JOURNAL OF BACTERIOLOGY, Oct. 1976, p. 86-98

Vol. 128, No. 1
Printed in U.S.A.

Mutant Strains (*nit*) of *Salmonella typhimurium* with a Pleiotropic Defect in Nitrogen Metabolism

JAMES BROACH,[1] CYNTHIA NEUMANN, AND SYDNEY KUSTU*

*Department of Biochemistry, University of California, Berkeley, California 94720, and Department of Bacteriology, University of California, Davis, California 95616**

Received for publication 12 April 1976

We have isolated mutant strains (*nit*) of *Salmonella typhimurium* that are defective in nitrogen metabolism. They have a reduced ability to use a variety of compounds including glutamate, proline, arginine, *N*-acetyl-glucosamine, alanine, and adenosine as sole nitrogen source. In addition, although they grow normally on high concentrations of ammonium chloride (>1 mM) as nitrogen source, they grow substantially more slowly than wild type at low concentrations (<1 mM). We postulate that the inability of these strains to utilize low concentrations of ammonium chloride accounts for their poor growth on other nitrogen sources. The specific biochemical lesion in strains with a *nit* mutation is not known; however, mutant strains have no detectable alteration in the activities of glutamine synthetase, glutamate synthase, or glutamate dehydrogenase, the enzymes known to be involved in assimilation of ammonia. A *nit* mutation is suppressed by second-site mutations in the structural gene for glutamine synthetase (*glnA*) that decrease glutamine synthetase activity.

Several laboratories interested in regulation of bacterial nitrogen metabolism have characterized mutant strains with pleiotropic defects in the utilization of various nitrogen sources. In certain cases the biochemical lesions have been defined. Mutant strains (*asm*) of *Klebsiella pneumoniae* that have low glutamate synthase (EC 2.6.1.53) activity were first described by Nagatani et al. (20), and similar strains of *K. aerogenes* were characterized further by Brenchley et al. (4). These strains fail to grow on low concentrations of NH_4Cl as nitrogen source and consequently are unable to use a variety of other compounds as nitrogen source (4, 20). The phenotype is consistent with the proposed role of glutamate synthase in assimilation of low concentrations of ammonia (18).

Prival et al. (23) characterized mutant strains (*glnA*) of *K. aerogenes* with low glutamine synthetase (EC 6.3.1.2) activity. Unlike the wild type, these mutants are unable to derepress synthesis of the histidine and proline degradative enzymes in response to nitrogen limitation. Tyler et al. (36) proposed that glutamine synthetase activates transcription of genes for these degradative enzymes. (It is important to note that *Salmonella typhimurium* cannot derepress synthesis of the histidine- and proline-degradative enzymes in response to nitrogen limitation [24; J. Broach, unpublished data] but can do so only in response to carbon limitation [5, 24].)

We have isolated mutant strains of *S. typhimurium* with a pleiotropic defect in nitrogen metabolism different from that in strains with an *asm* or *glnA* mutation; in this paper we describe their phenotype and preliminary biochemical and genetic characterization. Since the biochemical lesion in these strains is not known, we call the mutated gene *nit* for nitrogen utilization. Mutations in *nit* are suppressed by second-site mutations in *glnA*.

MATERIALS AND METHODS

Chemicals. [^{3}H]proline (3.0 Ci/mmol) was obtained from New England Nuclear Corp. ICR372 and ICR191E were kindly donated by R. M. Peck, R. K. Preston, and H. J. Creech, Chemotherapy Laboratory of the Institute for Cancer Research in Philadelphia. Malate dehydrogenase (pig heart, 1,000 U/mg) was obtained from Sigma Chemical Co.

Media and growth of bacterial strains. Minimal medium refers to the following salts mixture: 1 g of K_2SO_4; 17.7 g of $K_2HPO_4 \cdot 3H_2O$; 4.7 g of KH_2PO_4; 0.1 g of $MgSO_4 \cdot 7H_2O$; and 2.5 g of NaCl per liter. Compounds added to this medium to serve as carbon and nitrogen sources are indicated in the text. A mixture of trace elements (2) was added at a concentration of 1 ml/liter as noted. Medium E of Vogel and Bonner (37), a minimal medium that contains 0.2% citrate and 17 mM NH_4^+, was used in some experiments. Nutrient broth medium contained 8 g of nutrient broth (Difco) and 5 g of NaCl per liter. Cultures were incubated at 37°C in a New Brunswick rotary shaker with aeration. Bacterial growth was monitored tur-

[1] Present address: Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724.

bidimetrically by measuring optical density at 650 nm except as noted. For determination of growth rates, inoculum cultures were grown overnight in medium E + 0.4% glucose and were diluted 1:100 or 1:50 into minimal medium containing the supplements specified. Residual NH_4^+ carried over with the inoculum allowed growth to an optical density at 650 nm of 0.035 to 0.07. For determination of growth rates at low concentrations of NH_4Cl, inoculum cultures were grown in minimal medium + 0.4% glucose + 5 mM NH_4Cl so that no NH_4^+ would be carried over with the inoculum. Growth tests on petri plates were done by placing a filter paper disk containing the compound to be tested on a plate seeded with 10^8 bacteria (13, 25).

Strains and genetic analysis. All strains constructed for this work were derived from *S. typhimurium* strain LT2 and are listed in Table 1. Strains *nit-9* and *nit-11* were obtained from a mutagenized culture of strain LT2 by enriching for cells unable to use a mixture of glutamate and aspartate as nitrogen source. An overnight nutrient broth culture of strain LT2 was diluted 1:10 into medium E containing 0.1 mg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (nitrosoguanidine) per ml and incubated for 10 min at 37°C with aeration. Cells were collected by centrifugation, washed twice, and grown in medium E + 0.4% glucose. Penicillin enrichment was carried out in minimal medium containing 0.4% glucose and 10 mM glutamate + 10 mM aspartate for 8 h. Cells were washed and grown in medium E + 0.4% glucose, and samples of this culture were spread on minimal plates containing 0.4% glucose, 0.1 mM NH_4Cl, and glutamate + aspartate + alanine at 1 mM each. Plates were incubated at 37°C for 48 h; small colonies were tested for their ability to grow on various compounds as nitrogen source, and strains that grew well on NH_4Cl and D-serine but not on glutamate or aspartate were retained. In general, strain *nit-9* grew slightly better on various compounds as nitrogen source than did strain *nit-11*, suggesting that the *nit-9* and *nit-11* mutations are different.

Strain SK51 (*nit-12 dhuA1 hisJ5601*) was isolated as part of another study by enriching an ICR-mutagenized culture of strain TA1650 (*dhuA1 hisJ5601*) for cells unable to grow on arginine as the nitrogen source. The ability of this strain to grow on various organic compounds as nitrogen source was tested only on petri plates.

Revertants of strains *nit-9* and *nit-11* were induced on minimal plates containing 0.4% glucose and 10 mM glutamate or arginine as nitrogen source; nitrosoguanidine (1 μl of a 1-mg/ml solution) or diethylsulfate (1 μl) was placed at the edge of a plate seeded with 10^8 bacterial cells in soft agar.

"Spontaneous revertants" of strains *nit-9* and *nit-11* that displayed partial or conditional glutamine auxotrophy (see Results) were tested for the presence of a *glnA* mutation (14). P22 phage grown on these strains was used to transduce strain SK35 (*glnA60 hisF645*) or strain TA2190 (*glnA54 hisF645*) to growth in the absence of glutamine, and transductants were scored for the partial or conditional glutamine auxotrophy of the donor. (Strain SK35 has an absolute requirement for glutamine; the *glnA* mutation in this strain was ICR induced and does not revert [K. Bisharat and S. Kustu, unpublished data].)

Strains SK128 (Nit^+) and SK129 (Nit^+) were obtained by transducing strains *nit-9* and *nit-11*, respectively, to growth on glutamate as nitrogen source with P22 phage grown on strain LT2. Phage-sensitive clones of these strains were isolated as described previously (25).

Strain *glt-611* was obtained from a nitrosoguanidine-mutagenized culture of strain TA1772 (a wild type) by selecting for growth on 10 mM glutamate as sole carbon and nitrogen source. Mutant strains of *E. coli* selected by the same procedure have elevated glutamate transport (8, 10). Strains SK46 (*glt-611*) and SK47 (*glt-611 nit-9*) were obtained by transducing strains LT2 and *nit-9*, respectively, to growth on glutamate as carbon source (with 10 mM NH_4Cl as nitrogen source) with P22 phage grown on strain *glt-611*. The following properties of strains *glt-611* and SK46 are consistent with their having elevated glutamate transport. These strains grow on glutamate as sole carbon or carbon and nitrogen source; they have improved growth on glutamate as nitrogen source in the presence of glucose. On the other hand, like strain LT2, they do not grow on aspartate, arginine, asparagine, or glutamine as sole carbon and nitrogen source; tests on petri plates indicate that their growth on aspartate, arginine, or proline as nitrogen source is not improved.

Strains TA2186 (*nit-9*/F'*lac*$^+$*pro*$^+$) and TA2187 (*nit*$^+$/F'*lac*$^+$*pro*$^+$) were obtained by episome transfer from strain TR132 to strains *nit-9* and LT2, respectively; selection was for growth on minimal medium with lactose as carbon source.

The donor strain SK50 (*nit-11 thy201* HfrK5) used for conjugation mapping was derived from strain *nit-11* by L. Kier, using methods described previously (14). The origin of transfer for HfrK5 is around 67 min; *metG* (67 min) is transferred early, and *his* (65 min) is transferred late (26). With one exception, recipient strains carried single auxotrophic mutations spaced at 10- to 15-min intervals around the chromosome; several recipient strains with mutations in the 33-min region (obtained from Alper and Ames [1]; Table 1) contained deletion mutations. Matings were carried out directly on petri plates (medium E + 0.4% glucose) as described previously (14), and recombinants were scored for ability to grow on glutamate or arginine as nitrogen source.

P22 phage was P22 *int4* (30) or P22 HT *int*$^-$ (27); this phage was obtained from G. Roberts, who introduced the *int*$^-$ mutation. Mapping by transduction was done using the recipient strains listed in Table 1 and phage grown on strain *nit-9*. Transductants were scored for growth on glutamate as nitrogen source; those that grew were scored positive even though they did not grow as well as strain LT2. Ambiguity in scoring was apparently due to the fact that the presence of phage in transductants interfered with their growth on poor nitrogen sources.

Procedures used for isolating strains sensitive to phage P1 and for transductions with phage P1*kc* were those described by Enomoto and Stocker (6)

TABLE 1. *Bacterial strains*

Strain	Genotype	Parent (recipient, donor)	Operation that produced strain
nit-9		LT2	Mutagenesis, nitroso-guanidine
nit-11		LT2	Mutagenesis, nitroso-guanidine
SK51	*nit-12 dhuA1 hisJ5601*[a]	TA1650	Mutagenesis, ICR
SK128	Wild type	*nit-9*, LT2	Transduction
SK129	Wild type	*nit-11*, LT2	Transduction
TA1772[b]	Wild type		
glt-611		TA1772	Mutagenesis, nitroso-guanidine
SK46	*glt-611*	LT2, *glt-611*	Transduction
SK47	*nit-9 glt-611*	*nit-9*, *glt-611*	Transduction
TA2183	*nit-9 glnA57*	*nit-9*	Spontaneous
TA2184	*nit-9 glnA58*	*nit-9*	Spontaneous
TA2185	*nit-9 glnA59*	*nit-9*	Spontaneous
TA2190[c]	*glnA54 hisF645*		
SK15	*hisF645*	TA2190, *nit-9*	Transduction
SK16	*glnA57 hisF645*	TA2190, TA2183	Transduction
SK17	*glnA58 hisF645*	TA2190, TA2184	Transduction
SK18	*glnA59 hisF645*	TA2190, TA2185	Transduction
SK35	*glnA60 hisF645*	TA831	Mutagenesis, ICR
TR132[d]	*aroP505 gal1501 hags10 ilv405 proA46 purC7 rha465 str*/F'*lac*$^+$ *pro*$^+$		
TA2186	*nit-9*/F'*lac*$^+$ *pro*$^+$	*nit-9*, TR132	Episome transfer
TA2187	*nit*$^+$/F'*lac*$^+$ *pro*$^+$	LT2, TR132	Episome transfer
SK50[e]	*nit-11 thy201* HfrK5		
leu1083[f]			
proAB47[f]			
purE54[f]			
pyrC7[f]			
pyrD135[g]			
trpD9[f]			
TA831[b]	*hisF645*		
TA1665[f]	Δ(*gal bio uvrB chl dhb*)		
TA1674[f]	Δ(*gal bio uvrB chl dhb aroG nicA*)		
TR2405[h]	*nic506 hisD*$^-$		
deoK14[g]			
JL379[g]	*pyrC1502 purC7*		
aroE135[f]			
SK75	*nit-9 galE1797*	*nit-9*	Spontaneous
TR2411[h]	*nic506 his01242 hisB2147 galE542 hut*$^+$		
TR2582[h]	*pyrD23 his01242 hisC3734 galE542 hut*$^+$		
TR2584[h]	*purB73 his01242 hisC3734 galE542 hut*$^+$		
TR2588[h]	*pyrC7 his01242 hisC3737 galE542 hut*$^+$		
SK77	*aroA124 galE1798*	*aroA124*	Spontaneous
SK78	*aroA148 galE1799*	*aroA148*	Spontaneous

[a] *dhuA* and *hisJ* mutations affect the high-affinity histidine permease.
[b] Obtained from G. F.-L. Ames.
[c] Characterized in reference 14.
[d] Obtained from J. Roth.
[e] Constructed by L. Kier as described in Materials and Methods.
[f] Obtained from B. N. Ames.
[g] Obtained from J. L. Ingraham.
[h] Obtained from G. Roberts.

and Ornellas and Stocker (21). Strain SK75 (*nit-9 galE1797*) was the donor in each cross; recipient strains are listed in Table 1. Transductants were scored on glutamate as nitrogen source. Some recipient strains obtained from G. Roberts were *his*$^-$ and *hut*$^+$. Transductants obtained from these strains were scored on plates containing 0.06 mM histidine in addition to other supplements; this level was sufficient to satisfy auxotrophy but not to serve as nitrogen source for *hut*$^+$ strains.

Testing for auxotrophy. Strains *nit-9* and *nit-11* were tested for auxotrophy on minimal plates containing 0.4% glucose and 10 mM glutamate as nitrogen source. Paper disks containing the following compounds were placed on these plates: 0.5 to 1 μmol of each L-amino acid; 0.25 to 1 μmol of each purine and pyrimidine base; 1 μmol of *N*-acetylglucosamine; and >2 μg of the following vitamins: pyridoxine, pyridoxamine, nicotinamide, nicotinic acid, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, folic acid, thiamine, riboflavin, biotin, and pantothenate. Growth was observed only around compounds that could serve as nitrogen source, and the response to these compounds was tested further in liquid culture. The optical density of an overnight culture of strain *nit-9* or *nit-11* grown on glutamate plus the supplement to be tested (0.5 mM concentration) was compared to the sum of the optical densities of cultures grown on glutamate or the supplement alone. If the two were equal and the optical density of the supplemented culture was considerably less than that of strain LT2 grown on glutamate, it was concluded that the supplement was serving as an alternative nitrogen source.

Purification of glutamine synthetase. Glutamine synthetase was purified from strains LT2 and *nit-9* by the method of Woolfolk et al. (38). The enzyme was purified about 300-fold and in both cases was approximately 50% pure as judged by disk gel electrophoresis.

Enzyme assays. Cells were grown to late exponential or early stationary phase in the media indicated. Cell extracts were prepared in 0.05 M potassium phosphate buffer, pH 7.5, as described previously (14) except that cells were broken in a French pressure cell at 20,000 lb/in^2. $MnCl_2$ (1 mM) was added to that portion of extracts used for glutamine synthetase assays. A second extraction buffer was used for some glutamate synthase assays: 10 mM imidazole-hydrochloride, pH 7, containing 1 mM EDTA; 5 mM 2-mercaptoethanol; and 100 mM KCl. Protein was determined by the method of Lowry et al. (15) after precipitation with 5% trichloracetic acid.

Glutamine synthetase. Total activity and degree of adenylylation of glutamine synthetase in crude extracts were determined using the γ-glutamyl transfer assay of Stadtman et al. (31) with the modifications described (14). In the presence of 0.3 mM Mn^{2+}, this assay is a measure of total activity independent of degree of adenylylation. In the presence of 0.3 mM Mn^{2+} + 60 mM Mg^{2+}, activity of adenylylated subunits is inhibited (31). Thus, the ratio of activities $+Mg^{2+}/-Mg^{2+}$ provides an estimate of degree of adenylylation.

Activity of purified enzyme preparations was determined by biosynthetic assays in which the release of inorganic phosphate from adenosine 5'-triphosphate (29) or the conversion of [^{14}C]glutamate to [^{14}C]glutamine (33) was measured.

Glutamate synthase and glutamate dehydrogenase (EC 1.4.1.4). Activities of glutamate synthase and glutamate dehydrogenase were determined at 28°C by measuring oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (decrease in absorbance at 340 nm). Reaction mixtures contained: 50 mM tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.8; 0.25 mM NADPH; 5 mM α-ketoglutarate; crude extract containing between 0.04 and 0.4 mg of protein; and either 5 mM glutamine or 5 mM NH_4Cl for glutamate synthase or glutamate dehydrogenase assay, respectively. Activities were corrected for NADPH oxidation in the absence of glutamine or NH_4Cl and are expressed as micromoles of NADPH oxidized per minute per milligram of protein.

Aspartate ammonia-lyase (EC 4.3.1.1). Aspartate ammonia-lyase was assayed at 37°C by measuring release of ammonia from aspartate as described by Marcus and Halpern (16).

Aspartate aminotransferase (EC 2.6.1.1). Aspartate aminotransferase activity was measured at 25°C by coupling to malate dehydrogenase and following oxidation of reduced nicotinamide adenine dinucleotide (NADH) at 340 nm. Reaction mixtures contained: 60 mM phosphate buffer, pH 7.4; 40 mM aspartate; 0.1 mM pyridoxal phosphate; 0.25 mM NADH; 1 U of malate dehydrogenase; crude extract containing between 0.05 and 0.2 mg of protein; and 4 mM α-ketoglutarate. Activities are expressed as micromoles of NADH oxidized per minute per milligram of protein and were corrected for NADH oxidation in the absence of α-ketoglutarate or aspartate.

Proline oxidase and β-galactosidase (EC 2.1.23). Proline oxidase was measured in toluenized whole cells as described by Dendinger and Brill (5); cells were grown in medium E + 10 mM proline or medium E + 10 mM proline + 0.4% glucose. The activity of β-galactosidase was measured in toluenized cells as described by Pardee et al. (22). Strains TA2187 (*nit*$^+$/F'*lac*$^+$ *pro*$^+$) and TA2186 (*nit-9*/F'*lac*$^+$ *pro*$^+$) were grown in medium E or medium E + 0.4% glucose; 30 min before sampling, isopropyl-β-D-thiogalactoside was added to the medium at a final concentration of 0.1 mM.

Proline transport. High-affinity proline transport was measured by the protein incorporation assay described by Ames for histidine transport (2). Rates of transport of proline at concentrations from 0.01 to 0.5 μM were determined; K_m and V_{max} values were obtained from double-reciprocal plots of these data. Transport at high concentrations of proline was measured by a starved-cells assay (2). A culture growing exponentially in minimal medium plus glucose and ammonium chloride was washed, suspended in minimal medium without additions, and incubated at 25°C without aeration. After approximately 2 h, samples were removed and assayed for transport of [^{3}H]proline at 23°C; transport was linear for at least 3 min. Between 1.5 and 2.5 h of starvation, the rate of transport was constant. Rate is expressed as micromoles per minute per gram (dry weight) of cells, assuming 75 μg (dry weight)/10^8 cells (2).

RESULTS

Phenotype of *nit* strains. By the procedures described in Materials and Methods, we isolated three mutant strains (*nit-9*, *nit-11*, and

SK51) of *S. typhimurium* that have impaired ability to utilize a variety of nitrogen sources. Table 2 summarizes the ability of two of these strains and their parent (strain LT2) to grow on minimal medium containing glucose as carbon source and various nitrogen sources. The mutant strains failed to grow on compounds such as glutamate and arginine, which served as poor nitrogen sources for the wild type (doubling time, 240 min or longer); they grew well on compounds such as D- and L-serine and cytidine, which served as good nitrogen sources for the wild type (doubling time, 55 to 75 min); and they grew poorly on compounds such as asparagine and alanine, which served as "intermediate" nitrogen sources for the wild type (doubling time, 100 to 150 min). Representative growth curves of strain *nit-9* and wild type on a poor nitrogen source (glutamate), a good nitrogen source (D-serine), and an intermediate nitrogen source (alanine) are shown in Fig. 1.

TABLE 2. *Doubling times of strains with a nit mutation on various compounds as nitrogen source*[a]

Nitrogen source[b]	Doubling time (min)		
	LT2	*nit-9*	*nit-11*
Glutamate	303	>840	>840
Arginine	450	No doubling[c]	No doubling
Proline	540	No doubling	–[d]
Aspartate	260	630	700
N-acetylglucosamine	462	900	No doubling
Asparagine	98/154[e]	211/645[e]	–
Alanine	145	188[f]	235[f]
Adenosine	65	93	105
D-Serine	71	70	–
L-Serine	49	76	–
Cytidine	55	72	–
Glutamine	56	63/153[g]	65/148[g]
NH_4Cl	54	56	–

[a] Strains were grown in minimal medium containing added trace elements with 0.4% glucose as carbon source.

[b] Glutamate, arginine, aspartate, asparagine, adenosine, and cytidine were present at a concentration of 10 mM; *N*-acetylglucosamine, alanine, D- and L-serine, glutamine, and NH_4Cl were present at 20 mM; and proline was present at 50 mM.

[c] Optical density of the culture does not double in 24 h.

[d] –, Growth rate not determined.

[e] The culture shifts from the faster to the slower doubling time at an optical density at 650 nm of 0.2.

[f] The mutant strain has a lag phase about 3 h longer than that of the wild type before achieving this doubling time (see Fig. 1C).

[g] The culture shifts from the faster to the slower doubling time at an optical density at 650 nm of 0.6.

(Tests on petri plates indicate that the mutant phenotype is essentially the same with citrate or ribose as carbon source.)

In addition to growing poorly on some organic nitrogen sources, the mutant strains grew poorly on NH_4Cl if the concentration was low (0.1 to 0.5 mM), having both a decreased growth rate and decreased cell yield (Table 3). However, at higher NH_4Cl concentrations (2 to 10 mM) they grew almost as rapidly (within 10%) as the wild type (Table 3) and had essentially normal cell yield.

Mutagen-induced revertants and P22-mediated transductants of strains *nit-9* and *nit-11* selected for growth on glutamate as nitrogen source simultaneously regained the ability to use all other nitrogen sources listed in Table 2 and the ability to grow normally on low concentrations of NH_4Cl (Table 3). The same applies for spontaneous revertants of strain SK51. These results indicate that pleiotropic defects in nitrogen metabolism in *nit* strains are probably due to a single mutation. (Revertants of strains *nit-9* and *nit-11* were induced with mutagens because a high proportion of "spontaneous revertants" of these strains had suppressor mutations unlinked to the *nit* locus [see below].)

***nit* strains can metabolize compounds that they fail to use as nitrogen source.** Strains *nit-9* and *nit-11* grew well on proline and *N*-acetylglucosamine as sole sources of carbon or carbon and nitrogen (Table 4), even though they could not use these compounds as sole nitrogen source. Furthermore, strain SK47 (*glt-611 nit-9*) (see Materials and Methods for strain construction) utilized glutamate as sole carbon or carbon and nitrogen source, but not as sole nitrogen source in the presence of glucose (Table 4). Thus, the *nit* mutation specifically prevents utilization of certain compounds as nitrogen source. The results suggest that enzymes required for degradation of these compounds are structurally intact in strains with a *nit* mutation (although regulation of their synthesis or activity may be altered). This hypothesis is confirmed below for the enzymes of glutamate and proline degradation.

Glutamate degradation. In *Escherichia coli*, glutamate is degraded to α-ketoglutarate by sequential action of aspartate aminotransferase and aspartate ammonia-lyase (16). Activities of these enzymes in strains with a *nit* mutation were normal under the several growth conditions tested (Table 5). Activities of aspartate aminotransferase and aspartate ammonia-lyase in strain LT2 were the same on medium containing glutamate as nitrogen source (conditions under which strain *nit-9*

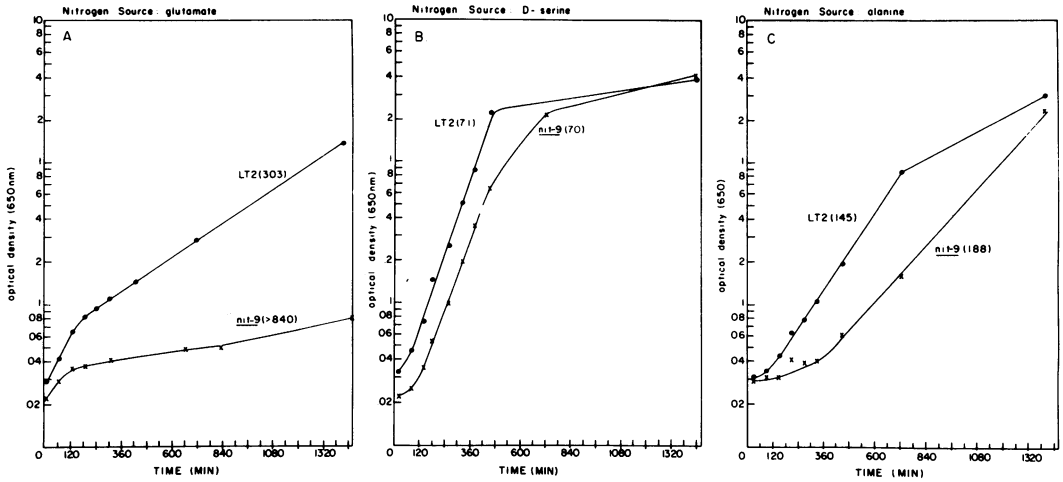

FIG. 1. *Growth of strains nit-9 and LT2 on various compounds as nitrogen source. Cells were grown in minimal medium plus trace elements with 0.4% glucose as carbon source. Doubling times (minutes) are given in parentheses. Nitrogen source: (A) 10 mM glutamate; (B) 20 mM* D-*serine; (C) 20 mM alanine.*

TABLE 3. *Doubling times of strains with a nit mutation and their Nit⁺ transductants on various concentrations of NH_4Cl as nitrogen source*[a]

Concn of NH_4Cl (mM)	Doubling time (min)				
	LT2	*nit-9*[b]	*nit-11*[b]	SK128 (Nit⁺)[c]	SK129 (Nit⁺)[c]
0.1[d]	74	214	160	86	74
0.2[d]	76	151	130	87	76
0.3	75	117	92		
0.5	74	95	78		
2.0	66	68	65		
10.0[d]	65			70	63

[a] Strains were grown in minimal medium containing 0.4% glucose as carbon source (trace elements were not added). Optical density was monitored at 420 nm.

[b] Cell yield was 60 to 70% that of wild type at NH_4Cl concentrations up to 0.5 mM and 80 to 100% that of wild type at higher concentrations.

[c] Cell yield was normal at all NH_4Cl concentrations.

[d] Temperature for this experiment was 35°C.

could not be tested) as they were on medium containing NH_4Cl as nitrogen source. This indicates that activities of these enzymes do not limit growth of strain *nit-9* on glutamate as nitrogen source. (The activity of aspartate ammonia-lyase in strain LT2 was about 15% that of aspartate aminotransferase; aspartate ammonia-lyase activity was elevated 5- to 10-fold in strains growing on glutamate as carbon and nitrogen source [Table 5]. All activities were similar to those reported for *E. coli* [9, 16].)

The following observation indicates that strains with a *nit* mutation can transport glutamate rapidly. Strain SK47 (*glt-611 nit-9*), which failed to use glutamate as nitrogen source, grew on glutamate as sole carbon and nitrogen source more rapidly than did strain SK46 (*glt-611*) (Table 4). (In *E. coli*, mutations similar to *glt-611* cause an increased rate of glutamate transport [8, 10]. We have not directly measured the rate of glutamate transport in our strains.)

Enzymes of proline degradation: proline oxidase. Proline is degraded to glutamate by sequential action of the enzymes proline oxidase and 1-pyrroline dehydrogenase (EC 1.5.1.12), which are induced by proline and are subject to catabolite repression by glucose. Proline oxidase levels of strain *nit-9* grown in the presence and absence of glucose are similar to those of strain LT2 (data not shown), which suggests that induction and glucose repression of this enzyme are normal (the same applies for β-galactosidase). Kinetic constants for high-affinity proline transport are the same in strains *nit-9* and LT2 (K_m = 0.3 μM; V_{max} = 0.9 μmol/min per g [dry weight] of cells); in addition, the rate of proline transport at a high external proline concentration (0.1 mM) is also the same in the two strains (2.5 μmol/min per g [dry weight] of cells).

Conditionally expressed auxotrophy. We tested the possibility that *nit* strains might be auxotrophic in medium containing a poor nitrogen source. (This phenotype can result from a mutation that inactivates a biosynthetic glutaminase such as that associated with anthranilate synthetase [11, 39].). However, this is not the case because *nit* strains cannot grow on glutamate as nitrogen source even in the presence of exogenous L-amino acids, purines, py-

TABLE 4. *Doubling times of strains with a nit mutation on various compounds as sole nitrogen, carbon, and nitrogen, or carbon source*[a]

Carbon (C) and nitrogen (N) sources[b]	Doubling time (min)				
	LT2	*nit-9*	*nit-11*	SK46 (*glt-611*)	SK47 (*nit-9 glt-611*)
Glucose (C) *N*-acetylglucosamine (N)	462	900	No doubling		
N-acetylglucosamine (C and N)	55	64[c]	64[c]		
N-acetylglucosamine (C) NH_4Cl (N)	52	52	52		
Glucose (C) Proline (N)	540	No doubling			
Proline (C and N)[d]	+	+	+		
Proline (C)[d] NH_4Cl (N)	+	+	+		
Glucose (C) Glutamate (N)	303	>840	>840	102	1,120
Glutamate (C and N)				210[e]	115
Glutamate (C) NH_4Cl (N)				225[e]	126

[a] Strains were grown in minimal medium supplemented with the indicated carbon and nitrogen source (trace elements were not added).

[b] *N*-acetylglucosamine and glutamate were present at a concentration of 20 mM. Proline was present at 50 mM, NH_4Cl at 10 mM, and glucose at 0.4%.

[c] The mutant strain has a lag phase 2 to 3 h longer than that of the wild-type strain before achieving this doubling time.

[d] Since strain LT2 had a very low growth yield on proline (or proline + NH_4Cl) in liquid medium, qualitative tests were done on petri plates as described in Materials and Methods.

[e] The culture has a faster doubling time initially.

rimidines, *N*-acetylglucosamine, vitamins (see list in Materials and Methods), or the following combinations of compounds: arginine and uracil; serine and pyridoxine; isoleucine, valine, and leucine; and the aromatic amino acids and aromatic vitamins. (The *nit* strains grow only on supplements (e.g., serine) that serve as alternative nitrogen sources [see Materials and Methods].)

Enzymes of glutamine and glutamate biosynthesis. Lack of evidence for a defect in degradation of poor nitrogen sources by *nit* strains coupled with the observation that they grow poorly on low concentrations of NH_4Cl as nitrogen source suggests that they have a defect in assimilation of low concentrations of ammonia. We therefore assayed the enzymes that catalyze synthesis of glutamate and glutamine, the primary products of ammonia assimilation.

Glutamate synthase and glutamate dehydrogenase. Assimilation of ammonia into glutamate could be catalyzed by glutamate synthase in conjunction with glutamine synthetase (reactions 1 and 2) and, at high ammonia concentrations, could also be catalyzed by glutamate dehydrogenase (reaction 3):

$$\alpha\text{-ketoglutarate} + \text{glutamine} + \text{NADPH} \rightarrow 2\ \text{glutamate} + \text{NADP} \quad (1)$$

$$\text{glutamate} + NH_3 + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i \quad (2)$$

$$\alpha\text{-ketoglutarate} + NH_3 + \text{NADPH} + \text{ATP} \rightarrow \text{glutamate} + \text{NADP} + \text{ADP} + P_i \quad (1 + 2)$$

$$\alpha\text{-ketoglutarate} + NH_3 + \text{NADPH} \rightarrow \text{glutamate} + \text{NADP} \quad (3)$$

TABLE 5. *Aspartate aminotransferase and aspartate ammonia-lyase activities in strains with a nit mutation after growth on various carbon and nitrogen sources*[a]

Carbon (C) and nitrogen (N) sources	Strain	Aspartate aminotransferase[b]	Aspartate ammonia-lyase[c]
Glucose (C) NH_4Cl (N)	LT2	0.57	0.08
	nit-9	0.58	0.05
	SK46 *(glt-611)*	0.50	0.045
	SK47 *(nit-9 glt-611)*	0.61	0.04
Glucose (C) Glutamate (N)	LT2	0.60	0.07
	SK46	0.93	0.06
Glutamate (C and N)	SK46	0.52	0.42
	SK47	1.31	0.48
Citrate (C) NH_4Cl (N)	LT2	–[d]	0.08
	nit-9	–	0.11

[a] Media, growth conditions, and preparation of extracts are described in Materials and Methods. Extracts were centrifuged at 40,000 × *g* for 75 min to decrease NADH oxidase activity.

[b] Activity was measured by coupling to malate dehydrogenase as described in Materials and Methods and is expressed as micromoles of NADH oxidized per minute per milligram of protein at 25°C.

[c] Activity is in micromoles of ammonia released per minute per milligram of protein at 37°C.

[d] –, Not determined.

(ADP is adenosine 5'-diphosphate; P_i is inorganic phosphate.)

Activities of glutamate synthase and glutamate dehydrogenase in crude extracts of strain *nit-9* were normal (Table 6), as were the apparent K_m values of glutamate synthase for glutamine and α-ketoglutarate (1.3 and 0.1 mM, respectively). (The levels of glutamate synthase and glutamate dehydrogenase in *Salmonella* do not vary with the concentration of NH_4Cl in the growth medium [3]. The activities of these enzymes in strain LT2 decrease when cells are grown in medium containing glutamate rather than NH_4Cl as nitrogen source [3; S. Kustu, unpublished data].)

Glutamine synthetase. The basal and derepressed levels of glutamine synthetase (38) in strain *nit-9*, i.e., levels in cells grown in medium containing, respectively, 20 and 2 mM NH_4Cl as nitrogen source, were similar to those for strain LT2 (Table 7). Moreover, glutamine synthetase activity of *nit* strains was normal under several other growth conditions (Table 7). The fact that glutamine synthetase specific activity of strain *nit-9* (0.59) on minimal medium containing 2 mM NH_4Cl as nitrogen source was about as high as that of strain LT2 (0.81) grown on medium containing glutamate as nitrogen source suggests that ability to derepress glutamine synthetase is not the factor that limits growth of strain *nit-9* on glutamate as nitrogen source.

The degree of covalent modification (adenylylation) of glutamine synthetase in crude cell extracts was estimated as described in Materi-

TABLE 6. *Glutamate synthase and glutamate dehydrogenase activities in extracts of strains nit-9 and LT2*

Strain	Glutamate synthase[a]	Glutamate dehydrogenase[a]
LT2	0.18	0.33
nit-9	0.15	0.33

[a] Strains were grown in medium E + 0.4% glucose. Preparation of extracts and assay conditions are described in Materials and Methods. Activity is in micromoles of NADPH oxidized per minute per milligram of protein at 28°C.

TABLE 7. *Glutamine synthetase activity in strains with a nit mutation after growth on several carbon and nitrogen sources*[a]

Carbon (C) and nitrogen (N) sources	Strain	Total activity[b]	Ratio[c] $+Mg^{2+}/-Mg^{2+}$
Glucose (C) 2 mM NH_4Cl (N)	LT2	0.76	1.5
	nit-9	0.59	1.55
Glucose (C) 10 mM NH_4Cl (N)	LT2	0.27	1.4
	nit-9	0.41	1.6
	SK46 *(glt-611)*	0.18	1.3
	SK47 *(nit-9 glt-611)*	0.17	1.4
Glucose (C) 20 mM NH_4Cl (N)	LT2	0.11	1.25
	nit-9	0.12	1.1
Glucose (C) 10 mM glutamate (N)	LT2	0.81	1.3
	SK46	0.70	1.5
50 mM glutamate (C and N)	SK46	0.07	0.3
	SK47	0.12	0.4

[a] Preparation of extracts is described in Materials and Methods. Activity was measured using the γ-glutamyltransfer assay (31).

[b] Activity in the presence of 0.3 mM Mn^{2+} as divalent cation, which is a measure of total activity independent of degree of adenylylation (14, 31).

[c] This ratio provides an approximate measure of the degree of adenylylation of glutamine synthetase. For strain LT2 this ratio has varied from about 0.5 for (partially) adenylylated enzyme to 1.5 for unadenylylated enzyme depending on the growth conditions (14).

als and Methods. Glutamine synthetase from both mutant and wild-type strains grown on medium containing NH_4Cl as nitrogen source is largely in the unadenylylated or "active" (17, 28) form (Table 7), as is the enzyme from wild type grown on a medium containing glutamate as nitrogen source. These data indicate that the *nit* phenotype is probably not caused by an increase in the degree of adenylylation of glutamine synthetase.

Glutamine synthetase was purified from strains *nit-9* and LT2, and apparent K_m values for glutamate and NH_4Cl in the biosynthetic reaction were determined (see Materials and Methods). Values summarized in Table 8 indicate that substrate affinities of glutamine synthetase from strain *nit-9* are the same as those of enzyme from strain LT2. (Several K_m values were determined with Mn^{2+} as divalent cation as well as with Mg^{2+}. For the *E. coli* glutamine synthetase, Mn^{2+}-dependent activity reflects solely activity of adenylylated subunits and Mg^{2+}-dependent activity that of unadenylylated subunits [7, 12].)

A *nit* mutation is suppressed by mutations in *glnA*. Growth defects of *nit* strains were suppressed by second-site mutations in the structural gene for glutamine synthetase, *glnA*. Most spontaneous mutants selected from strains *nit-9* and *nit-11* for recovery of ability to grow on glutamate (or arginine) as nitrogen source had second-site mutations causing partial glutamine auxotrophy. (The same applies for some spontaneous mutants selected from strain SK51.) Plate tests indicated that mutants selected on glutamate (arginine) also recovered ability to grow on arginine (glutamate) and aspartate; however, they grew very poorly in comparison with the wild-type strain. They required glutamine for optimal growth even on medium containing NH_4Cl as nitrogen source. Mutations causing the glutamine requirement in 10 independent strains were shown to be cotransducible with known mutations in *glnA* as described in Materials and Methods.

Three suppressor strains that have a heat-sensitive glutamine requirement (TA2183, TA2184, and TA2185) were studied further. Mutations in these strains are 80 to 90% co-transducible with known mutations in *glnA* (14). Glutamine auxotrophy resulted directly from the *glnA* mutation and not from an interaction of altered products of *glnA* and *nit*, because strain SK17 (*glnA58 hisF645*) and strain TA2184 (*glnA58 nit-9*) had similar heat-dependent glutamine auxotrophy. (The same applies for strains SK16 and TA2183 and strains SK18 and TA2185.) Glutamine auxotrophy and suppression of *nit* were due to a single mutation

TABLE 8. *Kinetic constants of glutamine synthetase purified from strains nit-9 and LT2*[a]

Substrate	Divalent cation[b]	Apparent K_m (mM)[c]	
		LT2	*nit-9*
NH_4Cl[d]	Manganese	0.25	0.22
	Magnesium	0.4	—[e]
Glutamate[f]	Manganese	2.2	2.3
	Magnesium	8.7	8.7

[a] Glutamine synthetase was purified from cells grown in medium E + 0.4% glucose as described in Materials and Methods. Activity in the γ-glutamyltransfer assay (14, 31) indicated that enzyme from both strains was partially adenylylated.

[b] For the *E. coli* glutamine synthetase, biosynthetic activity in the presence of $MnCl_2$ or $MgCl_2$ represents catalysis by adenylylated or unadenylylated subunits, respectively (7, 12).

[c] The apparent K_m value for a given substrate was determined by measuring biosynthetic activity at various concentrations of that substrate with the others present in excess.

[d] Biosynthetic activity measured by release of inorganic phosphate from ATP.

[e] Not determined.

[f] Biosynthetic activity measured by conversion of [^{14}C]glutamate to [^{14}C]glutamine.

because transduction of strains TA2183, TA2184, and TA2185 to glutamine prototrophy with P22 phage grown on strain LT2 yielded transductants with the phenotype characteristic of *nit* mutants. This indicates, in addition, that the *glnA* gene is separated from the *nit* gene by at least the length of the P22 genome.

Analysis of the heat stability of glutamine synthetase from strains TA2183, TA2184, and TA2185 provides further evidence that they have mutations in *glnA*. Relative to strain LT2, the mutants had 5- to 10-fold lower glutamine synthetase activity when they were grown at 37°C than when they were grown at 23°C (Table 9). This reduction in glutamine synthetase activity at 37°C is commensurate with their heat-sensitive glutamine auxotrophy. Glutamine synthetase in crude extracts of these mutants is more labile to heat denaturation than is the wild-type enzyme (Fig. 2). Increased heat lability persists in purified preparations and is independent of the degree of adenylylation of the enzyme (F. Cimino, personal communication). Thus the primary structure of glutamine synthetase is altered in these strains.

Mapping of the *nit* gene. Reciprocal crosses by transduction with P22 phage, performed as described previously (14), indicate that the three *nit* mutations studied are closely linked on the *Salmonella* chromosome (data not shown). Conjugation mapping of the *nit-11* mutation was carried out as described in Materials

TABLE 9. *Glutamine synthetase activity of strains with suppressor mutations in glnA*[a]

Strain	Percent of activity in strain LT2		
	Growth at 23°C		Growth at 37°C, assay at 37°C
	Assay at 23°C	Assay at 37°C	
TA2183 (*glnA57 nit-9*)	65	58	7
TA2184 (*glnA58 nit-9*)	30	27	5
TA2185 (*glnA59 nit-9*)	34	29	—[b]

[a] Cells were grown at 23°C in medium E + 0.4% glucose or at 37°C in medium E + 0.4% glucose + 3 mM glutamine. Preparation of extracts is described in Materials and Methods. Total glutamine synthetase activity was determined at the indicated temperature by the γ-glutamyltransfer assay and is expressed as a percentage of the activity in extracts of strain LT2 grown and assayed under the same conditions. Activities in extracts of strain LT2 were 0.09 and 0.29 μmol of γ-glutamyl hydroxamate formed/min per mg of protein for growth at 23°C and assay at 23°C and 37°C, respectively, and 0.17 μmol/min per mg for growth and assay at 37°C.

[b] Not determined.

and Methods. There was 30% co-inheritance of *nit* with *pyrC* (60/200) and *pyrD* (44/150) at 42 min and none with deletions of the *gal-chl* region (0/100) at 33 min, indicating that the *nit* gene does not lie under these deletions. There was 0.5% co-inheritance (1/200) of *nit* with *trpD* (52 min) (the limited range of co-inheritance may reflect selective pressure against inheritance of the Nit⁻ character even on medium containing a high concentration of NH_4Cl). There was no (0/150) P22-mediated co-transduction of *nit* with the following markers: *nicA* (33 min), *deoK* (37 min), *pyrC* and *pyrD* (42 min), and *aroE* (46 min). There was no (0/200) P1-mediated co-transduction of *nit* with the following markers: *nicA* (33 min), *pyrC* and *pyrD* (42 min), *purB* (43 min), and *aroA* (45 min).

DISCUSSION

We have isolated mutant strains of *S. typhimurium* with pleiotropic defects in nitrogen metabolism; evidence has been presented that these defects are due to mutation of a single gene (*nit*). Mutant strains have reduced ability to use a variety of compounds including glutamate, proline, arginine, *N*-acetylglucosamine, alanine, and adenosine as sole nitrogen source. In addition, they grow poorly on low concentrations of NH_4Cl, suggesting that the lesion is in assimilation of low concentrations of ammonia rather than in metabolism of organic nitrogen sources.

The following evidence indicates that degradative pathways are intact in strains with a *nit* mutation. (i) Catabolic pathways for compounds that *nit* mutants fail to use as nitrogen source (e.g., glutamate and *N*-acetylglucosamine) have no common steps. Thus *nit* does not encode a degradative enzyme. (ii) Degradative enzymes for glutamate and proline are present in mutant extracts in normal amounts. Glucose catabolite repression of proline oxidase appears normal. (iii) The mutant strains grow well on glutamate, proline, and *N*-acetylglucosamine as sole carbon source, confirming that they are able to transport and degrade these compounds as rapidly as the wild type.

We postulate that the rate of release of ammonia from various compounds may determine whether strains with a *nit* mutation can use them as nitrogen source. The mutant strains grow best on compounds that the wild type uses best (doubling times of 55 to 75 min); they grow at intermediate rates on compounds that the wild type uses less well (doubling times of 100 to 150 min) and fail to grow on compounds that the wild type uses poorly (doubling times longer than 240 min). The *nit* strains can use all compounds used by the wild type as sole carbon

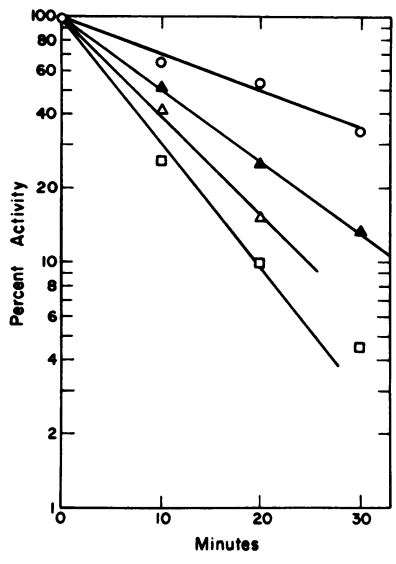

FIG. 2. *Heat lability of glutamine synthetase from strains with suppressor mutations in glnA. Extracts of strains LT2 (○), TA2183 (glnA57 nit-9, ▲), TA2184 (glnA58 nit-9, □), and TA2185 (glnA59 nit-9, △) with a protein concentration of 20 mg/ml were heated at 63°C. At the indicated times, samples were withdrawn and assayed for total activity at 37°C using the γ-glutamyltransfer assay. Results are presented as percentage of initial activity remaining at the indicated time.*

and nitrogen source, although they fail to use some of the same compounds as nitrogen source in the presence of glucose. The rate of release of ammonia from these compounds (e.g., *N*-acetylglucosamine and glutamate) is more rapid when they are used as carbon and nitrogen source than when they are used as nitrogen source. For example, the wild type grows slowly on *N*-acetylglucosamine as nitrogen source (doubling time of 462 min) but rapidly on *N*-acetylglucosamine as carbon and nitrogen source, i.e., in the absence of glucose (doubling time of 55 min). Thus, the rate of degradation of *N*-acetylglucosamine (and hence the rate of release of ammonia) increases in the absence of glucose. Similarly, the high aspartate ammonia-lyase activities of organisms grown on glutamate as carbon and nitrogen source suggest that the rate of glutamate degradation also increases in the absence of glucose. It is important to note that *Salmonella* is able to increase activities of various degradative enzymes in response to carbon limitation (5, 24) but, unlike *Klebsiella,* is unable to do so in response to nitrogen limitation (24; J. Broach, unpublished data).

Although our evidence indicates that strains with a *nit* mutation have a defect in assimilation of low concentrations of ammonia, we have detected no change in the activity of enzymes known to be involved in this process. Activities of glutamate synthase and glutamate dehydrogenase in extracts of mutant strains are normal. In addition, one of the compounds that the mutant strains fail to use as nitrogen source is glutamate, the product of reactions catalyzed by these enzymes. These observations suggest a defect beyond glutamate synthesis. The fact that *nit* mutants are not auxotrophic on poor nitrogen sources and that the *nit* gene is not linked by P22-mediated transduction to any known gene for a biosynthetic glutaminase (J. Broach; unpublished data) suggests a defect before any specific biosynthetic pathway and focuses attention on synthesis of glutamine. We have investigated the activity and regulation of glutamine synthetase in detail. The following results indicate that there is no alteration in the primary structure of glutamine synthetase and suggest that there is no change in regulation of its activity. (i) The *nit* gene is unlinked to *glnA*, the structural gene for glutamine synthetase, as judged by P22-mediated transduction. Rather it appears to lie at about 42 min on the *Salmonella* chromosome (the *glnA* gene is at 125 min [14]). (ii) Kinetic properties of purified glutamine synthetase from strain *nit-9* are the same as those of the enzyme from wild type. (iii) Like the wild type, *nit* strains derepress synthesis of glutamine synthetase when they are grown on low concentrations of NH_4Cl and convert the enzyme to the unadenylylated or "active" form (17, 28).

The Nit phenotype is suppressed by second-site mutations in the structural gene for glutamine synthetase (*glnA*) that decrease glutamine synthetase activity. (We find this surprising because the Nit phenotype itself mimics the consequences of decreased glutamine synthetase activity.) We do not know whether suppression is due to a change in catalytic activity of glutamine synthetase (e.g., an increase in affinity for ammonia) or to a less direct regulatory effect (14).

Other mutations that cause pleiotropic defects in nitrogen metabolism similar to but distinguishable from those caused by a *nit* mutation have been described in both *Salmonella* and *Klebsiella*. Mutations at the *asm* locus of *Klebsiella* result in low glutamate synthase activity, loss of ability to grow on low concentrations of NH_4Cl as nitrogen source, and loss of ability to use a number of other compounds as nitrogen source (4, 20). In contrast, the *nit* mutation does not affect glutamate synthase activity, and *nit* mutants fail to use a different spectrum of nitrogen sources. Notably, *nit* mutants fail to use glutamate, one of the few compounds that *asm* mutants can use. Mutants of *S. typhimurium* that grow poorly on low concentrations of NH_4Cl and on several organic compounds as nitrogen source were isolated by resistance to methionine sulfoximine (32). Unlike *nit* mutants, these strains have low glutamate synthase activity. Moreover, *nit* mutants remain sensitive to methionine sulfoximine (S. Kustu, unpublished data).

A second type of mutant of *K. pneumoniae* that is unable to use N_2 and various other compounds as nitrogen source was described by Tubb (35). The spectrum of compounds that this mutant fails to use is similar to that of *nit* mutants. However, in contrast to results for *nit* mutants, Tubb finds that his mutant is unable to derepress synthesis of glutamine synthetase in response to nitrogen limitation and proposes that this regulatory defect is the primary lesion (35). In agreement with Magasanik and his colleagues (4, 23, 36), Tubb proposes that decreased ability to synthesize glutamine synthetase results in failure to transcribe nitrogenase and various degradative enzymes (35). This explanation cannot account for the phenotype of *nit* mutants because they are able to derepress synthesis of glutamine synthetase and they have normal levels of degradative enzymes.

In conclusion, our evidence suggests that the *nit* mutation interferes with normal utilization of low concentrations of ammonia as nitrogen source rather than with degradation of other compounds. The biochemical lesion remains undefined. We are currently considering several possibilities, among them the following: (i) that mutant strains have a regulatory defect in ammonia assimilation that was not detected under growth conditions we used and (ii) that they have a defect in ammonia (NH_4^+) transport and fail to maintain normal intracellular ammonia pools. Knowledge of the biochemical lesion in these strains and of the basis for suppression of their growth defects by *glnA* mutations should contribute to our understanding of the regulation of bacterial nitrogen metabolism.

ACKNOWLEDGMENTS

S. K. thanks J. L. Ingraham for his continued interest in this study and for his thoughtful advice and criticism. We thank B. N. Ames, in whose laboratory this work was begun, for many valuable suggestions, and S. W. Artz for criticisms of the manuscript.

This study was supported by Public Health Service grants GM21307-01 to S. K. and GM19993 to B. N. Ames, both grants from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Alper, M. D., and B. N. Ames.** 1975. Positive selection of mutants with deletions of the *gal-chl* region of the *Salmonella* chromosome as a screening procedure for mutagens that cause deletions. J. Bacteriol. **121**:259–266.
2. **Ames, G. F.** 1964. Uptake of amino acids by *Salmonella typhimurium*. Arch. Biochem. Biophys. **104**:1–18.
3. **Brenchley, J. E., C. A. Baker, and L. G. Patil.** 1975. Regulation of the ammonia assimilatory enzymes in *Salmonella typhimurium*. J. Bacteriol. **124**:182–189.
4. **Brenchley, J. E., M. J. Prival, and B. Magasanik.** 1973. Regulation of the synthesis of enzymes responsible for glutamate formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:6122–6128.
5. **Dendinger, S., and W. J. Brill.** 1970. Regulation of proline degradation in *Salmonella typhimurium*. J. Bacteriol. **103**:144–152.
6. **Enomoto, M., and B. A. D. Stocker.** 1974. Transduction by phage P1kc in *Salmonella typhimurium*. Virology **60**:503–514.
7. **Ginsburg, A., J. Yeh, S. B. Hennig, and M. D. Denton.** 1970. Some effects of adenylylation on the biosynthetic properties of the glutamine synthetase from *Escherichia coli*. Biochemistry **9**:633–649.
8. **Halpern, Y. S., and M. Lupo.** 1965. Glutamate transport in wild-type and mutant strains of *Escherichia coli*. J. Bacteriol. **90**:1288–1295.
9. **Halpern, Y. S., and H. E. Umbarger.** 1960. Conversion of ammonia to amino groups in *Escherichia coli*. J. Bacteriol. **80**:285–288.
10. **Halpern, Y. S., and H. E. Umbarger.** 1961. Utilization of L-glutamic and 2-oxoglutaric acid as sole sources of carbon by *Escherichia coli*. J. Gen. Microbiol. **26**:175–183.
11. **Jackson, E. N., and C. Yanofsky.** 1974. Localization of the two functions of the phosphoribosyl anthranilate transferase of *Escherichia coli* to distinct regions of the polypeptide chain. J. Bacteriol. **117**:502–508.
12. **Kingdon, H. S., B. M. Shapiro, and E. R. Stadtman.** 1967. Regulation of glutamine synthetase. VIII. ATP:glutamine synthetase adenylyltransferase, an enzyme that catalyzes alterations in the regulatory properties of glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. **58**:1703–1710.
13. **Kustu, S. G., and G. F.-L. Ames.** 1973. The *hisP* protein, a known histidine transport component in *Salmonella typhimurium*, is also an arginine transport component. J. Bacteriol. **116**:107–113.
14. **Kustu, S. G., and K. McKereghan.** 1975. Mutations affecting glutamine synthetase activity in *Salmonella typhimurium*. J. Bacteriol. **122**:1006–1016.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Marcus, M., and Y. S. Halpern.** 1969. The metabolic pathway of glutamate in *Escherichia coli* K-12. Biochim. Biophys. Acta **177**:314–320.
17. **Mecke, D., and H. Holzer.** 1966. Repression und Inaktivierung von Glutamin-synthetase in *Escherichia coli* durch NH_4^+. Biochim. Biophys. Acta **122**:341–351.
18. **Meers, J. L., D. W. Tempest, and C. M. Brown.** 1970. "Glutamine (amide): 2-oxoglutarate aminotransferase oxido-reductase (NADP)," an enzyme involved in the synthesis of glutamate by some bacteria. J. Gen. Microbiol. **64**:187–194.
19. **Miller, R. E., and E. R. Stadtman.** 1972. Glutamate synthase from *Escherichia coli*. J. Biol. Chem. **247**:7407–7419.
20. **Nagatani, H., M. Shimizu, and R. C. Valentine.** 1971. The mechanism of ammonia assimilation in nitrogen fixing bacteria. Arch. Mikrobiol. **79**:164–175.
21. **Ornellas, E. P., and B. A. D. Stocker.** 1974. Relation of lipopolysaccharide character to P1 sensitivity in *Salmonella typhimurium*. Virology **60**:491–502.
22. **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.
23. **Prival, M. J., J. E. Brenchley, and B. Magasanik.** 1973. Glutamine synthetase and the regulation of histidase formation in *Klebsiella aerogenes*. J. Biol. Chem. **248**:4334–4344.
24. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**:6288–6296.
25. **Roth, J. R.** 1970. Genetic techniques in studies of bacterial metabolism. Methods Enzymol. **17A**:1–35.
26. **Sanderson, K. E., H. Ross, L. Ziegler, and P. H. Mäkelä.** 1972. F^+, Hfr, and F′ strains of *Salmonella typhimurium* and *Salmonella abony*. Bacteriol. Rev. **36**:608–637.
27. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110**:378–381.
28. **Shapiro, B. M., H. S. Kingdon, and E. R. Stadtman.** 1967. Regulation of glutamine synthetase: a new form of the enzyme with altered regulatory and kinetic properties. Proc. Natl. Acad. Sci. U.S.A. **58**:642–649.
29. **Shapiro, B. M., and E. R. Stadtman.** 1970. Glutamine synthetase (*Escherichia coli*). Methods Enzymol. **17A**:910–922.
30. **Smith, H. O., and M. Levine.** 1967. A phage P22 gene controlling integration of prophage. Virology **31**:207–216.
31. **Stadtman, E. R., A. Ginsburg, J. E. Ciardi, J. Yeh, S. B. Hennig, and B. M. Shapiro.** 1970. Multiple molecular forms of glutamine synthetase produced by enzyme catalyzed adenylylation and deadenylylation reactions. Adv. Enzyme Regul. **8**:99–118.

32. **Steimer-Veale, K., and J. E. Brenchley.** 1974. Characterization of *Salmonella typhimurium* strains sensitive and resistant to methionine sulfoximine. J. Bacteriol. **119**:848–856.
33. **Tiemeier, D. C., and G. Milman.** 1972. Chinese hamster liver glutamine synthetase. J. Biol. Chem. **247**:2272–2277.
34. **Trotta, P. P., K. E. B. Platzer, R. H. Haschmeyer, and A. Meister.** 1974. Glutamine-binding subunit of glutamate synthase and partial reactions catalyzed by this glutamine amidotransferase. Proc. Natl. Acad. Sci. U.S.A. **71**:4607–4611.
35. **Tubb, R. S.** 1974. Glutamine synthetase and ammonium regulation of nitrogenase synthesis in *Klebsiella*. Nature (London) **251**:481–485.
36. **Tyler, B., A. B. Deleo, and B. Magasanik.** 1974. Activation of transcription of *hut* DNA by glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. **71**:225–229.
37. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–102.
38. **Woolfolk, C. A., B. Shapiro, and E. R. Stadtman.** 1966. Regulation of glutamine synthetase. I. Purification and properties of glutamine synthetase from *Escherichia coli*. Arch. Biochem. Biophys. **116**:177–192.
39. **Zalkin, H., and T. Murphy.** 1975. Utilization of ammonia for tryptophan synthesis. Biochem. Biophys. Res. Commun. **67**:1370–1377.