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Identification of the Forms of Vitamin B_6 Present in the Culture Media of "Vitamin B_6 Control" Mutants

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An *Escherichia coli* mutant resistant to isoniazid (WG497) contained 0.6 μmole of extracellular pyridoxamine and pyridoxamine phosphate in the early stationary phase. A suppressed lysine mutant (AT1024) contained 1.4 μmoles of pyridoxal phosphate under the same conditions. The internal concentration of vitamin B_6 was one-half of normal for AT1024 and increased fivefold for WG497.

Two mutants of *Escherichia coli* which appear to have mutations in the regulation of vitamin B_6 levels have been recently reported. One strain (WG497) derived from *E. coli* B is resistant to high levels of isoniazid (L. J. Arcement, W. Korytnyk, and W. B. Dempsey, Bacteriol. Proc., p. 121, 1968). The other strain, AT1024, derived from *E. coli* K-12, is a genotypic *lys* mutant which is phenotypically Lys^+ owing, it is thought, to the overproduction of vitamin B_6 (3). The purpose of this report is to identify the nature of the forms of vitamin B_6 found in the culture media after growth of these mutants.

Wild-type *E. coli* B (WG1) and WG497 were grown in glucose minimal medium (5). AT1024 (*thr-4*, *leu-8*, *proA2*, *thi-1*, *strA20 lys9*, F^-) and its parent strain AB712 (*thr-4*, *leu-8*, *proA2*, *thi-1*, *strA20*, F^-) were grown in glucose minimal medium containing L-proline (160 mg/liter), L-threonine (40 mg/liter), L-leucine (40 mg/liter), and thiamine (0.2 μg/liter). WG8004 (a phage-resistant derivative of the wild-type K-12 strain ATCC 14948) was grown in glucose minimal medium (5).

For growth experiments reported here, a 50- to 100-ml portion of medium was inoculated with the appropriate strain and incubated overnight at 37 C with vigorous shaking. In the morning, the cells were centrifuged, washed once with 20 ml of 0.9% NaCl, and then used to inoculate 1 liter of medium. The organisms were grown in their respective media until the cells appeared to just stop doubling at an exponential rate. For identification of vitamin B_6 compounds, portions of the cultures were then centrifuged for 10 minutes at 5,000 × *g* at room temperature, and the supernatant fluid was passed through a 0.45-μm nitrocellulose filter. A 10-ml amount of this filtrate was diluted to 25 ml with water, and a portion was applied to the sequential cation-anion exchange columns described by Bain and Williams (2). Elution was as described by those authors (2), and identification and quantitation of vitamin B_6 were by yeast bioassay (1) by using the modifications previously described (4). The various forms of vitamin B_6 were identified by comparison of their elution pattern with that of known compounds (Fig. 1).

The distribution of vitamin B_6 in several culture samples is shown in Table 1. These measurements were made by the methods previously described (4) on separate cultures similarly grown and harvested. We found during these studies that AT1024 and AB712 stopped exponential growth at a cell density of 0.3 to 0.4 mg/ml, in other words before reaching maximum cell density for the amount of glucose supplied. In this state, AT1024 continued to synthesize vitamin B_6 for several hours after growth had stopped. The other strains grew to a cell density of 1 mg/ml before ceasing growth and net vitamin B_6 synthesis.

The results of these analyses show that the two presumed control mutants are different from their respective wild-type *E. coli* strains both in the amounts of vitamin B_6 found in the medium and in the amounts found in the cells. The results also show that the two mutants are different from each other in the amounts of vitamin B_6 in the cells and in the kinds of vitamin B_6 found in the medium. A very significant difference between the two mutants can be clearly seen if the

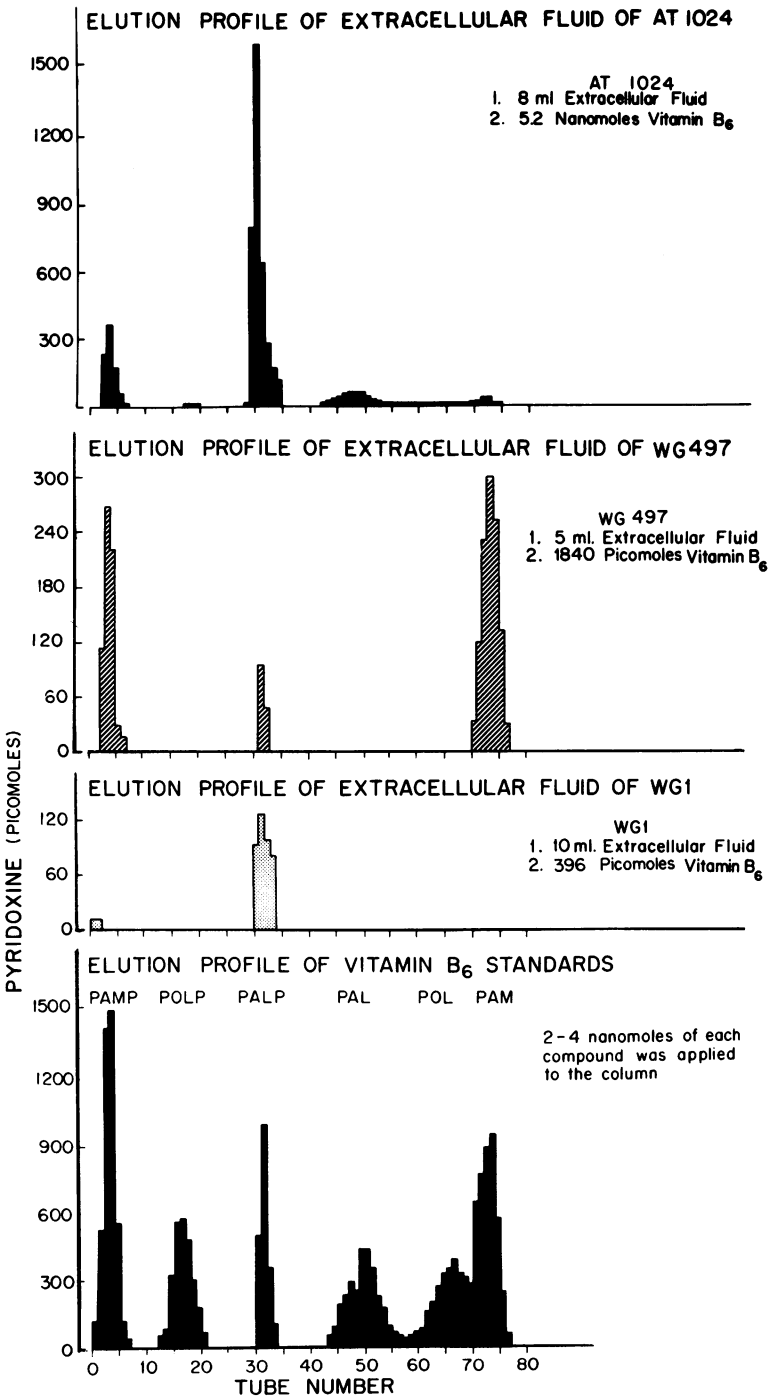

FIG. 1. *Patterns of elution of vitamin B_6 compounds. In each of the three experiments shown, the number 1 refers to the net amount of cell-free culture medium applied to the columns and the number 2 refers to the total amount of vitamin B_6 applied. The abbreviations used for the known compounds are: PAMP, pyridoxamine-5'-phosphate; POLP, pyridoxol-5'-phosphate; PALP, pyridoxal-5'-phosphate; PAM, pyridoxamine; POL, pyridoxol; PAL, pyridoxal.*

TABLE 1. *Total nanomoles of vitamin B_6 per milliliter of culture containing 1 mg (dry weight) of cells per milliliter during the early stationary phase*

Sample analyzed	*E. coli* B		*E. coli* K-12		
	WG497	WG1	WG8004	AB712	AT1024
Culture	1.5 ± 0.2[a]	0.53 ± 0.10	0.57 ± 0.04	0.50 ± 0.08	1.9 ± 0.3
Cell-free culture medium	0.73 ± 0.1	0.16 ± 0.04	0.25 ± 0.03	0.22 ± 0.06	1.5 ± 0.2
Cells	0.74 ± 0.08	0.37 ± 0.05	0.32 ± 0.03	0.29 ± 0.06	0.44 ± 0.05
Toluene extract of cells	0.27 ± 0.04	0.05 ± 0.008	0.12 ± 0.04	0.11 ± 0.01	0.066 ± 0.04

[a] Standard deviations are shown.

TABLE 2. *Extracellular and toluene-extractable pool concentrations of vitamin B_6 in cells and in the medium*

Strain no.	Strain	Cell (μM)	Medium (μM)	Cell/medium
WG497	B	90 ± 10	0.73 ± 0.1	123
WG1	B	17 ± 3	0.16 ± 0.04	106
WG8004	K-12	40 ± 3	0.25 ± 0.03	160
AB712	K-12	37 ± 3	0.22 ± 0.06	168
AT1024	K-12	22 ± 3	1.5 ± 0.2	14.6

data of Table 1 are used to calculate the molar concentrations of vitamin B_6 both in the cells and in the medium. In this calculation, we assume (i) that all forms of vitamin B_6 are equally diffusible, (ii) that 3 ml of cell water is associated with 1 g of dry cell mass, and (iii) that toluene-extractable vitamin B_6 is identical with the free or unbound vitamin B_6 of the cell. The results of these calculations point out clearly that the ratio of the toluene-extractable pool to external vitamin B_6 is nearly the same for WG497 and wild-type *E. coli* B, whereas in AT1024 the ratio is greatly reduced (Table 2). One interpretation of these findings is that WG497 is a true "control" mutant in which the increase in extracellular vitamin B_6 is merely a reflection of an increased intracellular vitamin B_6 arising perhaps from a mutation in an enzyme needed for regulating the vitamin B_6 level of the cell. By this type of reasoning, AT1024 is less likely to be a "control" mutant because the increase in extracellular vitamin B_6 does not reflect any increase in cellular vitamin B_6. Instead the cellular vitamin B_6 is one-half that found in both prototrophic and genetically related K-12 strains. There would be a likely explanation for this if AT1024 had altered the permeability of its membrane.

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