycloserine were 1×10^{-5} M and 1.4×10^{-5} M in the presence of D-alanine or of L-alanine respectively. The ratio, K_m/K_i, is 50 for D-alanine-1-C^{14} and 5 for L-alanine-1-C^{14}, which emphasizes the effectiveness of D-cycloserine as a competitor in the accumulation of D-alanine. L-Cycloserine has no significant inhibitory effect at concentrations similar to those of D-cycloserine (Fig. 8). Penicillin (1 μg per ml) did not inhibit accumulation of D-alanine-1-C^{14} by cells.

DISCUSSION

These experiments demonstrate that protoplasts of *S. faecalis*, like the intact cells, accumulate glycine, L-alanine, and D-alanine, apparently *via* a single transport system, either in free form or in a form that yields the free amino acids on extraction with hot water. Like other transport systems so far studied, this system requires an energy source, possesses a high temperature coefficient, and is saturated by low external concentrations of amino acids. The affinity of this transport system for these amino acids is not altered by removal of the cell wall. These results, like those of others (Mitchell and Moyle, 1956), indicate that the transport systems of the cell are located in the cell membrane.

Two major differences appear in the properties of this transport system, however, when the cell wall is removed. Firstly, the accumulation of amino acids is now stimulated by K^+ and inhibited by Na^+. Secondly, the accumulation of amino acids is greatly increased by high concentrations of pyridoxal. Neither effect is observed in intact cells of the same organism. Stimulation of amino acid uptake by K^+ and its inhibition by Na^+ has previously been observed in mammalian cells (Riggs *et al.*, 1958; Ricklis and Quastel, 1958); its basis is not fully understood. The suggestion that K^+ ion and ATP interact with structural elements of the membrane to change its conformation to one more favorable for operation of transport systems (Abrams *et al.*, 1960) is not inconsistent with our findings if one assumes that the rigid cell wall, when present, holds the membrane in the favored conformation. Alterna-

tively, intact cells may not lose and gain K^+ ion so readily as protoplasts. In either case one must postulate a change in properties of the membrane as a result of removal of the cell wall.

Similar considerations apply to the effects of pyridoxal, which are seen only with the protoplasts, and only when pyridoxal is present at unphysiologically high levels. In both the present work with *S. faecalis* and previous work with *L. casei* (Leach and Snell, 1960) and *L. arabinosus* (Holden, 1959a), intact cells have been found to accumulate amino acids at the same rate whether they are grossly deficient in vitamin B_6 or whether they are grown with an excess of this vitamin. In the present study, even the unphysiologically high levels effective with the protoplasts show relatively unimportant effects in the intact cells.[1] Again, the protoplasts resemble mammalian cells, in which similar effects of pyridoxal have been reported (Christensen, 1960). Pyridoxal and its phosphate are known not only to bind tightly to some proteins (*e.g.*, serum albumin) but also to affect their conformation, as indicated by their rotatory powers (Dempsey and Christensen, 1962). These compounds also exert nonspecific effects on the catalytic properties of certain other proteins (Nishimura *et al.*, 1962). It is possible that the effects of pyridoxal on amino acid absorption by protoplasts reflect changes in the conformation of the membrane induced by its presence perhaps similar to those changes reported for phlorizin, which binds to mammalian cell membranes (Rodriguez and Osler, 1958) and stimulates metabolic uptake of certain oligosaccharides by protoplasts of *S. faecalis* (Abrams, 1960). If, however, as our experiments indicate, the same single transport mechanism acts on glycine, L-alanine, and D-alanine in cells and protoplasts, then the differential effect of pyridoxal on uptake of D- and L-alanine by the protoplasts would indicate either (*a*) interaction of pyridoxal with a component of the transport system in such a manner as to effect a marked change in its optical specificity, or (*b*) interaction of pyridoxal with the amino acid with uptake of the "extra" amino acid as a complex with pyridoxal by an optically specific system which is different from that operative on the free amino acids and which is largely inoperative in the intact cells. The former mechanism would be consistent with a conformational change in the membrane; the latter would not require such a change, but does not exclude it. Additional possibilities also exist, and our data are insufficient to establish which possible explanation is most likely. The marked inhibition of uptake of glycine by 5-deoxypyridoxal, observed in both intact cells and protoplasts, cannot be interpreted with certainty as indicating a role of vitamin B_6 in the normal uptake of amino acids, since the concentration required is, again, rather high. The fact that this inhibition is competitive with substrate again suggests complex formation, this time between pyridoxal analog and amino acid, and that this complex is not readily absorbed by the cell.

The inhibition of uptake of D-alanine by D-cycloserine is not unexpected in view of the structural similarity of these compounds and the inhibition of several other reactions of D-alanine by this compound (Shockman, 1959; Strominger, 1962). Its inhibition of L-alanine uptake is the expected result if indeed a single uptake system accumulates both D- and L-alanine. It is not clear, however, why L-cycloserine does not also inhibit a system which has an even higher affinity for L-alanine than for D-alanine. The result suggests that more is involved than simple competition for a receptor site on the membrane.

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