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# Characterization of Pyridoxine Auxotrophs of *Escherichia coli*: P1 Transduction

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Pyridoxine mutants of *Escherichia coli* B, previously divided into a minimum of six groups by cross-feeding tests, were characterized by transduction studies performed with phage P1bt. The results of these studies allowed division of pyridoxine mutants into five unlinked groups and set the minimum number of enzymes between pyridoxal phosphate and a metabolite common to other pathways at six or seven, with the probable maximum at ten. One group was shown to be linked to *thr*, *leu*, and *pyrA*.

Vitamin $B_6$ auxotrophs of *Escherichia coli* B have been characterized primarily by cross-feeding tests (5). These tests, although convenient and reproducible, are more subject to interpretative error than other syntrophism tests, because the extent and intensity of the feeding observed is often vanishingly small. In addition, certain ambiguities exist in the published test results which do not appear resolvable by cross-feeding procedures. The need for a different and less ambiguous method to characterize the auxotrophs led to the experiments reported here.

The nutritional tests were originally interpreted as showing that pyridoxine auxotrophs of *E. coli* were distributed among a maximum of nine phenotypes. The first tested mutant of each of these nine types was termed the prototype mutant; the bulk of the work presented here is concerned with these prototypes. One of the prototypes, WG2 (formerly B-$B_6$-2), has been shown to be blocked in pyridoxol 5′-phosphate oxidase (4), but the others have not been characterized to this extent. In our experiments, phage P1bt grown on each of the prototypes was used to infect each of the other prototypes. The number of transductants obtained was compared to those from experiments in which P1bt grown on wild-type *E. coli* B was used. On the basis of these experiments, the prototype mutants have now been divided into five genetically unliked groups.

## MATERIALS AND METHODS

**Organisms.** All pyridoxine (pdx) mutants (3) of *E. coli* B were isolated in this laboratory either as described previously (5) or by a new procedure (strain no. WG1000 and higher; W. B. Dempsey, *in preparation*). The *thr*$^-$, *leu*$^-$, *pyrA* (determining growth requirement for both arginine and uracil) mutants were isolated by penicillin enrichment after NTG mutagenesis (1). WG3001 and WG3002, both *lac*$^-$ Hfr Hayes derivatives, were gifts of W. A. Newton. The organisms used in transductions are described in Table 1.

**Media.** Minimal medium was 0.063 M potassium phosphate, *p*H 7.0, which was 0.4 mM in $MgSO_4$, 0.01 mM in $FeSO_4$, and 15 mM in $(NH_4)_2SO_4$. Lactose and glucose were autoclaved separately and added to a final concentration of 0.2%.

Because mutants WG2 and WG533 specifically require pyridoxal (4), and because this compound is virtually absent even in rich media, it was necessary to add pyridoxal, final concentration $6 \times 10^{-7}$M, to all media used to grow these strains. For convenience, this compound was also routinely added to LB medium, MacConkey agar, Tryptic Soy agar, and lactose minimal agar.

Try-Mg medium was composed of 1% Tryptone (Difco), 1% NaCl, and 0.2% $MgCl_2 \cdot 6H_2O$; it was used without *p*H adjustment.

LB medium was the L broth of Bertani (2) used at *p*H 7.4. $CaCl_2$ (final concentration $2.5 \times 10^{-3}$M) was added only when phage adsorption was to be facilitated.

Soft agar used in titering was that described by Lennox (11), but at *p*H 7.4. SAH was soft agar, with agar reduced to 0.39%. LA medium was LB medium made 1% in agar. ML medium was the L broth described by Lennox (11), used at *p*H 7.4 with 1.7% agar.

Lactose tetrazolium agar was 2.55% Antibiotic Medium 2 (Difco), made 0.005% in 2,3,5 triphenyltetrazolium chloride, and autoclaved at 112 C for 20 min. Sterile lactose (final concentration 0.6%) was added after autoclaving.

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TABLE 1. *Description of E. coli strains used*

| Strain | No. | Old name | Genotype |
|---|---|---|---|
| B | WG1 | — | Wild type |
| | WG2 | B-$B_6$-2 | *pdxH2* |
| | WG3 | B-$B_6$-3 | *pdxB3* |
| | WG5 | B-$B_6$-5 | *pdxF5* |
| | WG25 | B-$B_6$-25 | *pdxG25* |
| | WG53 | B-$B_6$-53 | *pdxG53* |
| | WG73 | B-$B_6$-73 | *pdxE73* |
| | WG139 | B-$B_6$-$\mu$ | *pdxC139* |
| | WG15 | B-$B_6$-15 | *pdxD15* |
| | WG140 | B-$B_6$-1B | *pdxC140* |
| | WG571 | — | *thr*$^-$ |
| | WG620 | — | *pyrA*$^-$ |
| | WG1001 | — | *leu*$^-$ |
| B × K-12 hybrids | WG532 | — | *pdxF5, lac*$^-$, *str*$^r$ |
| | WG534 | — | *pdxG25, lac*$^-$, *str*$^r$ |
| | WG531 | — | *pdxG53, lac*$^-$, *str*$^r$ |
| | WG573 | — | *pdxE73, lac*$^-$, *str*$^r$ |
| | WG535 | — | *pdxB3, lac*$^-$, *str*$^r$ |
| | WG536 | — | *pdxD15, lac*$^-$, *str*$^r$ |
| | WG533 | — | *pdxH2, lac*$^-$, *str*$^r$ |
| | WG538 | — | *pdxC139, lac*$^-$, *str*$^r$ |
| | WG537 | — | *pdxC140, lac*$^-$, *str*$^r$ |
| K-12 | WG3001 | NG750 | *lac*$^-$ Hfr |
| | WG3002 | 3000×84 | *lac*$^-$ Hfr |

MacConkey agar and Tryptic Soy agar were commercial preparations. Streptomycin was used at 240 $\mu$g/ml.

**Preparation of lactose-negative pyridoxine mutants.** Streptomycin-resistant colonies of each prototype were isolated from ML medium plates, containing 240 $\mu$g of streptomycin sulfate per ml, on which $10^{11}$ cells freshly grown in LB medium had been spread. Mutants were purified by two consecutive single-colony isolations on the same agar. Introduction of *lac*$^-$ was accomplished by conjugation with WG3001. For this, each of the streptomycin-resistant prototypes was grown to 2 to 4 × $10^8$ cells per ml in 10 ml of LB medium at 37 C. Then, 2 × $10^8$ to 4 × $10^8$ cells of WG3001 actively growing in LB medium were added and mixed; the mixtures were allowed to incubate without shaking at 37 C for 120 to 140 min. The mixtures were diluted 1:$10^4$ in saline, and several 0.1-ml samples from each mixture were spread on lactose tetrazolium agar plates. An average of one lactose-negative colony was found per plate. The lactose-negative colonies were purified by two consecutive single-colony isolations on MacConkey agar containing streptomycin. The *lac*$^-$ allele in each strain was tested for identity with that of WG3001 by mating WG3001 1:1 with the *lac*$^-$ *E. coli* B strains and looking for Lac$^+$ recombinants among $10^8$ cells plated on lactose minimal agar containing streptomycin. With one exception, *lac*$^-$ strains which showed no Lac$^+$ recombinants were found for all strains used here. WG531 was the single exception; it readily yielded recombinants with WG3001 but only a few with WG3002.

**Preparation of phage stocks.** An initial stock of phage P1bt grown on *E. coli* B/r by R. B. Helling was obtained from L. C. Bertani. A single randomly selected plaque resulting from the infection of wild-type *E. coli* B with this phage stock was picked and used to infect *E. coli* B again. A randomly selected plaque from this second infection was picked and used according to the method of Swanstrom and Adams (14) to prepare the stock of phage grown on wild-type *E. coli* B from which all other stocks were prepared.

All phage stocks used were prepared by infecting the chosen strain with phage grown on wild-type *E. coli* B and then using these first-cycle phage to infect the chosen strain again. These second-cycle phage were used for our experiments. Phage stocks were routinely concentrated and purified by alternate low- and high-speed centrifugations and were stored at 5 C in 2% ammonium acetate at concentrations of 5 × $10^{10}$ to $10^{12}$ plaque forming units (PFU)/ml.

Some of the procedures for using P1bt phage with the *E. coli* B strain of this study were adapted from those of Bertani (2), Luria et al. (12), Lennox (11), Gross and Englesberg (6), and Helling (8). The methods below are those found empirically to give, per phage, the highest yields and greatest numbers of transductants with this particular strain of *E. coli* B.

Comparative studies with *E. coli* B/r (supplied by R. B. Helling) showed a 2- to 100-fold difference in phage yield between the two strains, depending upon which conditions were employed. In every case, *E. coli* B/r gave a higher yield. In transductions, the system described here was, on the average, several times less efficient than that described by Gross and Englesberg (6) for *E. coli* B/r.

The most efficient preparation method for P1bt on *E. coli* B was as follows. A 1-ml amount of an overnight culture of *E. coli* B grown aerobically at 37 C in LB medium was added to 30 ml of melted SAH at 48 C. Then 3 × $10^8$ P1bt phage were added in 1 ml of 2% ammonium acetate, and the solution was poured on a 500-cm$^2$ surface of fresh LA medium containing $CaCl_2$, final concentration 2 × $10^{-3}$ M, and incubated 5 hr at 37 C. Harvesting was done according to Helling (8). The average yield was 5 × $10^{11}$ PFU; an occasional yield reached $10^{12}$ PFU.

Phage stocks prepared on prototype mutants were then used to attempt to transduce the parental bacterial strain to Pdx$^+$. Only those stocks which were unable to give Pdx$^+$ transductants in this test were used further. Stocks grown on the rapidly reverting mutant WG5 (formerly B-$B_6$-5) frequently gave Pdx$^+$ colonies in this test. Phage stocks were prepared on WG5 by sequential steps of lysis of freshly isolated clones of this mutant until a lysate was obtained which failed to produce Pdx$^+$ recombinants.

**Transduction method.** Transduction was done in 2% ammonium acetate (R. B. Helling, *personal communication*). For this, cells were grown overnight in standing LB medium, subcultured 1:15 into 30 ml of LB medium in tubes (25 by 150 mm), and grown in still culture at 37 C to a cell density of 4.5 × $10^8$/ml.

The entire culture was centrifuged at 4,000 × *g* for 10 min at room temperature; the cells were suspended in 2 ml of 2% ammonium acetate at 37 C, and 0.35 ml of 0.1 M $MgCl_2$ at 37 C was added. Portions (1 ml) were then put into separate tubes; 1 ml of 2% ammonium acetate was added to one tube for a control, and $3 \times 10^9$ phage in 1 ml of 2% ammonium acetate were added to the other tube. Then 0.2 ml of 0.5 M $CaCl_2$ at 37 C was added to both tubes and mixed. After 5 min at 37 C, both tubes were centrifuged as above; the cells were suspended in 0.7 ml of Try-Mg at 37 C, and a portion from each tube was spread on selective agars prewarmed to 37 C. This method gave a mean ratio of pyridoxine-independent transductants to phage of $3 \times 10^{-6}$. (When LB medium replaced ammonium acetate, the ratio varied from $5 \times 10^{-7}$ to $10^{-9}$. Similar low ratios were found when Try-Mg was replaced with 0.9% saline, $MgCl_2$ alone, or with LB medium.) This method was used in all transductions of bacteria which contained a *lac*$^-$ allele. The selective agars used in this case were glucose-minimal and lactose-minimal containing pyridoxal. In these experiments, an average of 90% of phage was adsorbed.

All transductions with a single phage stock were performed on all nine prototype mutants at the same time to keep age and concentration of phage a constant. This ideal could not be consistently realized with WG532 because of the high reversion rate of the *pdxC5* allele. For transductions of mutants containing this allele, the usual procedure was simply repeated daily with cultures derived from freshly reisolated colonies until cultures were found in which reversion(s) had not occurred or had occurred sufficiently late in growth to keep the revertant frequencies at a reasonable level. Whereas all other transductants were counted after 48 hr at 37 C, transductants of WG532 had to be counted after 20 hr, again because the rate of spontaneous mutation to Pdx$^+$ made 48-hr plates difficult to count.

Transduction of organisms which did not contain a *lac*$^-$ allele was generally done as described above, but $3 \times 10^9$ phage were added in 0.05 ml instead of in 1.0 ml, and 0.05 ml of 2% ammonium acetate was added to the control. For transductions involving two types of phage, the above procedure was followed except that the cells were suspended in 4 ml of ammonium acetate after the initial centrifugation, and 0.7 ml of 0.1 M $MgCl_2$ was added. Samples (1 ml) of this mixture were then infected separately with each type of phage, and 1 ml was used for the control. To test the linkage of *pdx* to *thr*, *leu*, and *pyrA*, Thr$^+$, Leu$^+$, and Pyr$^+$ transductants on glucose-minimal plates containing pyridoxal, final concentration $6 \times 10^{-7}$ M, were put on identical plates, incubated, and then used to inoculate the same type of agar plate and a glucose-minimal agar plate by the replica-plate technique. Those transductants unable to grow without pyridoxal were scored.

**Miscellaneous.** Phage were titered with ATCC 11126 (*Shigella* sp.), by using the soft-agar method of Swanstrom and Adams (14). Bacteria were counted on Tryptic Soy agar plates. Bacteria were maintained in oil-covered Tryptic Soy agar stabs at room temperature and were routinely reisolated before use.

## RESULTS

Initial experiments in which the prototype mutants were transduced with phage grown on wild-type *E. coli* B (WG1) showed that significant differences existed between strains in the number of transductants obtained per $10^8$ phage (Table 2). This suggested that more meaningful data could be obtained if a common, unlinked mutant allele were present in all recipient strains, the transduction of which to wild phenotype could serve as an internal control for any differences in (i) the phage receptivity of the different strains of bacteria, (ii) the transducing potency of phage preparations, (iii) rates of recombination, and (iv) physical and chemical factors affecting the transduction procedure. Accordingly, the *lac*$^-$ allele of WG3001 was introduced into all but one prototype strain by conjugation.

The products of these matings were strain B × strain K-12 hybrids, and their use assumed that the probable variation between hybrids in the amount of deoxyribonucleic acid (DNA) received from the K-12 strain would not seriously affect the interpretation of the data acquired in transduction experiments. In addition, it assumed that introduction into the B strain of any peculiar K-12 genes, with one exception, could not reasonably be expected to affect the transduction experiments. The one exception could have arisen from the introduction of the gene which restricts this particular P1bt strain when it is grown on *E. coli* K-12 (efficiency of plating, $10^{-4}$). In each case, however, the *lac*$^-$ hybrids plated P1bt with an efficiency equal to that of the parental B strain (efficiency of plating, 0.04 to 0.9), suggesting that the K-12 restriction was not transferred to the particular hybrids used.

Each of the pyridoxine prototype mutants carrying the *lac*$^-$ allele was then transduced with phage grown either on WG1 or on the *lac*$^+$ pyridoxine prototype mutants; the number of complete Pdx$^+$ transductants obtained was normalized to the number of complete Lac$^+$ transductants (Table 3). Each figure in Table 3 was obtained by dividing the number of Pdx$^+$ transductants counted by the number of Lac$^+$ transductants counted in an identical sample from the same experiment. In each experiment, sufficient samples were taken to provide a minimum number of 1,000 Lac$^+$ transductants. The results shown in which the wild type is donor are the mean of six experiments, except for that with WG532 as the recipient, which is from a single experiment. Separate experiments showed that no linkage was detectable by transduction between *lac* and any of the *pdx* loci.

In all of the data above, "complete" trans-

TABLE 2. *Results of two-factor crosses with pyridoxine auxotrophs of E. coli B*[a]

| Recipient[b] | Donor[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WG3 (*pdxB3*) | WG139 (*pdxC139*) | WG140 (*pdxC140*) | WG15 (*pdxD15*) | WG73 (*pdxE73*) | WG5 (*pdxF5*) | WG25 (*pdxG25*) | WG53 (*pdxG53*) | WG2 (*pdxH2*) | WG1 (wild type) |
| WG3 (*pdxB3*) | 0 | 0 | 0.2 | 0.3 | 31 | 170 | 140 | 46 | 156 | 170 |
| WG139 (*pdxC139*) | 0 | 0 | 0 | 0.6 | 21 | 150 | 170 | 82 | 240 | 280 |
| WG140 (*pdxC140*) | 4 | 0 | 0 | 0.5 | 82 | 200 | 150 | 140 | 380 | 270 |
| WG15 (*pdxD15*) | 2 | 3 | 1 | 0 | 91 | 250 | 220 | 160 | 450 | 240 |
| WG73 (*pdxE73*) | 80 | 100 | 92 | 46 | 0 | 90 | 2 | 1 | 83 | 80 |
| WG5 (*pdxF5*) | — | — | 260 | 180 | 48 | 0 | 300 | 180 | — | — |
| WG25 (*pdxG25*) | 230 | 200 | 94 | 150 | 0 | 130 | 0 | — | 270 | 130 |
| WG53 (*pdxG53*) | 250 | 150 | 94 | 160 | 0 | 170 | 0 | 0 | 220 | 170 |
| WG2 (*pdxH2*) | 340 | 300 | 220 | 200 | 75 | 340 | 130 | 140 | 0 | 480 |

[a] The genotype symbols are defined in Table 5. Values are expressed as transductants per $10^8$ phage; — signifies no data obtained.

[b] The strain designation is expressed first; the allele designation is given in parentheses.

TABLE 3. *Results of transduction of pyridoxineless E. coli B × K-12 hybrids containing lac⁻ allele*[a]

| Recipient[b] | Donor[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | WG3 (*pdxB3*) | WG139 (*pdxC139*) | WG140 (*pdxC140*) | WG15 (*pdxD15*) | WG73 (*pdxE73*) | WG5 (*pdxF5*) | WG25 (*pdxG25*) | WG53 (*pdxG53*) | WG2 (*pdxH2*) | WG1 (Wild type)[c] |
| WG535 (*pdxB3*) | 0 | 0 | 0 | 0.01 | 2.2 | 2.1 | 1.5 | 1.8 | 3.7 | 3.8 ± 0.6 |
| WG538 (*pdxC139*) | 0 | 0 | 0 | 0.02 | 4.2 | 6.3 | 2.8 | 3.5 | 10 | 8.6 ± 2.5 |
| WG537 (*pdxC140*) | 0 | 0 | 0 | 0.04 | 5.5 | 5.2 | 2.1 | 3.7 | 10 | 9.8 ± 2.8 |
| WG536 (*pdxD15*) | 0 | 0.03 | 0 | 0 | 2.0 | 2.8 | 1.9 | 2.7 | 5.7 | 3.8 ± 1.7 |
| WG573 (*pdxE73*) | 2.4 | 1.7 | 2.9 | 2.4 | 0 | 3.8 | 0.01 | 0.06 | 3.6 | 5.0 ± 2.5 |
| WG532 (*pdxF5*) | 2.5 | 2.5 | 2.3 | 2.9 | 2.6 | 0 | 3.5 | 3.0 | 4.6 | 5.7 |
| WG534 (*pdxG25*) | 4.8 | 5.0 | 10 | 4.3 | 0.03 | 9.7 | 0 | 0 | 12 | 12 ± 4.3 |
| WG531 (*pdxG53*) | 3.6 | 5.0 | 5.3 | 3.7 | 0.02 | 4.2 | 0 | 0 | 7.0 | 6.8 ± 3.5 |
| WG533 (*pdxH2*) | 2.5 | 2.2 | 2.9 | 1.8 | 1.0 | 2.8 | 1.7 | 2.7 | 0 | 2.9 ± 1.4 |

[a] Values given express ratio of complete Pdx⁺ transductants per Lac⁺ transductant.

[b] The strain designation is expressed first; the allele designation is given in parentheses.

[c] With the one exception, numbers in this column are the mean ± standard deviation for six experiments.

ductants have been assumed to arise only from direct repair of a mutation by recombination of the host-cell DNA with DNA derived from the homologous region in the donor bacterial strain. It should be pointed out, however, that a complete transduction is only a phenotypic property, and as such it may derive occasionally from the introduction of any of a number of suppressor mutations. Although these latter might account for some of the data obtained, it is unlikely that they could account for more than a minor portion.

After the data of Table 3 were accumulated and the division of *pdx* prototype mutations into four unlinked groups of loci was seen, the linkage to these groups of each of the other independent *pdx* mutations isolated in this laboratory was measured by transduction frequencies. Nutrition tests had already established that a majority of the mutants were either phenotypically similar to the prototypes carrying mutations in group I (*pdxB3*, *pdxC139*, *pdxD15*, *pdxC140*) or the mutants carrying mutations in group II (*pdxG53*, *pdxG25*, *pdxE73*; reference 5). If the nutritional phenotype truly reflected genotype, then this majority of the mutants would carry mutations genetically linked to those in one of these two groups. Accordingly, P1bt grown on one member of both of the two groups of strains was used to transduce all of the previously untested mutants. In this test, linkage to the mutations in group II was suggested by a significantly lower number of transductants when WG25 served as genetic donor than when WG3 served as donor, and vice versa.

For those strains which showed about equal numbers of transductants with P1bt stocks grown on either WG3 or WG25, additional

transductions were performed with phage stocks grown on mutant strains carrying other *pdx* alleles until each mutant could reasonably be assigned to a linkage group (Table 4). From these tests, one *pdx*-allele was found which was apparently unlinked to any of the previously known groups. This allele, *pdxJ151*, in strain WG1027 was tested further both by performing reciprocal crosses with phage grown on strains containing *pdx* alleles representing each linkage group and by subjecting it to the cross-feeding tests similar to those described earlier (5). The former results are included in the data for group V (Table 4).

Testing of linkage by transduction frequencies as in Tables 2 and 4, instead of by ratio test as in Table 3, is subject to a large amount of potential error. This comes from assuming that phage adsorption, transducing potency, rate of recombination, and physical conditions are all sufficiently identical in each cross that every difference in frequency reflects only differences in proximity of one mutation to another. Because of this possibility of error, the assignment of the mutations into any group of loci is necessarily tentative.

A summary of what is known about the genetic interrelationships of *pdx* mutations of *E. coli* B is shown in Table 4. Many of the mutants tested (Table 4) had been subjected previously to a simplified cross-feeding test (5), and at that time they were assigned a probable phenotypic identity with one of the prototype mutants. The data in Table 4, together with the nutritional properties of all the mutants, were used to assign the genotype symbols to a majority of the *pdx* alleles in Table 5. In Table 5, the genotype symbol "A" has not been used to avoid confusion with the *pdxA1* isolated and mapped in *E. coli* K-12 by Taylor and Trotter (15).

The assignment of alleles to particular genotypes is done subject to possible future revision when the various enzymes in the biosynthetic sequence become known. At present, the assignments best fit all the available data.

The chromosomal positions of linkage groups III, IV, and V are presently unknown but, following the clue given by conjugational time of entry results, the position of group I was determined to be near *dsd* (W. B. Dempsey, *in preparation*); that of group II was determined to be near *pyrA* (45 min and 1 min, respectively, on the chromosomal map of *E. coli*; reference 15). For group II, Leu$^-$, Thr$^-$, Pyr$^-$, Pdx$^+$, strains WG571, WG620, and WG1001 were transduced with P1b$^c$ grown on Pdx$^-$ prototype mutants. Leu$^+$, Thr$^+$, or Pyr$^+$ transductants were tested for inheritance of Pdx$^-$ as an unselected trait (Table 6). Since Taylor and Trotter (15) have located *PdxA1* near *PyrA*, other mutations located in this vicinity have quite possibly occurred in the *pdxA* gene.

TABLE 4. *Classification of pdx alleles by transduction frequencies*[a]

| Linkage group[b] | Recipient *pdx* allele no. | Donor *pdx* allele no. | | | | |
|---|---|---|---|---|---|---|
| | | 3 | 25 | 5 | 2 | 73 |
| I (prototype alleles 3, 15, 139, 140) | 50 | 0.1 | 250 | — | — | — |
| | 60 | 2 | 300 | — | — | — |
| | 137 | 0.6 | 54 | 51 | 130 | — |
| | 142 | 0.1 | 320 | — | — | — |
| | 147 | 0.1 | 280 | — | — | — |
| | 150 | 0.1 | 260 | — | — | — |
| | 158 | 0.5 | 54 | — | — | — |
| II (prototype alleles 25, 53, 73) | 22 | 99 | 8 | — | — | 7 |
| | 67 | 76 | 0.1 | — | — | 1 |
| | 81 | 52 | 5 | — | — | 8 |
| | 132 | 51 | 0.1 | 110 | 250 | — |
| | 135 | 48 | 5 | 170 | 380 | — |
| | 136 | 28 | 5 | 160 | 280 | 2 |
| | 145 | 34 | 4 | 150 | 260 | — |
| | 146 | 49 | 7 | — | — | 17 |
| | 149 | 26 | 0.1 | — | — | — |
| | 152 | 48 | 0.3 | — | — | — |
| | 153 | 69 | 2 | — | — | — |
| | 155 | 57 | 1 | — | — | — |
| III (prototype allele 5) | 4 | 120 | 420 | — | — | — |
| | 131 | 140 | 240 | — | — | — |
| | 143 | 21 | 41 | 0 | 200 | — |
| | 144 | 110 | 390 | 0 | 200 | — |
| | 148 | 98 | 560 | 6 | 390 | — |
| IV (prototype allele 2) | 154 | | | | | |
| | 159 | 140 | 310 | 37 | 1 | — |
| V[c] | 151 | 142 | 138 | 234 | 124 | — |

[a] Values are expressed as number of transductants per $10^8$ phage.

[b] Unclassified alleles: pdx-141 and pdx-57.

[c] With allele 151 as donor, there were 34 transductants per $10^8$ phage with recipient allele 3, 151 with recipient allele 25, 59 with recipient allele 5, and 186 with recipient allele 2.

Transduction of one prototype mutant with phage grown on another prototype occasionally gave five types of apparent Pdx$^+$ transductants, namely those which grew in the following manner (as measured after 7 days of incubation): (i) colonies of normal size; (ii) small colonies 0.5 to 1 mm in diameter, maximum size; (iii) very small visible colonies 0.2 to 0.4 mm, maximum size; (iv) small round microscopic colonies of 0.1 to 0.2 mm, maximum size, only after 5 to 7 days of growth; and (v) small odd-shaped colonies from 0.1 to 2.0 mm, maximum size.

TABLE 5. *Definition of pyridoxine genotype symbols*

| Genotype symbol | Linkage group | Nutritional properties | Allele no. |
|---|---|---|---|
| B | I | Fed by mutants of genotype: C, D, E, F, G, H | 3, 59, 142, 147, 150 |
| C | I | Fed by mutants of genotype: D, E, F, G, H | 139, 140 |
| D | I | Fed by mutants of genotype: E, F, G, H | 15, 60, 137, 158 |
| E | II | Fed by mutants of genotype: F, G, H | 73, 67, 136, 145, 135 |
| F | III | Fed by mutants of genotype: G, H | 5, 144, 4, 131, 143, 148 |
| G | II | Fed by mutants of genotype: H | 152, 153, 155, 81, 146, 25, 53, 22, 132, 149 |
| H | IV | Requires pyridoxal or pyridoxamine | 2, 154, 159 |
| J | V | Fed by mutants of genotype: B, C, D, E, F, G, H | 151 |

TABLE 6. *Data locating group II near pyrA*

| Donor | | Recipient | | | | | |
|---|---|---|---|---|---|---|---|
| | | WG571 | | WG620 | | WG1001 | |
| Group | Strain | No. of $Thr^+$ transductants tested | No. of $Thr^+$ $Pdx^-$ | No. of $Pyr^+$ transuctants tested | No. of $Pyr^+$ $Pdx^-$ | No. of $Leu^+$ transductants tested | No. of $Leu^+$ $Pdx^-$ |
| I | WG3 | 157 | 0 | 212 | 0 | 163 | 0 |
| II | WG25 | 743 | 38 | 702 | 149 | 179 | 5 |
| | WG53 | 720 | 40 | 411 | 94 | 177 | 9 |
| | WG73 | 699 | 31 | 899 | 236 | 142 | 5 |
| III | WG5 | 157 | 0 | 180 | 0 | 166 | 0 |
| IV | WG2 | 147 | 0 | 193 | 0 | 154 | 0 |

Of these, only type (i) and type (iv) colonies were always randomly distributed on the plates. Types (ii), (iii), and (iv) colonies invariably were found only in the vicinity of type (i) colonies. Type (iv) colonies were present in 2- to 10-fold greater number than type (i) colonies.

Because of their slow growth, microscopic size, random distribution, and frequency, type (iv) colonies were thought to represent abortive transductants (7, 13). Accordingly, at least 10 of this type of clone from each cross were removed by touching them with a finely drawn capillary pipette partially filled with saline. This suspension was spread on a minimal area of 25 $mm^2$ on a fresh agar plate. After the plate was incubated for 1 week, the number of microclones was determined. In Table 7, 10 entries show an average of one or fewer microclones per clone spread. In no case did any of these clones show more than one microcolony-forming cell.

## DISCUSSION

The purpose of this investigation was to define by genetic means the number of loci determining pyridoxine biosynthesis in *E. coli* B. The results of the transductions (Table 2 and 3) show the existence of four unlinked groups of loci, and the survey of other pyridoxine-requiring mutants (Table 4) shows that all other mutants except one probably carry mutations linked to one of these groups of loci. From these results alone, a minimum of five enzymes can be postulated to exist on the pathway between a metabolite common to several pathways and pyridoxal 5-'phosphate. Pyridoxine kinase, another enzyme definitely not the product of the four unlinked groups, is known from biochemical studies (9, 10).

The number of enzymes actually involved in biosynthesis of pyridoxal phosphate is probably larger than five if the nutritional differences used in Table 5 to distinguish different alleles in the same group such as *pdxE73* and *pdxH25*, or *pdxC139* and *pdxB3*, are considered valid indications of different cistrons. If each nutritionally distinct mutant represents a mutation in a different cistron, group II mutations must be subdivided into two cistrons and group I mutations

TABLE 7. *Average number of microclones per microclone picked and spread*[a]

| Recipient[b] | Donor[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | WG3 (*pdxB3*) | WG139 (*pdxC139*) | WG140 (*pdxC140*) | WG15 (*pdxD15*) | WG5 (*pdxF5*) | WG25 (*pdxG25*) | WG53 (*pdxG53*) | WG2 (*pdxH2*) |
| WG535 (*pdxB3*) | — | — | — | — | 5 | 2 | 5 | 4 |
| WG538 (*pdxC139*) | — | — | — | — | <0.1 | 2 | 2 | — |
| WG537 (*pdxC140*) | — | — | — | — | 1 | 4 | 12 | — |
| WG536 (*pdxD15*) | — | >100 | — | — | >100 | — | >100 | >100 |
| WG532 (*pdxF5*) | — | — | — | — | — | — | — | >100 |
| WG534 (*pdxG25*) | 0.1 | — | 1 | 1 | 0.1 | — | 2 | 4 |
| WG531 (*pdxG53*) | 9 | — | 1 | 2 | 10 | — | — | >100 |
| WG533 (*pdxH2*) | — | — | — | 0.5 | 0.1 | — | 0.1 | — |

[a] Strains containing the *pdxE73* allele were uniformly "—" either as donor or recipient. (—) No microclones seen after transduction.

[b] The strain designation is expressed first; the allele designation is given in parentheses.

may represent three cistrons. These five, together with pyridoxol kinase, loci F, H, J and the mutant locus in WG141 (which appears to be nutritionally distinct but which reverts to $Pdx^+$ with such high frequency that it is not testable in genetic systems), indicate that 9 or 10 enzymes may be required to biosynthesize pyridoxal 5'-phosphate from a metabolite common to several pathways.

The results of the studies with pyridoxine alleles of *E. coli* B may be summarized in a simple linear sequence as follows:

$$\text{Common metabolite} \xrightarrow[V]{J} \xrightarrow[I]{B} \xrightarrow[I]{C} \xrightarrow[I]{D} \xrightarrow[II]{E} \xrightarrow[III]{F} \xrightarrow[II]{G} \text{Pyridoxol}$$

where the letters represent genotypes and the roman numerals linkage groups. Pyridoxol is then converted to pyridoxal 5'-phosphate in two steps as follows:

$$\text{Pyridoxol} \xrightarrow{\text{Pyridoxol kinase}} \text{Pyridoxol 5'-phosphate} \xrightarrow[IV]{H} \text{Pyridoxal 5'-phosphate}$$

The existence of these latter two enzymes in *E. coli* has been demonstrated and the sequence has been discussed (4). However, the nature of the reactions leading to pyridoxol is still unknown.

Abortive transductants have been explained as arising from the survival of an unincorporated, nonreplicating DNA fragment in a recipient cell (7, 13). This cell thus becomes a partial diploid for the selected region and is capable of growth but not exponential growth. At cell division, the daughter cell which does not inherit the DNA fragment is fated to cease growing when the pool of enzyme inherited from the mother cell, and capable of making the product, becomes too dilute to support growth.

Some clones were observed formed in certain crosses of two $Pdx^-$ mutants which may have been due to abortive transduction. If true abortive transduction occurs, then this shows that complementation occurs between certain pairs of pyridoxine mutation of *E. coli* B. This would supplement some of the earlier nutritional data for the existence of different loci. Where the number of microclones in Table 6 is greater than 1 but less than 100, the number of microclones seen cannot be explained by survival of a single nonreplicating DNA fragment but suggest instead that an occasional replication occurs and then ceases. The mechanism by which this could happen is unknown.

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