JOURNAL OF BACTERIOLOGY, May 1970, p. 524-530
Vol. 102, No. 2

Mutants of *Salmonella typhimurium* Lacking Phosphoenolpyruvate Carboxykinase and α-Ketoglutarate Dehydrogenase Activities

GUILLERMO CARRILLO-CASTAÑEDA AND MANUEL V. ORTEGA

Departamento de Genética y Biología Celular, Centro de Investigación y de Estudios Avanzados del I. P. N., Mexico 14, D.F., Mexico

Received for publication 4 March 1970

Two auxotrophic mutants (SM16 and SM51) of *Salmonella typhimurium*, which for aerobic growth, with hexoses as carbon source, required lysine and methionine (SM51 required also nicotinic acid), were isolated and characterized. The requirement for the amino acids disappeared in anaerobiosis. Neither lipoate nor 4-hydroxybenzoate was effective in supporting aerobic growth of the mutants. The lysine and methionine requirement for aerobic growth was due to the absence in the mutants of the enzymatic activities of the α-ketoglutarate dehydrogenase complex. The mutants could not use succinate as carbon source even after enrichment of the growth medium with acid-hydrolyzed casein and yeast extract. No phosphoenolpyruvate carboxykinase activity was found in the mutants, a phenomenon which explained their inability to use succinate. By interrupted conjugation and by transduction experiments, the positions of the three affected loci, *pck*, *suc*, and Nic, were located at approximately 17 to 19 min of the *S. typhimurium* chromosome; they were found to be closely linked. From different criteria, it appears as if the genetic lesions present in both mutants are due to deletion of a small chromosome fragment.

The enzyme phosphoenolpyruvate carboxykinase (EC 4.1.1.32), the gluconeogenic enzyme that links the tricarboxylic acid cycle with the glycolytic pathway, forming phosphoenolpyruvate by the guanosine triphosphate (GTP) [adenosine triphosphate (ATP), inosine triphosphate (ITP)]-linked decarboxylation of oxalacetate (30), is an indispensable enzyme for microorganisms using succinate as carbon and energy source for growth. Mutants of *Escherichia coli* which lack this activity (15) require for growth the presence of hexoses, pentoses, or glycerol in the incubation medium.

There is a group of mutants of *E. coli* (3, 6, 13, 34) and *Aerobacter aerogenes* (27) which, for aerobic growth with hexoses as the carbon source, exhibits a requirement for lysine and methionine. This requirement reflects a metabolic block in the formation, under aerobic conditions, of succinyl-coenzyme A (CoA), a compound which is necessary for the biosynthesis of lysine (8) and methionine (20). This phenotype can be due to different genetic lesions which produce either the loss of one or all of the components of the α-ketoglutarate dehydrogenase complex (EC 1.2.4.2.) (9, 13, 27), or to the inability to synthesize either lipoic acid (13, 31), or 4-hydroxybenzoic acid (6, 34). All these mutants requiring lysine and methionine for aerobic growth show the common characteristic of growing anaerobically with hexoses as the carbon source without the addition of the amino acids. This can be explained by the following. (i) In *E. coli*, 4-hydroxybenzoate is a precursor of ubiquinones, and these compounds play an important role in the aerobic respiration of the microorganism but not in its anaerobic metabolism (33); (ii) the α-ketoglutarate dehydrogenase complex, which requires lipoic acid for functioning, is not formed anaerobically (1); (iii) under anaerobic conditions, in which the microorganism becomes 4-hydroxybenzoate- and lipoate-independent (13, 34), the succinyl-CoA required for the synthesis of lysine and methionine is formed by the reduction of oxalacetate through the pathway involving malic dehydrogenase, fumarase, the aerobically repressed and anaerobically induced fumarate reductase (14), and succinic thiokinase activities.

In this paper we report the characterization of two mutants of *Salmonella typhimurium* which lack both the α-ketoglutarate dehydrogenase

and phosphoenolpyruvate carboxykinase activities, as well as the location on the bacterial chromosome of the loci encoding these enzymatic activities. Some characteristics of one of the mutants (SM51) have been presented in a brief preliminary report (G. Carrillo-Castañeda and M. V. Ortega, Bacteriol. Proc., p. 109, 1967).

MATERIALS AND METHODS

Bacterial and phage strains. The parental bacterial strain used was *S. typhimurium* LT2, kindly provided by the late M. Demerec. Mutant strains SM16 and SM51 were isolated as spontaneous auxotrophic mutants and were found to be resistant to a low concentration (20 μg/ml) of streptomycin. *S. typhimurium* SR305, HfrA (O-*thrE-ilv-metE*—), Gal⁻, His⁻; and *S. typhimurium* SU576, HfrA (same point of origin and direction of chromosome transfer as strain SR-305), *purC*⁻, both donated by K. E. Sanderson, were used in the conjugation experiments. For transduction, phage P22W, donated by N. Yamamoto, was used.

Culture media. The minimal medium of Davis and Mingioli (7), with citrate omitted, was used throughout, except for the conjugation and transduction experiments (*see* below). Additions to the minimal medium were as follows. Carbon sources were added at 0.4% (w/v), Yeast Extract (Difco) was used at 0.04% (w/v), L-lysine and L-methionine were each added at a concentration of 20 μg/ml, nicotinic acid was used at 1 μg/ml, DL-lipoic acid and 4-hydroxybenzoate were added at 100 ng/ml each. The enriched medium contained the carbon source and Yeast Extract at the indicated concentrations plus acid-hydrolyzed casein (Casamino Acids, Difco) at 0.2% (w/v). When solid medium was used, agar (Difco) was added at 1.5% (w/v) to the corresponding liquid medium.

Mutagens. The following mutagens were used: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (K & K Laboratories, Plainview, N.Y.), 5-bromodeoxyuridine and 2-aminopurine (Calbiochem, Los Angeles, Calif.), methyl methanesulfonate and ethyl methanesulfonate (Eastman Organic Chemicals, Rochester, N.Y.), and the acridine half-mustards ICR 191 and ICR 372, which were gifts of H. J. Creech (The Institute for Cancer Research, Philadelphia, Pa.).

Radioactive material. $NaH^{14}CO_3$, specific activity of 26.7 mc per mmole, was purchased from The Radiochemical Centre, Buckinghamshire, England.

Preparation of cell-free extracts. Cells were aerobically grown overnight at 37 C in 2-liter batches of either glucose or glycerol minimal medium supplemented with lysine, methionine, and yeast extract. The cells were harvested by centrifugation in the cold (4 C), washed once with cold saline solution (0.85% NaCl, w/v), resuspended in cold distilled water, and disrupted by sonic treatment during 4 to 5 min in a ultrasonic power unit (Measuring & Scientific Equipment, Ltd., London, England) set at 0.8 amp. Intact cells and debris were separated by centrifugation in the cold for 30 min at 10,000 × *g*. The cell-free extracts used for the determination of phosphoenolpyruvate carboxykinase and carboxylase activities were dialyzed for 8 hr at 4 C against 50 volumes of a 1 mM ethylenediaminetetraacetic acid-cysteine solution, *p*H 7.4. Those samples used for the determination of the activities of the α-ketoglutarate dehydrogenase complex were dialyzed overnight against 100 volumes of 20 mM tris(hydroxymethyl)aminomethane (Tris) buffer, *p*H 7.0. The protein content of the extracts was determined according to Lowry et al. (19).

Enzymatic determinations. The α-ketoglutarate dehydrogenase activity was determined by a slightly modified procedure of that of Hager and Kornberg (9). The complete system contained, in a final volume of 3 ml: 300 μmoles of potassium phosphate buffer (*p*H 7.4), 40 μmoles of sodium α-ketoglutarate, 3 μmoles of thiamine pyrophosphate, 26 μmoles of L-cysteine, 4.5 μmoles of nicotinamide adenine dinucleotide (NAD), 4 μmoles of CoA, 10 μmoles of $MgCl_2$, and extract (6 to 8 mg of protein). The reaction was carried out at 25 C, and the NAD reduction was followed spectrophotometrically at 340 nm by using a double-beam spectrophotometer (Hitachi Perkin-Elmer model Coleman 124).

Oxidative decarboxylation of α-ketoglutarate was determined manometrically, with ferricyanide as electron acceptor, by the method of Hager and Kornberg (9). The complete system contained, in a final volume of 3 ml: 100 μmoles of potassium phosphate (*p*H 6.0), 20 μmoles of sodium α-ketoglutarate, 0.5 μmoles of thiamine pyrophosphate, 25 μmoles of potassium ferricyanide, and extract (4 to 6 mg of protein). The reaction mixture was incubated at 30 C under a nitrogen atmosphere. Lipoyl reductase-transsuccinylase activity was assayed by the methods of Sanadi, Langley, and White (22) and of Hager and Kornberg (9). In the first case, the complete system contained, in 5.4 ml: 180 μmoles of potassium phosphate buffer (*p*H 7.4), 10 μmoles of sodium α-ketoglutarate, 0.5 μmoles of NAD, 2.4 mmoles of hydroxylamine, 13 μmoles of cysteine, and extract (6 to 8 mg of protein). Samples were incubated for 30 min at 30 C. In the second case, the complete system contained, in 3 ml: 300 μmoles of potassium phosphate buffer (*p*H 7.5), 100 μmoles of ATP, 0.6 μmoles of CoA, 60 μmoles of sodium succinate, 30 μmoles of reduced lipoic acid, and extract (6 to 8 mg of protein). At the end of the incubation period, 800 μmoles of hydroxylamine was added to the reaction mixtures. Reaction mixtures were incubated for 30 min at 30 C. In both cases, the succinyl hydroxamate formed was determined colorimetrically by the addition of the $FeCl_3$ reagent.

Dihydrolipoyl dehydrogenase was measured by the method of Sanadi, Langley, and Searls (21). The complete system contained, in a final volume of 3 ml: 75 μmoles of potassium phosphate buffer (*p*H 7.1), 0.39 μmoles of reduced lipoic acid, 0.48 μmoles of NAD, and extract (4 to 5 mg of protein). The reaction was carried out at 25 C, and the NAD reduction was followed spectrophotometrically at 340 nm, as in the case of the α-ketoglutarate dehydrogenase.

Succinic thiokinase activity was determined by the method of Kaufman (17). The complete system contained, in a final volume of 2 ml: 100 μmoles of Tris buffer (*p*H 7.4), 100 μmoles of potassium succinate, 10 μmoles of ATP, 130 nmoles of CoA, 20 μmoles of

L-cysteine, 10 μmoles of $MgCl_2$, 960 μmoles of hydroxylamine, and extract (3 to 4 mg of protein). The reaction mixtures were incubated for 30 min at 37 C, and the succinyl hydroxamate formed was determined colorimetrically.

Phosphoenolpyruvate carboxykinase was determined manometrically by measuring the production of CO_2 from oxalacetate in the presence of ATP. The reaction mixture contained, in a final volume of 3 ml: 80 μmoles of Tris buffer (*p*H 7.4), 20 μmoles of oxalacetate, 10 μmoles of ATP, 5 μmoles of $MnCl_2$, and extract (4 to 6 mg of protein). The reaction mixture was incubated at 30 C under a nitrogen atmosphere. A control test was run in which ATP was omitted from the incubation mixture; in this way, correction was made for the decarboxylation of oxalacetate which may have occurred either spontaneously or carried out by other enzymatic reactions.

Phosphoenolpyruvate carboxylase was determined by measuring the incorporation of $^{14}CO_2$ into oxalacetate, with phosphoenolpyruvate as substrate, and isolating the corresponding 2,4-dinitrophenylhydrazone (29). The reaction system contained, in a final volume of 3 ml: 80 μmoles of Tris buffer (*p*H 7.4), 10 μmoles of potassium phosphoenolpyruvate, 5 μmoles of $MnCl_2$, 20 μmoles of $NaHCO_3$ (containing 3×10^6 counts/min of $NaH^{14}CO_3$), and extract (4 to 5 mg of protein). The samples were incubated for 8 min at 30 C, under a nitrogen atmosphere. The purification and identification of the oxalacetate, 2,4-dinitrophenylhydrazone was carried out by the method of Kun and García-Hernández (18). The radioactivity present in the isolated and purified phenylhydrazone was determined with an ultrascalator (Nuclear-Chicago Corp., model C-186 A).

Conjugation experiments. Interrupted mating experiments were performed by the procedure of Sanderson and Demerec (24), except that the medium used for growing both the donor and recipient strains, as well as for mating, was glucose-nutrient broth. Appropriate dilutions of samples of the mating mixture, taken at various intervals and vigorously shaken for 1 min, were plated in triplicate on plates selective for recombinants. The plates were incubated at 37 C, and the recombinant-containing colonies were counted after 36 hr of incubation.

Transduction experiments. Phage P22W was propagated in the LT2 parental strain. The recipient bacteria and the phage (at a multiplicity of 3) were mixed in 3 ml of glucose-nutrient broth containing 1 mM NaCl. The suspension was incubated at 37 C for 10 min; at the end of the incubation period, 0.1-ml samples were taken and plated on 10 plates selective for transductants. The clones that developed were counted after incubation for 36 hr at 37 C.

RESULTS

Characterization of mutants SM16 and SM51. Under aerobic conditions, both mutant strains failed to grow, even in enriched medium, unless the carbon sources were hexoses (glucose, fructose, galactose, and mannitol), ribose, or glycerol; there was no growth when the carbon sources were succinate, fumarate, malate, or acid-hydrolyzed casein. Under aerobic conditions, in the presence of hexoses, ribose, or glycerol, mutants SM16 and SM51 required both lysine and methionine for growth; mutant SM51 also required nicotinic acid. Under anaerobic conditions, however, the mutants grew in minimal glucose medium (plus nicotinic acid for SM51) without any amino acid supplement. Even in the presence of lysine and methionine the aerobic growth of the mutants was slow. The addition of reducing agents such as ascorbic acid, mercaptoethanolamine, glutathione, or cysteine, improved the rate of growth; a similar effect was obtained by the addition of yeast extract. Lipoic acid or 4-hydroxybenzoate did not overcome the aerobic requirement for lysine and methionine.

Lack of α-ketoglutarate dehydrogenase activity. The aerobic and anaerobic growth characteristics shown by mutants SM16 and SM51 suggested that they might reflect a lesion affecting some or all of the activities of the α-ketoglutarate dehydrogenase complex: the α-ketoglutarate decarboxylase, the lipoyl reductase-transsuccinylase, and the dihydrolipoyl dehydrogenase activities. Accordingly, the activities of the whole α-ketoglutarate dehydrogenase complex and of each one of the three aforementioned enzymes, as well as that of the succinic thiokinase, were determined in the cell-free extracts of the LT2, SM16 and SM51 strains. The results are presented in Table 1. It should be pointed out that no lipoyl reductase-transsuccinylase activity could be demonstrated in any of the extracts by using the methods described by Sanadi et al. (22) and by Hager and Kornberg (9). Although the LT2 extract showed the α-ketoglutarate dehydrogenase, decarboxylase, dihydrolipoyl dehydrogenase, and succinic thiokinase activities, those of the mutants exhibited only the succinic thiokinase activity. These results permitted us to assign the *lysB*$^-$ (*suc*$^-$) genotype to both mutants.

Lack of phosphoenolpyruvate carboxykinase activity. The fact that mutants SM16 and SM51 failed to grow, even in enriched medium, with succinate, fumarate, or malate as carbon sources, was circumstantial evidence for the possible lack of phosphoenolpyruvate carboxykinase activity. This assumption was confirmed by the demonstration that cell-free extracts of both mutants did not have this activity. From the results presented in Fig. 1 it can be seen that, although the cell-free extract of the LT2 strain decarboxylated oxalacetate very efficiently, those of the mutant strains did not. Accordingly, the genotype *pck*$^-$ was assigned to both mutants.

The activity of the phosphoenolpyruvate

carboxylase present in the cell-free extracts was also determined and the results obtained are shown in Table 2. The cell-free extracts from the parental and mutant strains showed a very active phosphoenolpyruvate carboxylase; in the three extracts, the activity was equally inhibited (approximately 90%) by the presence of adenosine diphosphate (ADP), in agreement with the observations of Theodore and Englesberg (29). The enzymatic product of the phosphoenolpyruvate carboxylase activity was isolated as the 2,4-dinitrophenylhydrazone and purified. It migrated as a single component in chromatography, with an R_F identical to that of the authentic 2,4-dinitrophenylhydrazone of oxalacetate.

Chromosomal location of the affected loci. The positions of the affected loci in the bacterial chromosome of the mutants were located by interrupted mating experiments. Figures 2 and 3 show the kinetics of formation of prototrophic recombinants of mutants SM16 and SM51, respectively. The results obtained indicate that these three loci, *pck*, *lysB* (*suc*), and Nic, are located at approximately 17 to 19 min of the *S. typhimurium* chromosome. This time of entry coincides with that reported by Sanderson and Demerec (24) and Sanderson (23) for the *lysB* locus. Identical results were obtained when either HfrA strains SR305 or SU576 were used as donors. It should be pointed out that the two or three affected loci (depending on the recipient strain used) were corrected at what appears to be the same time, probably indicating a very close linkage.

TABLE 1. *Activities of the α-ketoglutarate dehydrogenase complex, its different enzyme components, and of succinic thiokinase in cell-free extracts of strains LT2, SM16, and SM51*[a]

Cell-free extract	Specific activities of			
	α-Keto-glutarate dehydro-genase[b]	α-Keto-glutarate decarboxy-lase[c]	Dihydro-lipoyl dehydro-genase[d]	Succinic thiokinase[e]
LT2	0.015	1.0	0.0096	0.0021
SM16	0.000	0.0	0.0000	0.0017
SM51	0.000	0.0	0.0000	0.0019

[a] Not including lipoyl reductase-transsuccinylase (*see* text).

[b] Micromoles of nicotinamide adenine dinucleotide reduced per minute per milligram of protein.

[c] Microliters of carbon dioxide evolved per minute per milligram of protein.

[d] Micromoles of lipoate oxidized per minute per milligram of protein.

[e] Micromoles of succinyl hydroxamate formed per minute per milligram of protein.

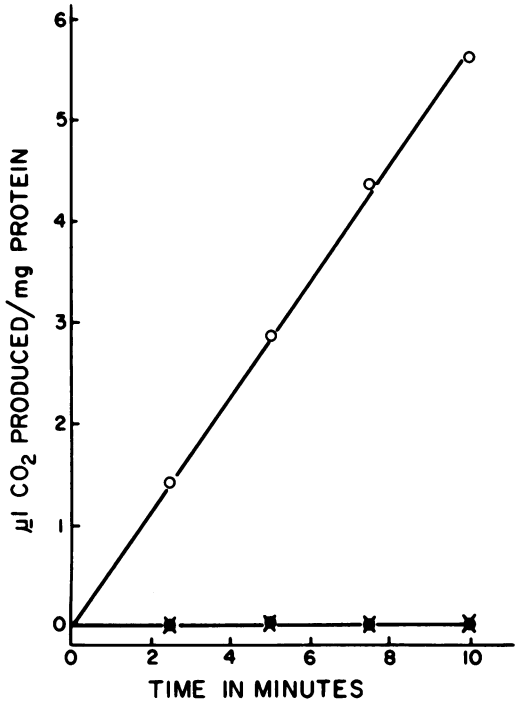

FIG. 1. *Phosphoenolpyruvate carboxykinase activity present in cell-free extracts of strains LT2, SM16, and SM51. Symbols:* ○, *activity of LT2 extracts;* ●, *activity of SM16 extracts;* ×, *activity of SM51 extracts.*

To substantiate the linkage relationship observed in conjugation, transduction experiments were carried out by using phage P22W propagated in the LT2 strain. The results obtained indicated that the affected characters of each mutant were corrected all together. For instance, of 546 transductants from strain SM51 selected for the character *lysB*$^+$ (*suc*$^+$), all were also Nic$^+$ and *pck*$^+$; of 732 transductants from strain SM16 selected for the character *lysB*$^+$ (*suc*$^+$), all were also *pck*$^+$. Similar results were obtained with transductants selected for any one of the other affected characters.

Nature of the genetic lesion. A study of the spontaneous reversion for any of the affected characters of both mutants demonstrated that all of them were very stable; the spontaneous reversion frequency for any of them was found to be less than 1×10^{-11}. There was no phenotypic correction of any of the lesions by streptomycin. With selective plates seeded with 10^{10} bacteria, no revertants for any of the affected characters could be induced by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, 5-bromodeoxyuridine, 2-aminopurine, methyl or ethyl methanesulfonate, or the acridine half-mustards

TABLE 2. *Phosphoenolpyruvate carboxylase activity present in cell-free extracts of strains LT2, SM16, and SM51*[a]

System	Specific activity[b]		
	LT2	SM16	SM51
Complete system	0.140	0.397	0.265
Complete system plus ADP[c]	0.013	0.037	0.022

[a] Oxalacetate formed isolated as the 2,4-dinitrophenylhydrazone.

[b] Expressed as nanomoles of carbon dioxide incorporated per minute per milligram of protein.

[c] In studying the inhibitory effect of adenosine diphosphate (ADP), 10 μmoles of this compound was added to the incubation mixtures.

ICR 191 and 372. These results, together with those of the transduction experiments, make probable the assumption that the lesion present in mutants SM16 and SM51 is a deletion. In support of this hypothesis, there is also the fact that no residual enzymatic activity encoded by the loci *pck* and *lysB* (*suc*) could be demonstrated.

DISCUSSION

The requirement for lysine and methionine for aerobic growth shown by mutants SM16 and SM51 is due to the absence of at least two of the three components of the α-ketoglutarate dehydrogenase complex, the α-ketoglutarate decarboxylase and the dihydrolipoyl dehydrogenase, although, probably, the lipoyl reductase-transsuccinylase is also missing. If this is the case, these mutants resemble the pyruvate dehydrogenase-less type III mutants of *E. coli* in which the three enzymatic activities of that complex are missing (12). With regard to the fact that it was not possible to demonstrate the presence of the lipoyl reductase-transsuccinylase in any of the extracts used, it should be pointed out that this anomaly has also been found in other systems and has been explained, indicating that when the reductase-transacylase of an α-ketoacid dehydrogenase complex is within the complex, it may be active only with the enzyme-bound lipoate. Only when the complex is dissociated may it utilize external lipoate as substrate (22). This could be the case for the failure in the determination of this activity with the cell-free extracts of strains LT2, SM16, and SM51.

We suggest that for those *S. typhimurium* mutants in which the *lysB*$^-$ character is due to the lack of one or more of the activities of the α-ketoglutarate dehydrogenase complex, the terms *lys* + *met* or *lysB* should be dropped and substituted by the term *suc*, as has already been done for similar *E. coli* mutants (28). By analogy with the equivalent loci of the pyruvate dehydrogenase complex of *E. coli*, the locus responsible for the α-ketoglutarate decarboxylase activity could be named *sucE* and that responsible for the lipoyl reductase-transsuccinylase activity, *sucF*. Accordingly, mutants SM16 and SM51 are *sucE*$^-$ and, probably, *sucF*$^-$ as well.

The fact that mutants SM16 and SM51 failed

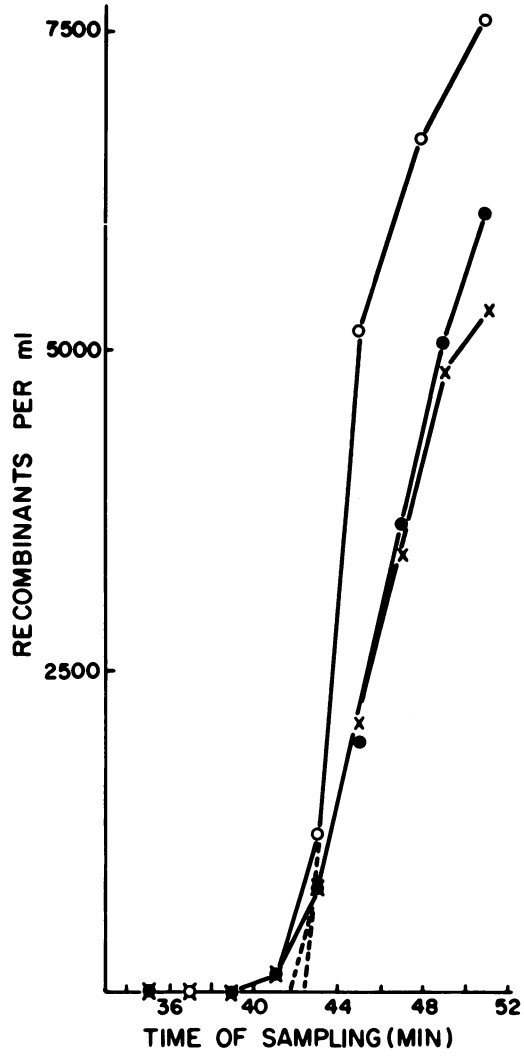

FIG. 2. *Kinetics of chromosome transfer by HfrA SU576 to recipient SM16. Symbols: ○, recombinants which grew in minimal glucose medium, lysB*$^+$ *and pck*$^{+,-}$*; ×, recombinants which grew in minimal succinate medium, pck*$^+$ *and lysB*$^{+,-}$*; ●, recombinants which grew in minimal succinate medium supplemented with lysine and methionine, pck*$^+$ *and lysB*$^{+,-}$.

to grow with succinate as carbon and energy source is explained by their lack of phosphoenolpyruvate carboxykinase activity. They showed a very active phosphoenolpyruvate carboxylase, and this phenomenon indicates that the reaction catalyzed by each enzyme is functionally irreversible; the anaplerotic function of the carboxylase (2) and the gluconeogenic of the carboxykinase (30) are not interchangeable (29).

The results from the conjugation and transduction experiments indicate that the genes coding for the α-ketoglutarate dehydrogenase and phosphoenolpyruvate carboxykinase activities are very closely linked although, on the basis of the characteristics of the mutants, it was not possible to elucidate their sequence in the *S. typhimurium* chromosome. With regard to the Nic^- character present in mutant SM51, the results obtained indicate that it is located very close to the *suc* and *pck* loci. As in the *E. coli* chromosome, the locus *nicA* is closely linked to the *suc* locus (28), it is possible that the Nic locus reported in this paper for *S. typhimurium* corresponds to the NicA locus of *E. coli*.

These results, and those indicating the nonreversibility, either spontaneous or induced, of any of the affected characters, indicate that the lesions present in mutants SM16 and SM51 probably are because of a deletion in their chromosomes.

Finally, the fact that both mutants show a low resistance to streptomycin can be explained by the following known facts. (i) For several bacterial species, the bactericidal effect of streptomycin is less under anaerobic than on aerobic conditions (5, 10); (ii) under the former conditions, there is an increase in the resistance to the antibiotic (11, 32); and (iii) mutants of *E. coli* and *Staphylococcus aureus* with low respiratory activity show marked resistance to streptomycin (4, 16). As a matter of fact, the last property has been used recently by Săsărman et al. (25, 26) for the selection, by the use of the streptomycin-related aminoglycoside antibiotic neomycin, of heminless mutants with low respiratory activity which show low sensitivity to this antibiotic. Mutants SM16 and SM51, which lack the α-ketoglutarate dehydrogenase activity, can be considered as having their metabolism shifted towards anaerobiosis (1), and, therefore, it is not surprising to find them resistant to low concentrations of streptomycin.

ACKNOWLEDGMENTS

We thank E. Englesberg for his advice and criticism. The support given by the Fundación Sears Roebuck de México to G. C. C. and the technical assistance of J. Chavez are gratefully acknowledged.

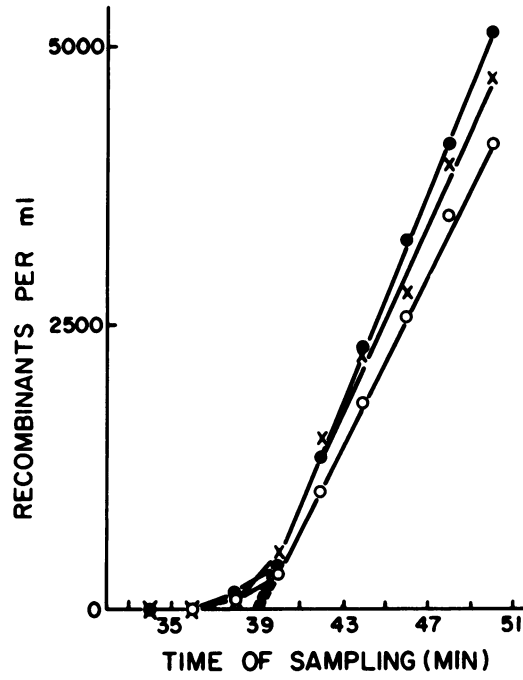

FIG. 3. *Kinetics of chromosome transfer by HfrA SR305 to recipient SM51. Symbols:* ○, *recombinants which grew in minimal glucose medium, lysB⁺, Nic⁺, and pck⁺;* ×, *recombinants which grew in minimal succinate medium, pck⁺, Nic⁺, and lysB⁺,⁻;* ● *recombinants which grew in minimal succinate medium supplemented with nicotinic acid, pck⁺, Nic⁺,⁻, and lysB⁺,⁻.*

LITERATURE CITED

1. Amarasinham, C. R., and B. D. Davis. 1965. Regulation of α-ketoglutarate dehydrogenase formation in *Escherichia coli*. J. Biol. Chem. 240:3664–3668.
2. Ashworth, J. M., and H. L. Kornberg. 1966. Anaplerotic fixation of carbon dioxide by *Escherichia coli*. Proc. Roy. Soc. (London) Ser. B 165:179–188.
3. Back, K. J. C., and E. G. Westaway. 1962. Studies on a mutant of *Escherichia coli* which requires both methionine and lysine for growth. J. Gen. Microbiol. 27:41–50.
4. Beljanski, M., and M. Beljanski. 1957. Sur la formation d'enzymes respiratoires chez un mutant d'*Escherichia coli* streptomycino-résistant et auxotrophe pour l'hemine. Ann. Inst. Pasteur. 92:396–412.
5. Bondi, A., Jr., C. C. Dietz, and E. H. Spaulding. 1946. Interference with the antibacterial action of streptomycin by reducing agents. Science 103:399.
6. Davis, B. D. 1955. Intermediates in amino acid biosynthesis. Adv. Enzymol. 16:247–312.
7. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 60:17–28.
8. Gilvarg, C. 1957. *N*-Succinyl-L-diaminopimelic acid, an intermediate in the biosynthesis of diaminopimelic acid. Biochim. Biophys. Acta 24:216–217.
9. Hager, L. P., and H. L. Kornberg. 1961. On the mechanism of α-oxo-glutarate oxidation in *Escherichia coli*. Biochem. J. 78:194–198.
10. Hancock, R. 1960. The bactericidal action of streptomycin on *Staphylococcus aureus* and some accompanying biochemical changes. J. Gen. Microbiol. 23:179–196.
11. Hancock, R. 1962. Uptake of ^{14}C-streptomycin by some

microorganisms and its relation to their streptomycin sensitivity. J. Gen. Microbiol. 28:493–501.
12. Henning, U., C. Herz, und K. Szolyvay. 1964. Polarisation und Disproportionalität der Synthese von Enzymkomponenten des Pyruvatdehydrogenase-Komplexes in *Escherichia coli* K12. Z. Vererbungsl. 95:236–259.
13. Herbert, A. A., and J. R. Guest. 1968. Biochemical and genetic studies with lysine + methionine mutants of *Escherichia coli*: lipoic acid and α-ketoglutarate dehydrogenase-less mutants. J. Gen Microbiol. 53:363–381.
14. Hirsch, C. A., M. Rasminsky, B. D. Davis, and E. C. C. Lin. 1963. A fumarate reductase in *Escherichia coli* distinct from succinate dehydrogenase. J. Biol. Chem. 238:3770–3774.
15. Hsie, A. W., and H. V. Rickenberg. 1966. A mutant of *Escherichia coli* deficient in phosphoenolpyruvate carboxykinase activity. Biochem. Biophys. Res. Commun. 25:676–683.
16. Jensen, J., and E. Thofern. 1953. Chlorhämin (Ferriporphyrinchlorid) als Bakterienwuchsstoff. I. Z. Naturforsch. 8b:599–603.
17. Kaufman, S. 1955. α-Ketoglutaric dehydrogenase system and phosphorylating enzyme from heart muscle, p. 714–722. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 1. Academic Press Inc., New York.
18. Kun, E., and M. Garcia-Hernandez. 1957. Identification and quantitative determination of ketoacids by paper chromatography. Biochim. Biophys. Acta 23:181–186.
19. 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.
20. Rowbury, R. J., and D. D. Woods. 1964. O-succinylhomoserine as an intermediate in the synthesis of cystathionine by *Escherichia coli*. J. Gen. Microbiol. 36:341–358.
21. Sanadi, D. R., M. Langley, and R. L. Searls. 1959. α-Ketoglutaric dehydrogenase. VI. Reversible oxidation of dihydrothioctamide by diphosphopyridine nucleotide. J. Biol. Chem. 234:178–182.
22. Sanadi, D. R., M. Langley, and F. White. 1959. a-Ketoglutaric dehydrogenase. VII. The role of thioctic acid. J. Biol. Chem. 234:183–187.
23. Sanderson, K. E. 1967. Revised linkage map of *Salmonella typhimurium*. Bacteriol. Rev. 31:354–372.
24. Sanderson, K. E., and M. Demerec. 1965. The linkage map of *Salmonella typhimurium*. Genetics 51:897–913.
25. Săsărman, A., and T. Horodniceanu. 1967. Locus determining normal colony formation on the chromosome of *Escherichia coli* K12. J. Bacteriol. 94:1268–1269.
26. Săsărman, A., M. Surdeanu, G. Szégli, T. Horodniceanu, V. Greceanu, and A. Dumitrescu. 1968. Hemin-deficient mutants of *Escherichia coli* K12. J. Bacteriol. 96:570–572.
27. Stouthamer, A. H. 1967. Mutant strains of *Aerobacter aerogenes* which require both methionine and lysine for aerobic growth. J. Gen. Microbiol. 46:389–398.
28. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
29. Theodore, T. S., and E. Englesberg. 1964. Mutant of *Salmonella typhimurium* deficient in the carbon dioxide-fixing enzyme phosphoenolpyruvic carboxylase. J. Bacteriol. 88:946–955.
30. Utter, M. F., and K. Kurahashi. 1954. Mechanism of action of oxalacetic carboxylase. J. Biol. Chem. 207:821–841.
31. Vise, A. B., and J. Lascelles. 1967. Some properties of a mutant strain of *Escherichia coli* which requires lysine and methionine or lipoic acid for growth. J. Gen. Microbiol. 48:87–93.
32. Williamson, G. M. 1958. Dihydrostreptomycin and anaerobiosis. Indirect evidence for two sites of action of dihydrostreptomycin. J. Gen. Microbiol. 19:584–591.
33. Wyn-Jones, R. G. 1967. Ubiquinone deficiency in an auxotroph of *Escherichia coli* requiring 4-hydroxybenzoic acid. Biochem. J. 193:714–719.
34. Wyn-Jones, R. G., and J. Lascelles. 1967. The relationship of 4-hydroxybenzoic acid to lysine and methionine formation in *Escherichia coli*. Biochem. J. 103:709–713.