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Isoleucine and Threonine Can Prolong Protein and Ribonucleic Acid Synthesis in Pyridoxine-Starved Mutants of *Escherichia coli* B

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Pyridoxineless mutants of *Escherichia coli* B stopped incorporation of nucleosides into trichloroacetic acid-insoluble material about 40 to 60 min after pyridoxine starvation was initiated, whereas incorporation of amino acids (measured the same way) slowed but did not stop for several hours. Both these incorporations and cell density were increased most effectively by the presence of either threonine or isoleucine. Arginine, glutamate, histidine, methionine, tryptophan, and tyrosine also caused significant but less dramatic increases. Inducibility of β-galactosidase continued beyond the point where nucleic acids appeared to stop their synthesis, suggesting that messenger ribonucleic acid synthesis continued beyond ribosomal ribonucleic acid synthesis. This inducibility was also increased by isoleucine and threonine. The overall results suggest that the threonine-isoleucine biosynthetic pathway is the most sensitive to starvation for pyridoxine.

The most likely principal function of pyridoxal phosphate in cultures of *Escherichia coli* growing exponentially in minimal medium is to serve as coenzyme in the biosynthesis of amino acids. In fact, pyridoxal phosphate occupies a rather unique place in metabolism, because the sequences of reactions by which all common amino acids in *E. coli* are synthesized (except for glutamate and proline) apparently require at least one enzyme that uses pyridoxal phosphate as a coenzyme (24). Considering these things, it would be reasonable to expect that pyridoxineless mutants, starved of pyridoxine, should behave with respect to protein and ribonucleic acid (RNA) synthesis in a manner similar to that described for amino acid auxotrophs starved for their required amino acids (8, 18).

The principal differences would be two. Firstly, starvation for coenzymes slows but does not stop cell growth until the cell density has nearly doubled (25). Secondly, starvation for pyridoxal phosphate should produce a kind of amino acid starvation in which the various amino acids become limiting at different rates. These rates would reflect the demand for each amino acid, their pool sizes, and the dissociation constant between pyridoxal phosphate and their appropriate biosynthetic enzymes. Presumably, growth stops when some one amino acid pool is exhausted and cannot be refilled.

It is the purpose of this report to describe some aspects of what happens to protein and RNA synthesis in pyridoxineless mutants starved for pyridoxine. This type of study has not been reported previously for *E. coli*. Reports of the effect of thiamine starvation in *Lactobacillus viridescens* have appeared (14, 16).

MATERIALS AND METHODS

Strains. *E. coli* B strains WG1468 (*pdx-200*, phage-resistant), WG1439 (*pdxH181*, phage-resistant), WG1473 (*pdx-204*, phage-resistant), and WG1027 (*pdxJ151*) were used. The allele *pdx-200* belongs to genetic linkage group I (3) represented at minute 46 on the chromosomal map drawn by Taylor (23). The allele *pdx-204* belongs to genetic linkage group II (2) represented at minute 1. Mutations in the other two strains have not been mapped but are known to be unlinked to each other and unlinked to those in group I or II (2). Three of the

strains were resistant to an unidentified virulent coliphage which appeared briefly in these laboratories 2 years ago. All of the other strains used have been described previously (2, 6, 7).

Media. Glucose minimal medium has been described (3). Vitamin B_6 was used in the pyridoxol form for all except group IV mutants. For these, pyridoxal was used. Both forms of the vitamin were at 6×10^{-7} M initial concentration. Lysis reagent was the sodium dodecyl sulfate, tris(hydroxymethyl)-aminomethane (Tris), ethylenediaminetetraacetate mixture of Brunschede and Bremer (1).

Radioisotope incorporation. The mutants in appropriately supplemented glucose minimal medium were grown overnight at 37 C with vigorous shaking. In the morning, a portion of the culture was centrifuged for 10 min at room temperature at 4,000 $\times$ *g*, and suspended in 50 ml of fresh identical medium to give an apparent absorbancy of 0.40 at 420 nm in 1-cm cuvettes. The culture was shaken at 37 C until the apparent absorbancy reached 1.25. A portion (33 ml) was centrifuged as above and suspended in 5 ml of fresh medium lacking glucose and pyridoxol but containing carrier amino acid or nucleoside (as appropriate). This was then added to 45 ml of identical medium to which had been added previously the radioactive amino acid or nucleoside, 0.5 ml of 20% glucose, and 30 nmoles of pyridoxol (if appropriate), and from which 100 μliters had been removed directly to 10 ml of scintillation fluid in order to measure total microcuries present at zero time. Likewise, before the bacteria were added, three 1-ml samples were removed as controls. Samples (1 ml) taken at intervals after addition of the bacteria, as well as the above controls, were added immediately to 1 ml of lysis reagent in tubes (13 by 100 mm) in a boiling-water bath, and both controls and bacterial samples were then treated as described by Brunschede and Bremer (1).

Determination of radioactivity and β-galactosidase activity. The procedures of Brunschede and Bremer were followed (1). Membrane filters (Millipore Corp.) from both controls and samples were placed in scintillation vials, dried overnight at room temperature, dissolved in 10 ml of Brays fluid and placed in a Beckman C5250 scintillation counter for measurement of counts per minute. Each sample was counted until 25,000 counts had been recorded; then 25 μliters of standard toluene (^{14}C or ^{3}H as appropriate) was added to the vial, and the vials were recounted. From individual efficiencies as determined by this internal standard, the disintegrations per minute were calculated. Net disintegrations per minute per sample were determined by subtracting the average value for the controls from that of the samples. β-Galactosidase inducibility was measured by the method of Pardee, Jacob, and Monod (20) by use of the assay derived by Kaempfer and Magasanik (15).

Relative growth measurements. The mutants tested were grown overnight at 37 C with vigorous shaking in 50 ml of glucose minimal medium containing vitamin B_6. Cultures of strain WG1145 always contained 500 μg of L-serine/ml in addition to all other materials. The overnight cultures were washed and diluted into 300 ml of identical medium. Shaking was resumed until a cell density of approximately 0.4 mg/ml was reached. The cells were collected, washed with 0.9% sodium chloride, and suspended in 1,200 ml of glucose-minimal medium lacking vitamin B_6. Portions of 50 ml were then dispensed into flasks containing 25 mg of the different amino acids, and growth, as measured by apparent absorbancy at 650 nm, was followed for 5 hr. For each strain, the increase in cell density during 4 hr of starvation for pyridoxine in the presence of amino acids was compared to the change seen in control flasks lacking amino acids.

Radioactive compounds. Uniformly labeled ^{14}C-L-leucine (260 mCi/mmole), *4,5-^{3}H*-L-leucine (55 Ci/mmole), uniformly labeled ^{14}C-L-lysine (101 mCi/mmole), *4,5-^{3}H*-lysine (250 mCi/mmole), *5-^{3}H*-uridine (280 Ci/mmole), and *8-^{3}H*-guanosine (7.6 Ci/mmole) were all obtained from New England Nuclear Corp.

Cell densities. For routine use, cell densities were determined in 18-mm culture tubes by comparing apparent absorbancy at 650 nm with a standard curve prepared with cultures whose dry weight per milliliter had been determined gravimetrically. For less dense cultures, such as those encountered in the β-galactosidase experiments, densities were determined from apparent absorbancy at 420 nm as measured in 1-cm cuvettes with a Cary model 14 double-beam spectrophotometer. An empirical curve relating apparent absorbancy at 420 nm to cell density was prepared by E. Sullivan. It agreed well with the curve of Koch and Deppe (17).

RESULTS

Protein and nucleic acid syntheses were measured as trichloroacetic acid-insoluble radioactivity. The isotope incorporation experiments were performed with three genetically distinct pyridoxineless mutants, with two different amino acids and two different nucleosides. This degree of variation was used for the following reasons. Firstly, the enzymatic defects in most pyridoxineless mutants have not been defined and therefore there cannot be at present a foolproof test that mutants classified phenotypically as Pdx are genotypically *pdx*. See, for example, the studies on "Km" mutants recently reported (10, 11, 13). Accordingly, the experiments were done with mutants from the three genetic classes which represent the majority of *pdx* mutants in *E. coli* B to establish the generality of the results. Secondly, there is no knowledge at present about possible involvement of particular amino acids or nucleosides in pyridoxine biosynthesis. Accordingly, two compounds of each class were chosen for these experiments in an effort to establish the generality of the results. In addition, all experiments were done at least

twice on general principles, with essentially identical results.

When pyridoxineless mutants were starved for pyridoxine, the rate of incorporation of amino acids into trichloroacetic acid-insoluble material decreased a short time after starvation began (Fig. 1-3). A similar but more dramatic effect was seen when incorporation of nucleosides was measured (Fig. 1-3). The cells recovered nearly normal incorporation of amino acids (Fig. 4) and nucleosides (Fig. 5) if vitamin B_6 was added to starving cultures.

It has been shown that pyridoxineless mutants starved of their pyridoxine decrease their growth over a period of 1 hr and gradually stop all net increases in cell mass (5). Figures 1-3 show that under similar conditions the incorporation of nucleosides into trichloroacetic acid-insoluble material also stopped about 40 to 60 min after pyridoxine starvation was started, whereas the incorporation of amino acids was reduced but did not show the rather abrupt stop observed in the nucleoside case.

The data in Fig. 1-5 suggest that the effects of pyridoxine starvation become noticeable after 40 to 60 min of pyridoxine starvation (generation time in this medium was 55 min). A similar time lag has been determined for the period before derepression of pyridoxine synthesis occurs (4). Accordingly, 40 min was assumed to be the time required for pyridoxine starvation to reduce the pyridoxal phosphate pool significantly.

To determine whether the internal concentration of any one particular amino acid was affected more than any other by pyridoxine starvation, the ability of individual amino acids to prolong incorporation of nucleosides and amino acids into trichloroacetic acid-insoluble material during pyridoxine starvation was measured. In essence, the experiments of Fig. 1-3 were repeated in the presence of individual amino acids, except that samples (in

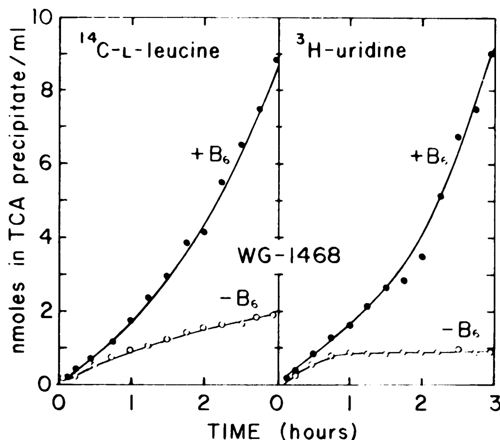

FIG. 1. *A measure of protein and nucleic acid synthesis in pyridoxine-starved and pyridoxine-fed cultures of strain WG1468. Pyridoxine was removed from exponentially growing cultures at time zero. Radioactivity in trichloroacetic acid-insoluble material present in lysed preparations of cells removed at 15-min intervals from both pyridoxine-fed ($+B_6$) and pyridoxine-starved ($-B_6$) cultures was measured. The radioactive material initially present in each experiment is indicated in the figure.*

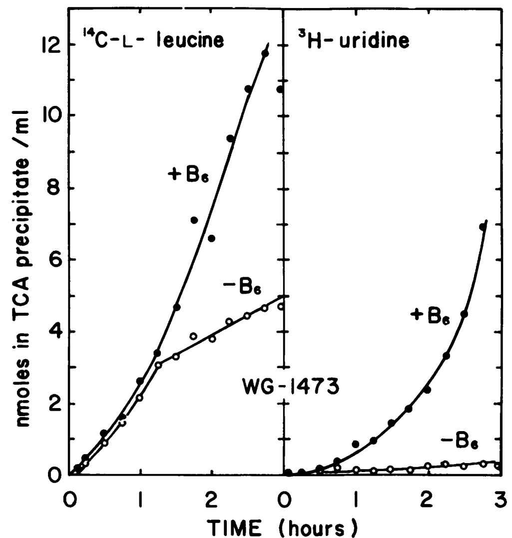

FIG. 2. *A measure of protein and nucleic acid synthesis in pyridoxine-starved and pyridoxine-fed cultures of strain WG1473. Details as in text and Fig. 1.*

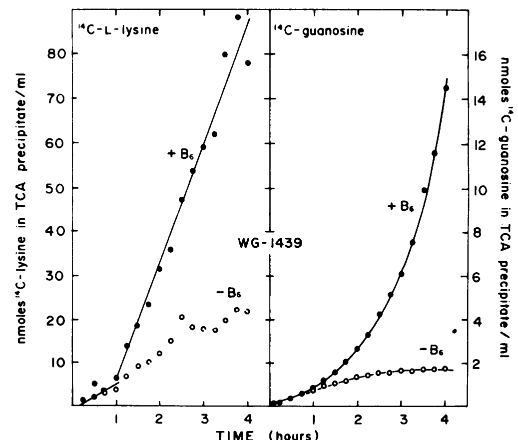

FIG. 3. *A measure of protein and nucleic acid synthesis in pyridoxine-starved and pyridoxine-fed cultures of strain WG1439. Details as in text and Fig. 1.*

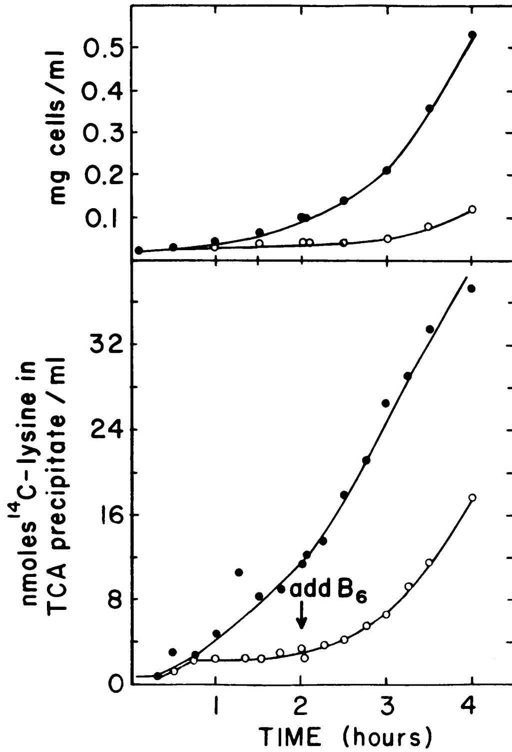

FIG. 4. *Effect upon lysine incorporation of the addition of pyridoxine to a pyridoxine-starved culture of strain WG1468. Details as in text and Fig. 1.* (●) *Pyridoxine-fed;* (O) *Pyridoxine-starved. Culture was made 6 × 10^{-7} M in pyridoxol at the time indicated by the arrow.*

triplicate) were withdrawn only at 0, 40, and 180 min. Table 1 shows the results of these experiments when incorporation of *4,5-^{3}H-leucine* was measured, and Table 2 shows the results when incorporation of ^{3}H-guanosine was measured. Both sets of data suggest that isoleucine and threonine were the most effective of the amino acids tested in allowing macromolecular synthesis to proceed during pyridoxine starvation. Several of the amino acids were then used in this same type of test but with another strain to test the generality of these observations (Table 3).

The generality of the stimulation by isoleucine and threonine of pyridoxine-starved pyridoxineless mutants was next tested by measuring the ability of the 18 amino acids to increase growth of pyridoxineless mutants of all types during pyridoxine starvation. The data in Table 4 show that representatives of all five genetic groups were stimulated to increased growth by threonine. In most cases, stimulation was also seen with isoleucine. Strains responding to both amino acids did not show any additive effect when both amino acids were tested simultaneously. Neither of these two amino acids had any noticeable effect upon growth of wild-type *E. coli* (WG1). Table 4 also shows that the amino acids glycine and methionine had a negative effect upon pyridoxine-starved mutants. This phenomenon will be described in a later report.

The continued incorporation of amino acids into trichloroacetic acid-insoluble material during pyridoxine starvation suggested that the cells might not have lost their ability to make meaningful protein during this time. To test whether inducible enzymes could be made during pyridoxine starvation, the inducibility of β-galactosidase during the course of pyridoxine starvation was measured by removing portions of the culture at several intervals, and incubating them for a short time with isopropyl thiogalactoside followed by assay for β-galactosidase activity (15, 20).

The results of these measurements on cultures simply starving for pyridoxine in the Tris-glycerol medium of Kaempfer and Maga-

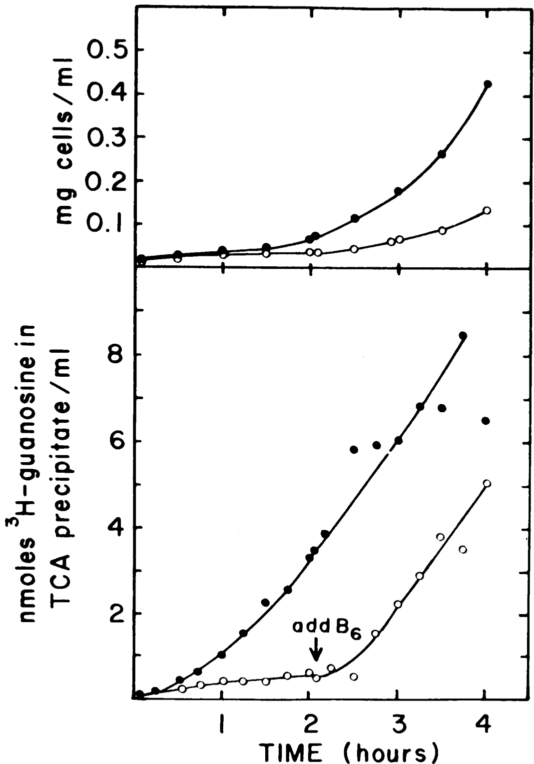

FIG. 5. *Effect upon guanosine incorporation of the addition of pyridoxine to a pyridoxine-starved culture of strain WG1468. Details as in Fig. 4.*

TABLE 1. *Effect of amino acids upon incorporation of 4,5-^{3}H-leucine by strain WG1468 during pyridoxine starvation*

Addition to medium	Amt incorporated[a]		Amt of cells[b]	
	In 0-40 min	In 40-180 min	At 40 min	At 180 min
Alanine	0.71	1.9	28	38
Arginine	0.75	2.0	29	39
Aspartic acid	1.2	1.9	31	38
Cysteine	0.10	0.50	26	30
Glutamic acid	0.95	1.6	30	39
Glycine	0.63	1.6	29	38
Histidine	0.78	2.0	31	41
Isoleucine	1.6	3.7	34	54
Leucine		0.6[c]	26	30
Lysine	0.93	1.7	31	39
Methionine	1.1	1.9	32	42
Phenylalanine	1.2	1.5	32	40
Proline	1.1	1.3	31	39
Serine	1.1	1.4	30	38
Threonine	1.8	4.8	36	37
Tryptophan	0.96	2.0	30	42
Tyrosine	0.92	2.3	32	40
Valine	1.1	1.6	31	40
None	1.1	1.3	30	40

[a] Expressed in nanomoles per milliliter (742 counts per min per nmole).
[b] Expressed in micrograms per milliliter. At time zero, the density was 28 μg/ml.
[c] Specific activity for this one experiment equal to 65 counts per min per nmole.

TABLE 2. *Effect of amino acids upon incorporation of ^{3}H-guanosine by strain WG1468 during pyridoxine starvation*

Addition to medium	Amt incorporated[a]		Amt of cells[b]	
	In 0-40 min	In 40-180 min	At 40 min	At 180 min
Alanine	0.68	0.66	30	42
Arginine	1.5	1.0	34	50
Aspartic acid	1.3	0.37	33	41
Cysteine	0.09	0.13	24	37
Glutamic acid	1.4	0.92	35	50
Glycine	1.0	0.69	34	42
Histidine	1.1	0.94	32	43
Isoleucine	1.6	2.5	33	60
Leucine	0.15	0.25	26	54
Lysine	1.3	0.81	34	60
Methionine	1.4	1.6	34	60
Phenylalanine	1.5	0.50	36	49
Proline	1.5	0.37	35	46
Serine	1.1	0.39	33	44
Threonine	1.8	2.2	36	42
Tryptophan	1.5	0.90	34	51
Tyrosine	1.3	0.84	36	46
Valine	0.85	0.50	32	42
None	1.4	0.47	36	48

[a] Expressed in nanomoles per milliliter (1,015 net counts per min per nmole).
[b] Expressed in micrograms per milliliter. At time zero, the density averaged 31 μg/ml.

sanik (15), as well as cultures starving for pyridoxine in the same medium containing isoleucine or threonine, are depicted in Fig. 6-8. The data show that inducibility of β-galactosidase diminished during pyridoxine starvation and that the presence of isoleucine in pyridoxine-starved cultures prolonged the duration and increased the extent of the inducibility. For two of the three mutants, threonine had a response similar to that of isoleucine; the third mutant (WG1468) was inhibited by threonine. The nature of the inhibition is not known but is under investigation. Similar results for these three mutants were observed when inducibility was measured in medium in which the Tris had been replaced by potassium phosphate. In all cases, the effect of isoleucine and threonine upon inducibility of β-galactosidase was eliminated by pyridoxine.

For several years, we have observed that cultures of most pyridoxineless mutants of *E. coli* B in late stages of pyridoxine starvation have a characteristic odor of diacetyl or acetoin very similar to that noticeable when isoleucine-valineless mutants are starved for their amino acids. A likely explanation of this may be re-

TABLE 3. *Effect of amino acids upon incorporation of 4,5-^{3}H-lysine by strain WG1027 during pyridoxine starvation*

Addition to medium	Amt incorporated[a]		Amt of cells[b]	
	In 0-40 min	In 40-180 min	At 40 min	At 180 min
Glycine	4.3	10.9	35	42
Isoleucine	5.0	16.2	39	83
Leucine	2.7	5.8	32	54
Lysine		5.7[c]	40	64
Methionine	4.4	11.8	36	58
Phenylalanine	5.4	7.3	40	66
Proline	5.4	6.6	40	66
Threonine	4.2	15.3	38	86
Valine	4.5	7.7	36	66
None	5.3	5.7	42	64

[a] Expressed in nanomoles per milliliter (207 net counts per min per nmole).
[b] Expressed in micrograms per milliliter. At time zero, the density was 33 μg/ml.
[c] Specific activity for this one experiment equal to 26 net counts per min per nmole.

lated to the conclusion drawn from these studies, namely, that threonine or isoleucine is the first amino acid to become limited by pyridoxine starvation.

DISCUSSION

Pyridoxal phosphate appears to serve in *E. coli* primarily as a necessary coenzyme in the

TABLE 4. *Ratio of cell density change after 4 hr of starvation for vitamin B_6 in the presence of additions to that in the absence of additions*

Addition	Pdx group and WG strain no.										
	WG1	I			II			III, WG1145	IV, WG1439	V	
		WG3	WG15	WG1468	WG73	WG25	WG1473			WG1027	WG1229
None	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alanine	0.9	1.2	1.0	0.9	1.0	1.0	1.0	1.3	0.9	1.0	1.6
Arginine	1.1	1.0	1.0	1.0	0.9	1.0	1.0	1.3	1.2	1.1	1.2
Aspartic acid	1.1	0.9	1.0	0.9	0.9	1.0	1.0	1.0	1.1	0.8	0.9
Cysteine	0.2	0.4	0.3	0.3	0.4	0.2				0.6	0.5
Glutamic acid	1.1	1.0	1.0	0.8	1.0	1.0	1.0	1.3	1.0	0.9	0.8
Glycine	0.9	0.3	0.5	0.7	0.5	0.3	0.2	1.1	0.7	0.0	0.2
Histidine	0.7	0.9	1.0	1.0	1.0	0.6	1.0	1.0	1.0	1.3	1.0
Isoleucine	1.0	1.2	1.3	1.3	1.2	0.5	1.3	1.7	0.7	1.5	0.8
Leucine	0.9	0.8	0.6	0.6	0.7	0.7	0.7	1.2	0.6	1.0	0.9
Lysine	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.2	1.0
Methionine	1.0	0.8	0.4	1.0	0.6	0.3	0.6	0.6	1.0	0.4	0.6
Phenylalanine	1.0	1.0	1.0	1.0	1.0	1.0	1.1	1.3	1.2	0.9	1.1
Proline	1.0	1.0	1.0	0.9	1.0	1.0	1.1	1.2	1.0	0.8	1.2
Serine	1.1	0.9	1.0	0.6	0.9	1.0	1.0	1.1	1.1	1.1	0.7
Threonine	1.0	1.3	1.4	0.7	1.1	1.1	1.3	1.7	1.2	1.5	1.0
Tryptophan	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.3	1.1	1.2	1.2
Tyrosine	0.8	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.0	0.9	1.2
Valine	1.0	0.9	1.0	1.0	0.9	1.0	0.9	1.3	0.9	1.0	1.0

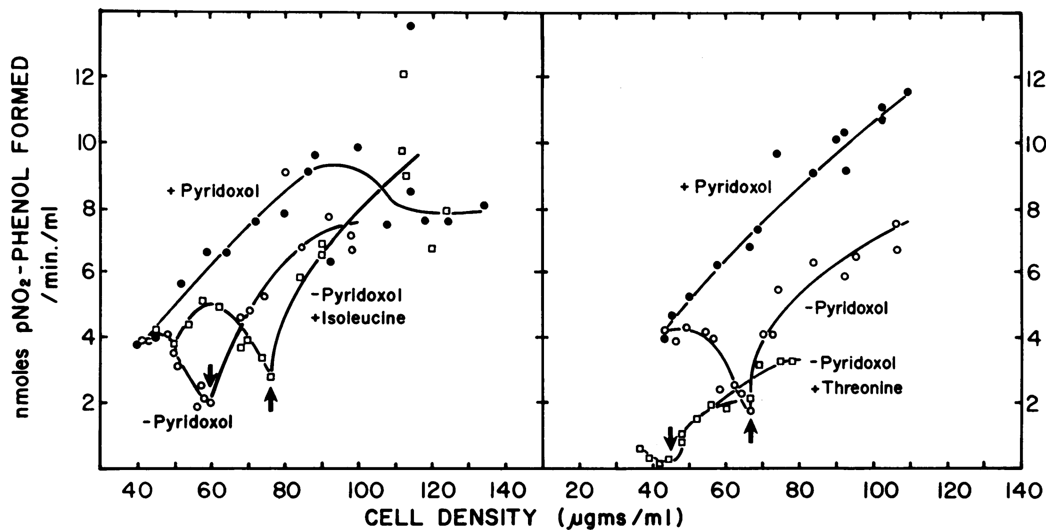

FIG. 6. *Effect of isoleucine and threonine upon inducibility of β-galactosidase during pyridoxine starvation of strain WG1468. A culture growing exponentially in the Tris-glycerol medium of Kaempfer and Magasanik and containing pyridoxol was centrifuged, washed in saline, and then split into three equal portions. One (closed symbols) was returned to fresh identical medium. The others (open symbols) were returned to medium lacking pyridoxine with (□) 100 µg of amino acid/ml or without (○) it. Samples were removed immediately and every 15 min thereafter for cell density and β-galactosidase assay. At the time indicated by the arrows (120 min), the starved cultures were made 6×10^{-7} M in pyridoxol.*

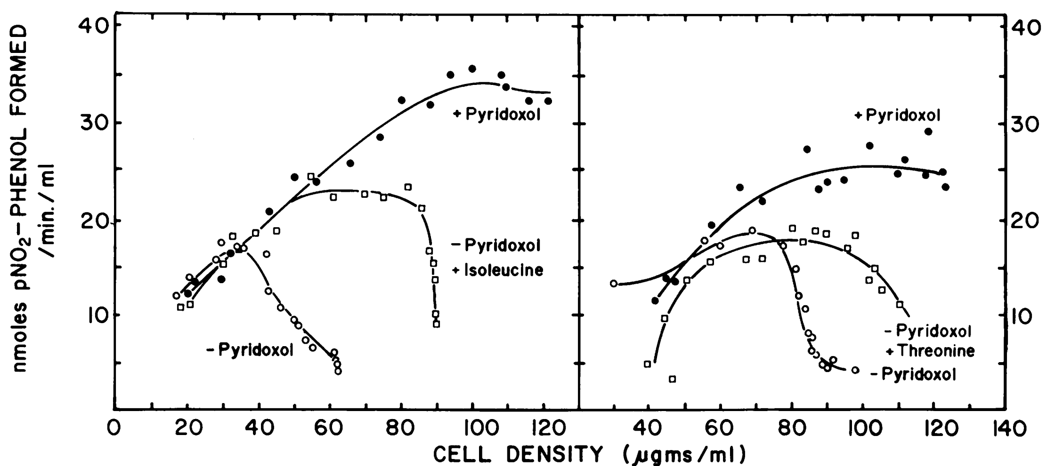

FIG. 7. *Effect of isoleucine and threonine upon inducibility of β-galactosidase during pyridoxine starvation of strain WG1473. Details as in Fig. 6 except that pyridoxol was not added back.*

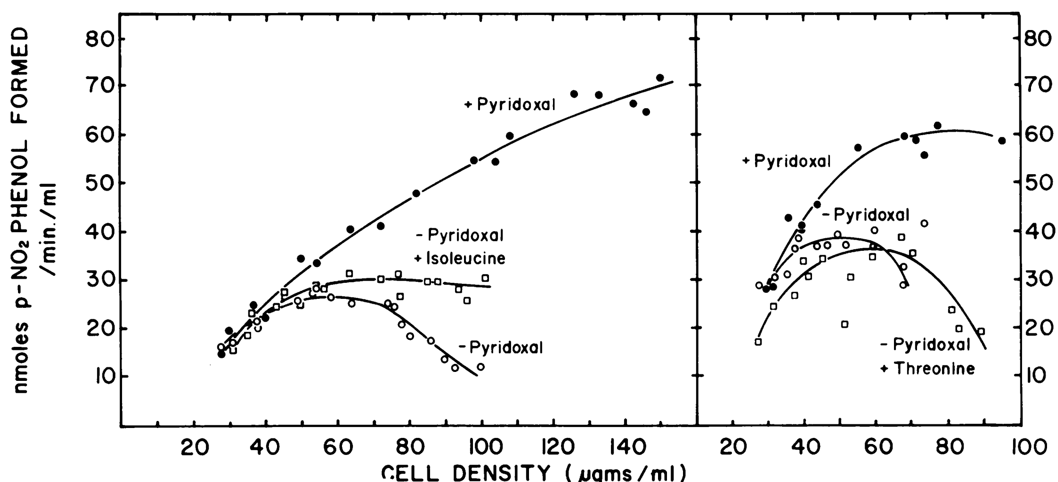

FIG. 8. *Effect of isoleucine and threonine upon inducibility of β-galactosidase during pyridoxine starvation of strain WG1439. Details as in Fig. 6 except that pyridoxol was not added back.*

biosynthesis of many amino acids. Accordingly, one would reasonably expect that starvation for pyridoxine would quite closely mimic starvation for amino acids and therefore that growth finally stops from a lack of one or more amino acids. One can propose the following sequence of events during pyridoxine starvation. At the instant starvation is initiated, the cell has adequate pools of both amino acids and pyridoxal phosphate. A few seconds later, as cell mass increases, newly made pyridoxal phosphate-requiring apoenzymes begin to deplete the pyridoxal phosphate pool by their conversion to holoenzymes. Once the pyridoxal phosphate pool is reduced, a new smaller pool may be maintained. This pool is fed by that pyridoxal phosphate which arises from the dissociation of holoenzymes with high dissociation constants, and is drained by the association of pyridoxal phosphate with newly made molecules of apoenzymes which have a very low dissociation constant for pyridoxal phosphate. This trend would presumably continue until one or more easily dissociated holoenzymes was completely in the apoenzyme form. Because apoenzymes are generally inactive, at some time near this point, growth of the cells would stop. The data presented in this study show that cell growth stops after 60 to 90 min of starvation for vitamin B_6.

Our results show that threonine and isoleucine both allow pyridoxine-starved cells to behave like pyridoxine-fed cells for a short while. The first and most obvious way in which

this could happen would be that discussed above, namely, that one or more of the enzymes in threonine and isoleucine biosynthesis has a high dissociation constant for pyridoxal phosphate and loses its pyridoxal phosphate during starvation for pyridoxine. There are three enzymes which could be affected, namely, threonine deaminase, transaminase B, and possibly threonine synthase. The last enzyme was shown to be unaffected in *E. coli* by pyridoxal phosphate (26), but has been established as a pyridoxal phosphate holoenzyme in *Neurospora* (9). It is also possible that there are valid explanations for the effect observed other than simple loss of coenzyme from holoenzyme. For example, it is possible and likely (21) that exogenously supplied threonine causes the induction of a degradative enzyme system which converts a portion of the exogenously supplied threonine to products other than isoleucine. One of these metabolic products might then be used to synthesize some vitamin B_6. Alternatively, the threonine could counteract a growth inhibitor which is accumulating under conditions of vitamin B_6 starvation. To keep speculations to a minimum, we will limit this discussion to the first case considered above without ruling out the other possibilities.

When threonine is in short supply because threonine synthetase is largely in apoenzyme form, the addition of external threonine would supply both the threonine and isoleucine that the cell needs. Isoleucine would, by serving as the feedback inhibitor of threonine deaminase, exert its effect by preventing what little threonine was being made from being converted to isoleucine. If instead threonine deaminase were the first enzyme to lose its pyridoxal phosphate during pyridoxine starvation, then growth in pyridoxine-starved cells would in effect be stopping because of a lack of isoleucine. In this case, the addition of threonine to the medium might allow growth by increasing the size of the threonine pool. Then by mass-action effect, enough threonine might be forced through the partially active deaminase to satisfy the isoleucine requirement. The third possibility, namely, that transaminase B is the first enzyme to lose its pyridoxal phosphate during pyridoxine starvation, would be explained in essentially the same manner as in the previous case. The lack of isoleucine would allow enough threonine to be deaminated to eventually provide a pool of α-keto-β-methyl valerate large enough to increase the rate of its conversion into isoleucine to adequate levels. At present, we have no data to support conclusively any one of these three enzymes as the first to lose its pyridoxal phosphate.

If, instead of the above, the results are considered in light of the model for control of the isoleucine-valine operon in *Salmonella typhimurium* presented by Hatfield and Burns (12), certain useful predictions seem possible. In their model, threonine deaminase exists in four different tetrameric forms with widely differing biological functions. An apotetramer (A) is reversibly converted to an immature holotetramer (B) in the presence of pyridoxal phosphate. B, in the presence of leucyl-transfer-RNA^{leu}, is reversibly converted to the holorepressor (C) of the isoleucine valine operon. B is irreversibly converted to the native threonine deaminase form (D) by isoleucine, valine, or threonine. Conversion of B to D is prevented by isoleucine plus valine or isoleucine plus threonine (12). Two simple cases based on this model are considered below.

Case 1. Transaminase B is effectively the first and only enzyme converted to apoenzyme by pyridoxine starvation and threonine deaminase is normal. In this case, the cell should primarily lack isoleucine. (Valine would be less limiting because a second transaminase for valine is known [22].) The response of the cell should be to derepress the isoleucine-valine operon through conversion of form B to D. Synthesis of α-keto-β-methyl valerate and α-keto isovalerate should then proceed rapidly, and they and their precursors would be expected to accumulate, giving rise to the observed odor of acyloins and their derivatives. Feeding isoleucine and valine (or isoleucine alone if the valine supplied by the second transaminase is adequate) should repress the operon by blocking conversion of B to D, thereby increasing conversion of B to C.

Case 2. Threonine deaminase is effectively the first and only enzyme dissociated from its pyridoxal phosphate by pyridoxine starvation and transaminase B is normal. In this case, the cell should lack only isoleucine. The response of the cell again should be the derepression of the isoleucine-valine operon, but this time by conversion of form B to A. In this case, the only acyloin-type intermediates present would be the precursors of valine, and these might not accumulate because the functional transaminase B would convert them to valine. Also, in this case, the feeding of isoleucine would not repress the operon because most of the threonine deaminase would be in the A form, which is not convertible to the C or holorepressor form in the absence of pyridoxal phosphate. In fact, in this case the operon might even derepress in the constant presence of isoleucine. This would allow case 1 and case 2 to

be distinguished easily. Experiments are in progress to test these possibilities.

Certain other points presented in the data bear further consideration. Apparent discrepancies can be found, for example, between the extent of growth of strains in Tables 1, 2, and 3 and those listed in Table 4. Two causes are likely. First, the data in Table 4 are averages of several experiments, whereas the data in the other tables are from single experiments. Second, the data in Table 4 were all obtained at 4 hr and the other data at 3 hr. This is a significant time difference for glycine and methionine and a trivial difference for others, because, after approximately 2 hr of pyridoxine starvation, lysis of starved cells by the former amino acids frequently begins (Dempsey, *in preparation*).

The experiments measuring β-galactosidase inducibility during pyridoxine starvation also tested whether the cessation of RNA synthesis seen during pyridoxine starvation included a cessation of messenger RNA synthesis. Figure 1 shows that strain WG1468 appears to stop RNA synthesis after 1 hr, and Fig. 6 shows that β-galactosidase inducibility in this strain does continue longer than this but decreases rapidly during the second hour. Figure 2 shows an apparent stoppage of RNA synthesis at a very early time in pyridoxine-starved cultures of strain WG1473, whereas Fig. 7 shows that β-galactosidase inducibility continues to increase for 1 hr before falling rapidly during the second hour. Nucleic acid synthesis continues for 2 hr after pyridoxal starvation of strain WG1439 (Fig. 3), and the inducibility of β-galactosidase increases during this time and does not begin to fall until the third hour of pyridoxine starvation (Fig. 8). We conclude from all of these results that messenger RNA can still be made by pyridoxine-starved *E. coli* cultures which have reached the point where there is little net synthesis of RNA.

The gradual fall of β-galactosidase inducibility in Fig. 6, 7, and 8 may be the result of depletion of all amino acid pools to the point where protein synthesis is reduced, or it may be the result of an increasingly effective catabolite repression of this enzyme by the gradually increasing amino acid deprivation caused by pyridoxine starvation (19). We have no firm data to distinguish between these alternatives, but in preliminary experiments we have observed apparent derepression of anabolic enzymes in strain WG1439 after 5 hr of pyridoxine starvation (Dempsey, *unpublished data*) and thus we favor the second of the two alternatives above.

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LITERATURE CITED

1. Brunschede, H., and H. Bremer. 1969. Protein synthesis in *Escherichia coli* after irradiation with ultraviolet light. J. Mol. Biol. **41**:25–38.
2. Dempsey, W. B. 1969. Characterization of pyridoxine auxotrophs of *Escherichia coli:* P1 transduction. J. Bacteriol. **97**:1403–1410.
3. Dempsey, W. B. 1969. Characterization of pyridoxine auxotrophs of *Escherichia coli:* chromosomal position of linkage group I. J. Bacteriol. **100**:295–300.
4. Dempsey, W. B. 1971. Control of vitamin B_6 biosynthesis in *Escherichia coli*. J. Bacteriol. **108**:415–421.
5. Dempsey, W. B. 1971. Role of vitamin B_6 biosynthetic rate in the study of vitamin B_6 synthesis in *Escherichia coli*. J. Bacteriol. **108**:1001–1007.
6. Dempsey, W. B. 1972. *Escherichia coli* mutants that require either pyridoxine or alanine. J. Bacteriol. **111**: 838–840.
7. Dempsey, W. B., and H. Itoh. 1970. Characterization of pyridoxine auxotrophs of *Escherichia coli*: serine and PdxF mutants. J. Bacteriol. **104**:658–667.
8. Edlin, G., and G. S. Stent. 1968. Synthesis of a specific messenger RNA during amino acid starvation of *Escherichia coli*. J. Mol. Biol. **37**:257–268.
9. Flavin, M., and C. Slaughter. 1962. Purification and properties of threonine synthetase of *Neurospora*. J. Biol. Chem. **235**:1103–1108.
10. Grimminger, H., and F. Lingens. 1969. Alternate requirement for pyridoxine or isoleucine in mutants of *Escherichia coli*. FEBS Lett. **5**:225–226.
11. Guirard, B. M., B. N. Ames, and E. E. Snell. 1971. *Salmonella typhimurium* mutants with alternate requirements for vitamin B_6 or isoleucine. J. Bacteriol. **108**: 359–363.
12. Hatfield, G. W., and R. O. Burns. 1970. Specific binding of leucyl transfer RNA to an immature form of L-threonine deaminase: its implication in repression. Proc. Nat. Acad. Sci. U.S.A. **66**:1027–1035.
13. Henderson, G. B., and E. E. Snell. 1971. Vitamin B_6-responsive histidine deficiency in mutants of *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U.S.A. **68**: 2903–2907.
14. Julien, J., H. Kersten, and B. Hess. 1969. Action of thiamine on protein and nucleic acid metabolism. II. Ribosome function and properties. Hoppe-Seyler Z. Physiol. Chem. **350**:1635–1646.
15. Kaempfer, R. O. R., and B. Magasanik. 1967. Effect of infection with T-even phage on the inducible synthesis of β-galactosidase in *Escherichia coli*. J. Mol. Biol. **27**:453–468.
16. Kersten, H., K. H. Averkamp, W. Braatz, P. Greif, W. Kersten, and B. Hess. 1969. Action of thiamine on protein and nucleic acid metabolism. I. Synthesis of ribosomes and messenger RNA during recovery from thiamine starvation in Lactobacillus viridescens. Hoppe Seyler Z. Physiol. Chem. **349**:1619–1634.
17. Koch, A. L., and C. S. Deppe. 1971. *In vivo* assay of pro-

tein synthesizing capacity of *Escherichia coli* from slowly growing chemostat cultures. J. Mol. Biol. **55**: 549-562.

18. Lavalle, R. and G. DeHauwer. 1968. Messenger RNA synthesis during amino acid starvation in *Escherichia coli*. J. Mol. Biol. **37**:269-288.
19. Magasanik, B. 1970. Glucose effects: inducer exclusion and repression, p. 189-219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, New York.
20. Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**:165-178.
21. Roberts, R. B., P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. 1955. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Wash. Publ. 607.
22. Rudman, D., and A. Meister. 1953. Transamination in *Escherichia coli*. J. Biol. Chem. **200**:591-604.
23. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
24. Umbarger, E., and B. D. Davis. 1962. Pathways of amino acid biosynthesis, p. 167-251. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 3. Academic Press Inc., New York.
25. Wilson, A. C., and A. B. Pardee. 1962. Regulation of flavin synthesis by *Escherichia coli*. J. Gen. Microbiol. **28**:283-303.
26. Wormser, E. H., and A. B. Pardee. 1958. Regulation of threonine biosynthesis in *Escherichia coli*. Arch. Biochem. Biophys. **78**:416-432.