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Transport of Vitamin B_{12} in *Escherichia coli*: Genetic Studies

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The chromosomal location of two genetic loci involved in the transport of cyanocobalamin (B_{12}) in *Escherichia coli* K-12 was determined. One gene, *btuA*, is believed to code for the transport protein in the cytoplasmic membrane, because a mutant with an alteration in this gene has lost the ability to accumulate B_{12} within the cell although normal levels of the surface receptors for B_{12} are present. The other locus, *btuB*, apparently codes for the surface receptor on the outer membrane. These mutants have lost the ability to bind B_{12} and have greatly reduced transport activity, although growth experiments have shown that they can utilize B_{12} for growth, but with decreased efficiency. This surface receptor for B_{12} also appears to function as the receptor for the E colicins, because *btuB* mutants are resistant to the E colicins, and mutants selected for resistance to colicin E1 are defective in B_{12} binding and transport. The gene order was determined by transduction analysis to be *cyc-argH-btuA-btuB-rif-purD*. In addition, mutations in *metH*, the gene for the B_{12}-dependent homocysteine methylating enzyme, were obtained in this study. This gene was localized between *metA* and *malB*.

The uptake of cyanocobalamin (B_{12}) by whole cells of *Escherichia coli* is a biphasic process consisting of an initial rapid phase, which is independent of the energy metabolism of the cell, followed by an energy-dependent phase (7). The initial B_{12}-binding sites are firmly embedded in the outer membrane of the cell envelope (19). Small amounts of B_{12}-binding activity are released from cells by osmotic shock, and these have been separated into two discrete fractions with molecular weights of 22,000 and >200,000 (17, 18). Strains of *E. coli* that are defective in either phase of B_{12} uptake have been isolated and described (8). Those cells lacking the initial phase have undetectable amounts of the receptor on the outer membrane but normal amounts of the smaller protein released by osmotic shock treatment. The mutant lacking the secondary phase while retaining the initial phase has normal amounts of all binding activities (19).

The accompanying paper suggests that the initial B_{12}-binding receptor also functions as a receptor for colicins E1 and E3 (9). Binding of B_{12} to an outer membrane fraction or to whole cells is inhibited by these colicins. Conversely, colicin binding to cells is competitively inhibited by B_{12}. Further, the mutants lacking the initial phase of B_{12} uptake are resistant to colicin action.

The objective of this paper is to define the chromosomal location of two genes involved in B_{12} transport and to give some indication of the role of the B_{12}-binding component in the transport process. The genotype *btu* is suggested to designate those genes involved in the uptake or utilization of B_{12}. This paper provides genetic evidence also suggesting the identity of gene *btuB*, the locus controlling the initial phase of B_{12} uptake, and *bfe*, which has been described as the locus specifying the receptor for the three colicins E and for the bacteriophage BF23 (11).

MATERIALS AND METHODS

Media. The complex medium was LB broth (13). The minimal medium A was that described by Davis and Mingioli (5). Required growth factors were added to minimal media to these concentrations: glucose, 0.5%; amino acids, 100 μg/ml; purines and pyrimidines, 40 μg/ml; thiamine, 0.1 μg/ml; and B_{12}, 3 ng/ml. In addition, selective plates contained, as appropriate, streptomycin (100 μg/ml), D-cycloserine (Calbiochem, 30 μg/ml), rifampin (Calbiochem, 10

μg/ml), or cystathionine (50 μg/ml). Utilization of maltose was scored by growth on plates supplemented with 0.4% maltose.

Bacterial strains. The properties of the *E. coli* K strains used in this study are listed in Table 1. Strain RK4101 was constructed by the transduction of a *purE*$^+$ derivative of strain KBT041 to *btuA*$^+$ by a P1 lysate grown on strain AT2535 (*argH1*). More than 70% of the *btu*$^+$ recombinants had received the donor *argH* allele. From one of these recombinants, a strain resistant to cycloserine and rifampin was isolated by sequential selection.

Transduction and mating procedures. Donor Hfr cells were grown with aeration into the logarithmic phase of growth at 37 C, then allowed to stand at 37 C for 60 to 120 min before mating (3). Cells were mixed in a 1:10 donor-recipient ratio and incubated at 37 C with gentle aeration. For determination of the time of entry of genetic markers, the mating mixture was gently diluted after 3 min with 10 volumes of prewarmed medium A containing 10% nutrient broth (10). At intervals after dilution, portions of the mating mixture were pipetted into 3 ml of soft agar (0.75% agar in medium A) and subjected to mechanical agitation in the vibratory blending device of Low and Wood (12). The mixture was poured onto selective plates.

Phage-mediated transductions were made by using phage P1kc. Lysates of donor strains with titers of 10^9 to 10^{11} infective particles per ml were prepared by the confluent plate lysis technique by using an inoculum of 10^3 to 10^4 infectious particles. Transduction crosses were carried out with a multiplicity of infection of 1 to 3 at 37 C for 20 min. The transduction mixture was plated directly onto recombinant-selective plates, which were then incubated for 36 to 48 h. $CaCl_2$ (2.5 mM) was present during phage adsorption.

Characterization of recombinant colonies. Recombinant clones obtained on the selective plates were transferred with sterile toothpicks to a grid on the same selective medium. This master plate was replicated to other selective plates to test for methionine auxotrophy, utilization of B_{12}, etc. Resistance to rifampin was scored on plates no more than 5 days old because of its loss of biological activity. Colicin resistance was scored by applying an appropriate dilution of colicin E1 with a sterile toothpick to recombinant cells which had just been applied to a nutrient agar plate. The colicin preparations were obtained by the methods of Di Masi et al. (9).

Mutants resistant to colicin E1 were selected by plating a mixture of 10^7 cells and colicin E1 in a soft-agar overlay on nutrient agar plates containing 100 μg of streptomycin per ml to kill any of the cells contaminating the colicin preparation. Colonies surviving after overnight incubation at 42 C were purified by streaking for single-colony isolation.

Assays. The assays for the uptake of ^{60}Co-CN-B_{12} have been previously described and they use a final B_{12} concentration of 4 nM (7). The activity of the cobalamin-dependent N^5-methyltetrahydrofolate-homocysteine transmethylase was assayed in sonic extracts of cells by the method of Taylor and Weissbach (18).

RESULTS

Three strains of *E. coli* defective in B_{12} uptake have been obtained after selection for the loss of ability to utilize B_{12} for methionine biosynthesis (8). One of these mutants, KBT041, retained the initial phase of uptake but lost the secondary, energy-dependent phase. The two other mutants, KBT026 and KBT069, appeared to have lost both phases, because they were inactive in the normal transport assay. The apparent complexity of the uptake process suggests the functioning of several genes, two of

TABLE 1. *List of bacterial strains used*

Strain	Genotype[a]	Source or reference
Ra-2	Hfr, *supE42*	CGSC4241[b]
E15	Hfr C	E. Lin
AT2535	F$^-$, *pyrB59, argH1, his-1, purF1, str-9*	CGSC 4517[b]
PA505MPE11	F$^-$, *arg, metA, malB*	M. Schwartz
KBT001	F$^-$, *leu, pro, lysA, trp, purE, metE, str, lac*	7
KBT041	As KBT001, also *btuA41*	7
KBT026	As KBT001, also *btuB26, metH*	7
KBT069	As KBT001, also *btuB69, metH*	7
RK4101	F$^-$, *leu, pro, lysA, metE, argHl, str, cyc, rif*	
RK4102	As RK4101, also *argH*$^+$, *btuA41*	
RK4103	As RK4101, also *argH*$^+$, *btuB26*	
RK4104	As RK4101, also *argH*$^+$, *btuB69*	
RK4105	As RK4101, also *argH*$^+$, *btuB26, metH*	
RK4106	As RK4101, also *argH*$^+$, *btuB69, metH*	
RK4107	As RK4101, also *argH*$^+$	

[a] The nomenclature used is that of Demerec et al. (6), and Taylor (16).

[b] These strains were obtained from the Coli Genetic Stock Center, Yale Univ., through the courtesy of B. J. Bachmann.

which (*btuA* and *btuB*) are defined by these mutant strains.

Mapping by conjugation. The loci affecting the utilization of B_{12} in strains KBT041 and KBT069 were roughly mapped by conjugation with various Hfr strains. Transfer of the Btu$^+$ phenotype was obtained from HfrC, but only low transfer occurred with HfrH, KL16, KL96, or AB313. The analysis of the number of recombinants for various donor markers from HfrC suggested that the loci affecting B_{12} utilization were located between *leu* and *metE*. Recombinants selected for B_{12} utilization (still Met$^-$) had inherited *leu*$^+$, *pro*$^+$, and *purE*$^+$ with frequencies of 38, 32, and 10%, respectively. This analysis was complicated by the entry of *metE*$^+$, which prevented the scoring of ability of recombinants to use B_{12}. However, Hfr Ra-2, which transfers *metE*$^+$ as a terminal marker, did transfer *btu*$^+$ to each of the recipients with high frequency.

The time of entry of *btu*$^+$, *leu*$^+$, and *pro*$^+$ from this Hfr was determined by mating interruption. Fig. 1 shows the number of recombinants for these markers as a function of the time of mating before mating interruption, with strain KBT041 (Fig. 1a) or strain KBT069 (Fig. 1b) as recipient. For both strains, the ability to utilize B_{12} entered very early, 13 to 14 min and 10 to 12 min before *leu*$^+$ for strains KBT041 and KBT069, respectively. The times of entry indicate that the mutations in strains KBT041 and KBT069 are distinct and are located in the region of the chromosome represented on the Taylor map by the regions near min 77 to 78 and 79 to 81, respectively.

Characterization of btuA. The mutation in strain KBT041 is designated *btuA41*. It affects the secondary phase of B_{12} uptake and does not apparently affect the structure of any of the B_{12}-binding components of the cell wall or periplasmic space (19). Preliminary experiments showed that this gene was closely linked by P1-mediated transduction with *argH*.

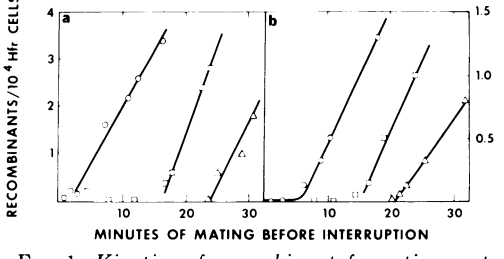


FIG. 1. *Kinetics of recombinant formation: entry of ability to utilize B_{12} for methionine biosynthesis (○), leu*$^+$ *(□), and pro*$^+$ *(△). The donor was Hfr Ra-2, and the recipient strains were KBT041 (a) and KBT069 (b). The Hfr cells were counterselected with streptomycin.*

Co-transduction of *btuA41* with *argH* and the orientation of this mutation relative to *cyc* and *rif* were investigated. Strain KBT041 (*arg*$^+$, *metE*, *btuA41*, *rif*$^+$, *cyc*$^+$) served as the donor; the recipient was strain RK4101. Transductants were selected for *arg*$^+$ and were scored for utilization of B_{12}, resistance to rifampin or cycloserine, and methionine auxotrophy. The data from the analysis of more than 800 *arg*$^+$ transductants is presented in Table 2. The *cyc* and *rif* alleles in this strain were located at equal distances to either side of *argH*, because both gave approximately 50% co-transduction with *argH* but only 25% co-transduction with each other. There was 83% co-transduction of *btuA* and *argH*. If *btuA* were between *cyc* and *argH*, then the minority recombinant classes (those requiring at least four crossover events) would be represented by classes 5 and 7 of Table 2. In fact, classes 7 and 8 occurred with lowest frequency, which is most consistent with the gene order *cyc-argH-btuA-rif*.

Other transduction experiments showed that *argH*, *btuA*, and *rif* were co-transduced with *purD* at frequencies of approximately 25, 30, and 50%, respectively. This is consistent with the findings of others that the gene order in this region of the chromosome is *metB-cyc-argH-btuA-rif-thi-purD* (11, 15).

The B_{12}-transport properties of four of the *arg*$^+$ *btuA* recombinants were tested. All four were identical to strain KBT041 in their possession of the initial phase of uptake and the complete absence of the secondary phase. Several *arg*$^+$ *btu*$^+$ recombinants had transport properties essentially identical to that of the wild-type strain. Thus, the inability of *btuA* strains to utilize B_{12} is associated with their inability to accumulate B_{12} within the cell.

KBT026 and KBT069 resistance to colicin E1. Transduction experiments in which P1 lysates grown on strains KBT026 or KBT069 were used to transduce strain RK4101 to *arg*$^+$ showed that 2 to 6% of the recombinants had received the Btu$^-$ phenotype. Other crosses showed that the locus controlling this phenotype gave roughly 25% co-transduction with *purD*.

At this time, the findings presented by Di Masi et al. (9) were made, which indicated a relationship between the receptor for colicin E1 and the binding component responsible for the initial phase of B_{12} uptake. Mutants lacking the initial phase (KBT026 and KBT069) are resist-

ant to colicin E1 and E3 but sensitive to colicin K, whereas both the parental strain and the secondary phase mutant, KBT041, are sensitive to all of these colicins. Accordingly, the *arg*$^+$ recombinants obtained in the transduction from strains KBT026 and KBT069 into strain RK4101 were tested for their resistance to colicin E1. The colicin resistance of the donor strains was 66% co-transducible with *argH* (Table 3). Analysis of the inheritance of the other unselected markers, *rif* and *cyc*, showed that the locus specifying sensitivity to colicin E1 was located between *argH* and *rif*. This location is quite similar to that of the locus *bfe*, which has been described as coding for the receptor for the phage BF23 and the three colicins E (11). The locus in these strains specifying colicin resistance is called *btuB*, although it may be identical to *bfe*.

The relationship between the *btuB* locus and the initial phase of B_{12} uptake was further indicated by the measurement of the transport properties of some of the *arg*$^+$ recombinants. Fifty-two colicin-resistant (Col-R) recombinants and 68 colicin-sensitive (Col-S) recombinants were assayed. All of the Col-R recombinants were defective in B_{12} uptake, averaging less than 5% of the wild-type level of either the initial or secondary phase of uptake. In contrast, all of the Col-S recombinants were normal in B_{12} uptake, exhibiting greater than 90% of the wild-type level of both the initial and secondary phases. This strongly suggests that the Col-R phenotype is related to the defect in the initial phase of B_{12} uptake.

Most of the *btuB* recombinants lacking the initial phase of uptake are able to utilize 3 ng of B_{12} per ml for growth. This allowed the genetic ordering of *btuA* and *btuB*. The recombinant strain RK4102 (*metE*, *btuA41*, *btuB*$^+$, *rif*), which is unable to utilize B_{12} at any concentration, was used as the recipient. The donor strains on which P1 lysates were prepared were strains KBT026 and KBT069 (*metE*, *btuB*, *rif*$^+$, *metX*), which are also unable to utilize B_{12}. Selection was made for the ability to utilize 3 ng of B_{12} per ml. Such recombinants were obtained at frequencies approximately equal to that obtained with P1 grown on the parental strain KBT001. As had been predicted from the co-transduction frequencies of *btuA* and *btuB* with *argH* (0.84 and 0.67, respectively), 77% of the *btuA*$^+$ recombinants had become Col-R (Table 4). Analysis of the inheritance of the donor *rif* and *cyc* markers further substantiated the assignment of *btuB* between *btuA* and *rif*. This indicates the distinctness but close linkage of *btuA* and *btuB*, two of the genes involved in B_{12} transport.

TABLE 2. *Ordering by co-transduction of btuA, rif, and cyc with argH, and the co-transduction frequencies*[a]

Progeny genotypes[b]					Colony formation	
No.	*cyc*	*arg*	*btuA*	*rif*	No. of colonies formed	Percentage of total colonies formed
1	D	*	D	D	231	28
2	R	*	D	D	250	31
3	D	*	D	R	120	15
4	R	*	D	R	80	10
5	D	*	R	R	66	8
6	R	*	R	R	46	6
7	D	*	R	D	13	1.6
8	R	*	R	D	6	0.7

Co-transduction frequencies

cyc — 0.53 — *argH* — 0.84 — *btuA* — 0.69 — *rif*

argH — 0.58 — *rif*

cyc — 0.43 — *argH*

cyc — 0.30 — *btuA*

[a] The donor was strain KBT041 (*cyc*$^+$, *arg*$^+$, *btuA41*, *rif*$^+$, *metE*), and the recipient strain was RK4101 (*cyc*, *argH*, *btuA*$^+$, *rif*, *metE*). A total of 812 *arg*$^+$ transductants were analyzed.

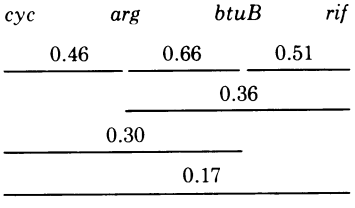
[b] Asterisk indicates the selected marker; D and R represent donor and recipient alleles, respectively, of the unselected genes.

metH mutation in KBT026 and KBT069. The cross described above indicates that the mutations in *btuB* in strains KBT026 and KBT069 are not responsible for their Btu$^-$ phenotype. This phenotype is 3% co-transducible with *argH*, compared to the 67% frequency for *btuB*. Enzyme assay for the *metH* gene product, the B_{12}-dependent homocysteine-N^5-methyltetrahydrofolate transmethylase, showed that both of these strains lacked this activity. In addition, six *arg*$^+$ recombinants of strain RK4101 that had received the Btu$^-$ phenotype from either strain KBT026 or KBT069 also lacked this activity, whereas seven *arg*$^+$ *btu*$^+$ recombinants from these crosses possessed wild-type levels of this activity. Four *arg*$^+$ *btu* recombinants from the crosses with strain KBT-041 (*btuA41*) as donor also possessed wild-type levels of this enzyme. Some of the *metH* recom-

TABLE 3. *Ordering by co-transduction of btuB, rif, and cyc with argH, and the co-transduction frequencies*[a]

Progeny genotypes[b]					Colony formation			
					Donor 1		Donor 2	
No.	*cyc*	*arg*	*btuB*	*rif*	No. of colonies formed	Percentage of total colonies formed	No. of colonies formed	Percentage of total colonies formed
1	D	*	D	D	71	24	24	8
2	R	*	D	D	44	15	55	19
3	D	*	D	R	45	15	35	12
4	R	*	D	R	35	12	79	27
5	D	*	R	R	50	17	34	12
6	R	*	R	R	41	14	59	20
7	D	*	R	D	4	1.4	1	0.4
8	R	*	R	D	0	0	1	0.4
1,2		*	D	D	115	38	62	31
3,4		*	D	R	67	22	75	38
5,6		*	R	R	114	38	57	29
7,8		*	R	D	4	1.3	6	3

Co-transduction frequencies[c]



[a] The same recipient, RK4101 (*argH, rif, cyc, btuB*$^+$, *metE*) was used in each experiment. Donor 1 was strain KBT026 (*btuB26*) and donor 2 was KBT069 (*btuB69*); both donors are *cyc*$^+$, *arg*$^+$, *rif*$^+$, *metE*.

[b] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes. The donor *btuB* alleles were scored by the resistance of recombinants to the action of colicin El.

[c] The co-transduction frequency between *argH* and *btuB* was 0.64 for *btuB26* and 0.67 for *btuB69*.

binants were *btuB*$^+$ and possessed 100% of the wild-type level of B_{12} uptake, whereas the *btuB* recombinants were completely defective in transport. Therefore, it appears that strains KBT026 and KBT069 were isolated as double mutants defective both in *metH*, which conferred the Btu$^-$ phenotype, and in *btuB*, which eliminated B_{12} transport.

Mapping of metH. The chromosomal location of *metH* has been described in *Salmonella typhimurium* as being between *metA* and *malB* (1). The *metH* mutation in our *E. coli* strains was mapped by transduction crosses in which the P1 lysate was prepared on strain PA505MPE11 (*metH*$^+$, *metE*$^+$, *metA*, *malB*). The recipients were strains RK4106 (*metE*, *metH69*, *btuB69*, *metA*$^+$, *malB*$^+$) and RK4105 (*metE*, *metH26*, *btuB26*, *metA*$^+$, *malB*$^+$). Transductants were selected for their ability to grow on B_{12}; cystathionine was added to the selection plates to allow the growth of any *metA* recombinants. Of the recombinants obtained in either cross, 74% had lost their methionine auxotrophy, i.e., had become *metE*$^+$. The genotypes of the remaining *metE metH*$^+$ recombinants are shown in Table 5. The data from the two crosses are essentially identical and are most consistent with the gene order *metA-metH-malB*, because of the absence of a minority recombinant class which any other proposed gene order would require. The *metH* locus appears to be roughly equidistant between *metA* and *malB*.

Role of the initial phase of transport on B_{12} utilization. The ability of mutants lacking the initial phase of transport to utilize B_{12} at 3 ng/ml, despite their inactivity in the transport assay, has been described above. Growth of various pairs of *btuB*$^+$ and *btuB* strains on different initial supplements of B_{12} was meas-

TABLE 4. *Ordering by co-transduction of btuB and rif with btuA, and the co-transduction frequencies*

Donor strain[a]	No. of colonies scored	Progeny genotypes[b]			Colony formation	
		btuA	*btuB*	*rif*	No. of colonies formed	Percentage of total colonies formed
KBT026	97	*	D	D	38	39
		*	D	R	44	45
		*	R	R	12	12
		*	R	D	3	3
KBT069	106	*	D	D	18	17
		*	D	R	57	54
		*	R	R	29	27
		*	R	D	2	2

Co-transduction frequencies

btuA — 0.77 — *btuB* — 0.35 — *rif*

btuA — 0.30 — *rif*

[a] The recipient strain in each case was RK4102 (*btuA41, rif, metE*). The donors were *btuA*$^+$, *btuB*, *rif*$^+$, *metE*. Selection was made for the ability to grow on B_{12} (3 ng/ml); inheritance of *btuB* was scored by resistance to colicin E1.

[b] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes.

ured to define the role of the initial phase in B_{12} utilization. The inoculum for these studies was grown in 10 ng of B_{12} per ml in order to avoid the long lag which resulted upon transfer of cells from a methionine-supplemented medium to a B_{12}-supplemented medium. The exponential growth rate constant was determined for cultures inoculated into minimal media containing initial B_{12} concentrations from 0.13 to 6.13 ng/ml. The observed growth rate constants for strains RK4107 (*metE, btuB*$^+$), RK4103 (*metE, btuB26* and RK4104 (*metE, btu69*) as a function of B_{12} concentration are shown in Fig. 2. Figure 3 presents an Eadie plot of this data in which the background growth of unsupplemented cultures is subtracted from the observed rate. Each point is the average of triplicate determinations. The *btuB*$^+$ strains had an average, uncorrected maximal doubling time with B_{12} of about 65 min, whereas the *btuB* strains averaged 74 to 79 min. All strains had the same doubling time (56 to 60 min) when supplemented with methionine. These strains showed marked differences in their apparent K_m for growth. The *btuB*$^+$ strains achieved half the maximal growth rate at a B_{12} concentration of 0.15 to 0.20 nM, compared with 0.85 to 0.90 nM for the *btuB* strains. Thus the loss of the initial phase decreased the affinity of the cell for B_{12} as assayed either by transport assay or by growth measurements.

Properties of Col-R mutants. The close relationship of the colicin receptor and the initial phase of B_{12} uptake was further demonstrated by the properties of mutants selected for colicin resistance. Out of 24 mutants resistant to colicin E1, 20 showed decreased utilization of B_{12} for growth and had the B_{12} uptake properties similar to strain KBT026 or strain KBT069. The remaining four isolates had normal uptake and B_{12} utilization, but at least three of these were still sensitive to colicin E3, in contrast to the former 20, which were resistant. These latter isolates were probably altered in some site of colicin action other than the receptor.

DISCUSSION

This paper has described two genetic loci, *btuA* and *btuB*, which are involved in the

TABLE 5. *Ordering by co-transduction of metA and malB with metH, and the co-transduction frequencies*

Recipient strain[a]	No. of colonies scored	Progeny genotypes[b]			Colony formation	
		metA	*metH*	*malB*	No. of colonies formed	Percentage of total colonies formed
RK4105	120	D	*	D	15	13
		D	*	R	25	21
		R	*	D	24	20
		R	*	R	56	47
RK4106	147	D	*	D	25	17
		D	*	R	32	22
		R	*	D	13	9
		R	*	R	77	52

Co-transduction frequencies

metA — 0.36 — *metH* — 0.29 — *malB*

metA — 0.15 — *malB*

[a] The same donor strain, PA505 MPE11 (*metA, malB, metH*$^+$, *metE*$^+$) was used in both crosses. The recipient strains were both *metA*$^+$, *malB*$^+$, *metH, metE*. Selection was made for ability to utilize B_{12}. Of the transductants appearing, 74% were *metE*$^+$. The data in this table are for the *metH*$^+$ *metE* transductants. No multiple integrational events need to be involved if the order is assumed to be *metA-metH-malB*.

[b] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes.

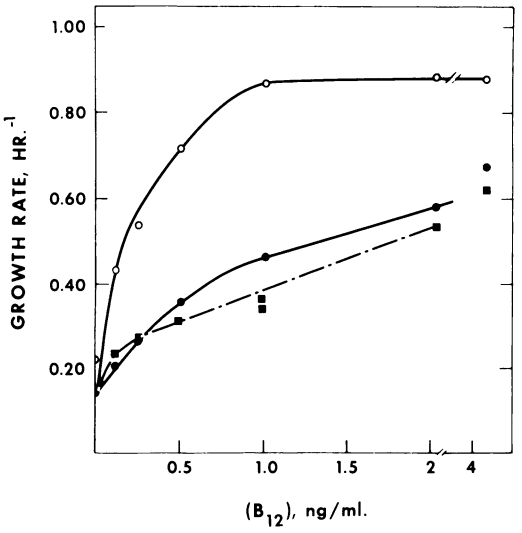


FIG. 2. *Effect of B_{12} supplementation on growth rate. The cells were grown overnight in minimal growth medium supplemented with 10 ng of B_{12} per ml and washed twice with medium A. Portions were inoculated into side-arm flasks containing minimal growth medium with the indicated concentrations of B_{12}, and were incubated at 37 C with shaking. The absorbance at 420 nm was plotted against time of incubation, and the growth rate was determined from the doubling time. The strains used were RK4107 (metE, btuB$^+$) (○), RK4103 (metE, btuB26) (●), and RK4104 (metE, btuB69) (■).*

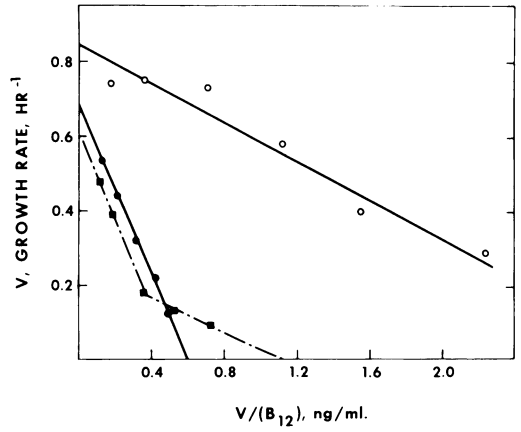


FIG. 3. *Eadie plot of the data of Fig. 2. The growth rate (V) is corrected for the growth rate of cultures not supplemented with B_{12}. Symbols are the same as in Fig. 2. Lines were drawn with the aid of a linear regression analysis.*

uptake of B_{12}. This uptake consists of two distinct, sequential phases (7). The first phase is the rapid binding of the B_{12} to specific receptor sites which are firmly attached to the outer membrane of the cell envelope (19). The dissociation constant for this binding is roughly 0.8 nM. Di Masi et al. (9) have presented evidence for the function of these outer membrane sites as receptors for the E colicins, as well as for B_{12}. Mutants lacking the initial phase of B_{12} uptake were resistant to the E colicins because of their lack of a functional receptor. The chromosomal location of the gene specifying colicin resistance (*btuB*) in these strains was 67% cotransducible with *argH*. This location is quite near to, if not identical with, the locus *bfe*, which has been described as specifying the receptor for the E colicins and bacteriophage BF23 (11). All the data presented would indicate that *btuB* may be identical to *bfe*, but this identity has not yet been rigorously established. The possession of the initial phase of B_{12} transport in these transductants was found to correlate well with their sensitivity to the E colicins. Conversely, mutants selected for their resistance to the E colicins had lost the initial phase of B_{12} uptake. The mutants selected for resistance to colicin E1 which retained the initial phase were, however, still sensitive to colicin E3 and presumably are tolerant to E1, rather than resistant.

The mutant strains lacking the initial phase (KBT026 and KBT069) were selected for their inability to utilize B_{12} for methionine biosynthesis. In fact, these strains are double mutants. There is mutation in *metH* that is responsible for their inability to utilize B_{12}, in addition to the mutation in *btuB* affecting their uptake of B_{12}. The *metH* mutation has no significant effect on B_{12} uptake or colicin resistance. The chromosomal location of *metH* was determined and is analogous to the location of *metH* in *S. typhimurium* (1).

Recombinant strains with a mutation in *btuB*, but *metH*$^+$, are able to utilize B_{12} for growth. However, they require roughly a fivefold-higher concentration of B_{12} to achieve the same growth rate as a *btuB*$^+$ strain. Paradoxically, these *btuB* strains possess no B_{12} transport activity as measured under standard assay conditions. The amount of B_{12} required to allow growth is much less than the amount that can be transported in the assay. Presumably, B_{12} can enter cells lacking the initial phase, but with decreased efficiency. The observed K_m for B_{12} for growth (0.9 nM) is in the range of the K_m for the secondary phase of uptake (0.4 nM) (P. M. DiGirolamo and C. Bradbeer, personal communication). Thus, the receptor plays a role in B_{12} transport, since its loss significantly decreases both the affinity of the cell for B_{12} and its maximal apparent rate of transport.

Mutation in *btuA* does not affect the initial phase of B_{12} uptake, but eliminates the energy-dependent accumulation of B_{12} in the cell. Such mutants are unable to utilize B_{12} for growth. This gene presumably codes for the B_{12} transport system in the cytoplasmic membrane, since this mutant possesses normal levels of all detectable B_{12}-binding activities (19). The gene is 83% co-transducible with *argH* and is closely linked to *btuB*. It is of interest to note that several other genes involved in membrane functions have been localized in this region of the chromosome. These include a gene (*bir*) implicated in biotin transport (2) and a cluster of genes (*mrb*) involved in murein synthesis (14).

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LITERATURE CITED

1. Ayling, P. D., and K. F. Chater. 1968. The sequence of four structural and two regulatory methionine genes in the *Salmonella typhimurium* linkage map. Genet. Res. **12**:341-354.
2. Campbell, A., A. del Campillo-Campbell, and R. Chang. 1972. A mutant of *Escherichia coli* that requires high concentrations of biotin. Proc. Nat. Acad. Sci. U.S.A. **69**:676-680.
3. Curtiss, R., L. G. Caro, D. P. Allison, and D. R. Stallions. 1969. Early stages of conjugation in *Escherichia coli*. J. Bacteriol. **100**:1091-1104.
4. Curtiss, R., L. J. Charamella, C. M. Berg, and P. E. Harris. 1965. Kinetic and genetic analyses of D-cycloserine inhibition and resistance in *Escherichia coli*. J. Bacteriol. **90**:1238-1250.
5. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
6. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-76.
7. DiGirolamo, P. M., and C. Bradbeer. 1971. Transport of vitamin B_{12} in *Escherichia coli*. J. Bacteriol. **106**:745-750.
8. DiGirolamo, P. M., R. J. Kadner, and C. Bradbeer. 1971. Isolation of vitamin B_{12} transport mutants of *Escherichia coli*. J. Bacteriol. **106**:751-757.
9. Di Masi, D. R., J. C. White, C. A. Schnaitman, and C. Bradbeer. 1973. Transport of vitamin B_{12} in *Escherichia coli*: common receptor sites for vitamin B_{12} and the E colicins on the outer membrane of the cell envelope. J. Bacteriol. **115**:506-513.
10. Hane, M. W. 1971. Some effects of nalidixic acid on conjugation in *Escherichia coli* K-12. J. Bacteriol. **105**:46-56.
11. Jasper, P. E., E. Whitney, and S. Silver. 1972. Genetic locus determining resistance to phage BF23 and colicins E_1, E_2 and E_3 in *Escherichia coli*. Genet. Res. **19**:305-312.
12. Low, B., and T. H. Wood. 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. **6**:300-303.
13. Luria, S. E., and J. W. Burrows. 1957. Hybridization between *Escherichia coli* and Shigella. J. Bacteriol. **74**:461-476.
14. Miyakawa, T., H. Matsuzawa, M. Mitsuhashi, and Y. Sugino. 1972. Cell wall peptidoglycan mutants of *Escherichia coli* K-12: evidence of two clusters of genes, *mra* and *mrb*, for cell wall peptidoglycan biosynthesis. J. Bacteriol. **112**:950-958.
15. Orias, E., T. K. Gartner, J. E. Lannan, and M. Betlach. 1972. Close linkage between ochre and missense suppressors in *Escherichia coli*. J. Bacteriol. **109**:1125-1133.
16. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
17. Taylor, R. T., S. A. Norrell, and M. L. Hanna. 1972. Uptake of cyanocobalamin by *Escherichia coli* B. Some characteristics and evidence for a binding protein. Arch. Biochem. Biophys. **148**:366-381.
18. Taylor, R. T., and H. Weissbach. 1971. N^5-methyltetrahydrofolate-homocysteine (vitamin B_{12}) methyltransferase, p. 379-388. *In* H. Tabor and C. W. Tabor, ed., Methods in enzymology, vol. 17. Academic Press, Inc., New York.
19. White, J. C., P. M. Di Girolamo, M. L. Fu, Y. A. Preston, and C. Bradbeer. 1973. Transport of vitamin B_{12} in *Escherichia coli*: location and properties of the initial B_{12}-binding site. J. Biol. Chem. **248**:3978-3986.