JOURNAL OF BACTERIOLOGY, Dec. 1970, p. 1318-1324
Vol. 104, No. 3
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Carbohydrate Accumulation and Metabolism in *Escherichia coli*: Characteristics of the Reversions of *ctr* Mutations[1]

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Received for publication 23 July 1970

The reversion behavior of pleiotropic carbohydrate mutants, previously designated as *ctr*, was studied. The mutants revert to complete restoration of the wild-type phenotype, as well as to a spectrum of partial wild-type phenotypes. Lac$^+$ reversions were found in the *lac* region (11 min) and some Mal$^+$ reversions occurred at *malB* (79 min), at a distance from the site of the *ctr* mutations (46 to 47 min). About one-third of Lac$^+$ and Mal$^+$ revertants were constitutive for uptake of their respective substrates, and one-third modified for inducibility. The remaining third were not distinguishable from wild type. Induction of a *ctr* mutation in a *lac* constitutive strain, either operator or repressor mutant, did not affect lactose metabolism. A polar-like *ctr* mutant, deficient in both enzyme I and heat-stable protein of the phosphoenolpyruvate-dependent phosphotransferase strain was also described. Partial revertants of *ctr* were still found to lack enzyme I.

In previous publications (12, 13; R. J. Wang and M. L. Morse, Bacteriol. Proc., p. 37, 1966; Wang and Morse, Bacteriol. Proc., p. 105, 1967) we described pleiotropic mutations (*ctr*) in *Escherichia coli* which affect the utilization of carbohydrates. Tests with radioactively labeled carbohydrates showed that the cells were incapable of concentrating the substance, the utilization of which was affected by the mutation. However, *ctr* mutants were able to grow utilizing glucose-6-phosphate, arabinose, and pyruvate, which argues against a nonspecific permeability effect and also indicates that the later metabolic pathways for the utilization of carbohydrates are intact. The chromosomal site of the *ctr* mutations was located at 46 to 47 min on the map of Taylor and Trotter (11) by bacterial matings and by transduction. This site was also shown to be the site of the mutation in *E. coli* strain MM6 which causes inability to grow on mannitol, fructose, mannose, succinate, and sorbitol. The biochemical defect in strain MM6 has been shown (9) to be the absence of enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system (4). In preliminary biochemical experiments, the metabolic defect in a *ctr* mutant could not be complemented by extracts of MM6, whereas extracts containing enzyme I did complement extracts of *ctr* mutants. From this preliminary observation it was concluded that *ctr* mutants are also lacking enzyme I. In support of this conclusion are the observations of the group in Moscow (1) that the mutation in *E. coli* K-12 strain P34, which they report as deficient in both enzyme I and the heat-stable protein (HPr) factor of the phosphotransferase system, maps in the neighborhood of 48 min.

Pleiotropic mutations affecting carbohydrate utilization in *Salmonella typhimurium* (8), *Staphylococcus aureus* (2), and *Aerobacter aerogenes* (3, 11) have been described. In each of these cases, the pleiotropic effect was owing to a defect in either enzyme I or HPr of the phosphotransferase system. In the case of *Salmonella*, the sites of the genes for enzyme I and HPr production were located. The site for the HPr gene corresponds to that of *ctr* in *E. coli*, whereas the site for the enzyme I gene corresponds to a site at about 5 min on the *E. coli* chromosomal map.

In considering the reversion behavior of these pleiotropic mutations, the steps of the phosphotransferase system should be recalled. Enzyme I transfers phosphate from phosphoenolpyruvate to the HPr and must therefore have sites to recognize these entities. HPr in the phosphorylated form is recognized by a number of membrane bound enzyme II forms, each specific for a

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particular carbohydrate or group of carbohydrates which transfer the phosphate group to the carbohydrates concerned. It is not known whether these enzymes have a common device for recognizing phosphorylated HPr. If not, the HPr molecule [molecular weight, 9,340 ± 230 (6)] must have several different sites for recognition by the specific enzyme II forms.

Reversion studies of enzyme I mutants of *Salmonella* (8) and *S. aureus* (2) have been reported. With one exception (8) enzyme I mutants revert only to the complete wild-type phenotype. In the exceptional case, a partial wild type (Glu$^+$ Mal$^+$) was also produced. The mechanism of this reversion was not elucidated, but it was noted that the reversions still lacked enzyme I, indicating that the reversion had occurred by a change in a cryptic alternate pathway. Reversion studies of HPr mutants have not been reported.

In this paper we present a study of the reversion characteristics of *ctr* mutants, which are to several phenotypes as well as to the complete wild type.

MATERIALS AND METHODS

Most of the cultures utilized and procedures employed have been previously described (12, 13). Some special cultures were synthesized, for example, the *metA cysG* strain used to locate Mal reversions, which was accomplished by a conventional cross between suitable parents.

Mutations were also induced as previously described, by using short exposures to nitrosoguanidine and platings on cholate indicator agar, also described earlier. Isolation of *ctr* mutants was accomplished on indicator agar containing glucose.

Phenotypic reversions were isolated from platings on cholate indicator agar containing the designated carbohydrate (1% concentration), usually selecting the revertants from isolated colonies.

The methods of studying carbohydrate uptake has also been described previously (12).

RESULTS

Spectrum of revertant phenotypes. The reversion behavior of at least nine independent *ctr* mutants has been studied. The *ctr* mutants themselves were heterogeneous, in that the set included one temperature-sensitive synthesis mutant and one mutant lacking HPr and enzyme I of the phosphotransferase system, in addition to the "standard" *ctr* mutant phenotype. The substrates for revertant selection included fructose, maltose, glucose, lactose, mannitol, mannose, glycerol, salicin, succinate, and in a few cases melibiose, although not all *ctr* mutants were studied on all selective carbohydrates. The different mutants did not appear to differ in their reversion pattern although no extensive effort was made to test this point. A cautionary note is made here, that if reversions are selected as papillae from growth films instead of from isolated colonies, a bias is introduced favoring the selection of complete reversion of the *ctr* phenotype. Selecting papillae from isolated colonies not only gives a better perspective of the reversion spectrum but also ensures that each revertant is independent of the others.

An example of the reversion pattern of one of the most extensively studied mutants, *ctr1*, is presented in Table 1. At least nine partial phenotypic reversion patterns are selectable on the various carbohydrates used, in addition to the complete reversion of the Ctr$^-$ phenotype to Ctr$^+$. Complete reversion of phenotype is obtainable in each of the selective environments, with the possible exception of succinate which was not studied extensively. In many instances, reversion phenotype was confirmed in broth plus *p*H indicator and 1.0% carbohydrate after initial characterization on cholate indicator agar. Since both broth and solid media are well buffered, reversion to weak utilization is discriminated against.

A composite of the reversion behavior of all strains is presented in Fig. 1, which indicates more than nine possible partial reversion routes. This reversion behavior contrasts very distinctly with previous work on pleiotropic mutants in

TABLE 1. *Reversion patterns of the ctr1 mutation*[a]

Selective medium	Reversion phenotypes observed
Mannitol	Ctr$^+$
Glucose	Ctr$^+$
	Glu$^+$
	Glu$^+$ Fru$^+$
Fructose	Ctr$^+$
	Fru$^+$ Man$^+$
Lactose	Ctr$^+$
	Lac$^+$
	Lac$^+$ Mal$^+$
Maltose	Ctr$^+$
	Mal$^+$
	Mal$^+$ Lac$^+$
	Mal$^+$ Glp$^+$
	Mal$^+$ Glu$^+$
	Mal$^+$ Glu$^+$ Fru$^+$

[a] The revertants were tested for ability to produce acid from glucose, lactose, maltose, mannitol, mannose, fructose, glycerol, and melibiose. The partial reversion phenotypes given represent only those substances for which there was change; i.e., Glu$^+$ is still Lac$^-$ Mal$^-$ Mtl$^-$ Man$^-$ Fru$^-$ Glp$^-$.

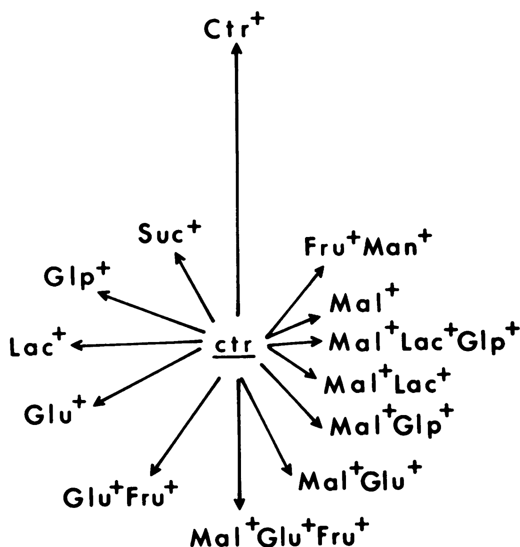

FIG. 1. *Reversion pattern of ctr mutants. The ctr phenotype is Glu⁻ Lac⁻ Mal⁻ Fru⁻ Man⁻ Mtl⁻ Glp⁻ Suc⁻. The changes shown represent partial reversion (Glu⁺ Lac⁻ Mal⁻ Fru⁻ Man⁻ Mtl⁻ Glp⁻ Suc⁻, etc.) as well as complete reversion (Ctr⁺).*

Salmonella (8). It is also very different from the reversion behavior of similar pleiotropic mutants of *S. aureus* which have been studied extensively in this laboratory.

Partial reversion by one step does not preclude a subsequent step. A *ctr* mutant can be carried through several sequential steps to obtain the nearly wild-type phenotype.

Genetic location of a Lac^+ and of a Lac^+ Mal^+ reversion. The site of the Lac^+ and Lac^+ Mal^+ reversions was sought in the following way. A strain diploid for the *lac* region was synthesized by crossing a F′ Lac^+ donor strain with an F^- *lacY lacZ* recipient to yield F^- y^- z^-/F^+ y^+ z^+, which is Lac^+ because of the dominance of y^+ and z^+. The diploid was treated with nitrosoguanidine and *ctr* mutants isolated on cholate-glucose indicator agar. These *ctr* mutants were Lac^- and unable to utilize the other carbohydrates described by the *ctr* phenotype.

Revertants were isolated on cholate-lactose medium. Of 24 independent revertants, 13 were concomitantly Mal^+.

The Lac^+ Mal^- and Lac^+ Mal^+ revertants were treated with acridine orange. After this treatment, the Lac^+ Mal^- revertants gave rise to Lac^- Mal^- descendents, whereas the Lac^+ Mal^+ revertants yielded only Lac^- Mal^+ progeny. These results (becoming Lac^-) can be understood as a result of the loss of the episome with its attached Lac^+ region. However, the site responsible for the Mal^+ phenotype is not attached to the episome since it was not eliminated by treatment with acridine orange.

The previous experiment does not indicate the site of the Lac^+ reversion since loss of the episome removes both permease and β-galactosidase activity.

The crosses given in Table 2 locate the site of the reversion in the genetic material attached to the episome by showing that transfer of the Lac genetic material attached to the episome in the reverted strain converts to Lac^+ both the parental *lacY lacZ* and the *lacY lacZ ctr* strains (obtained as a segregant from the diploid before reversion), whereas the Lac genetic material attached to the episome before reversion converts to Lac^+ after transfer only the parental *lacY lacZ* strains.

Similarly, since the episomal transfer from the Lac^+ Mal^+ revertant made only the parental *lacY lacZ* strain Lac^+ and not the *ctr* derivative, the site necessary for the Lac^+ Mal^+ reversion is not in the material attached to the episome.

Location of Mal^+ reversions. Some preliminary experiments by bacterial crosses to locate the site of Mal^+ reversions indicated that the site was distant from the chromosomal location of the *ctr* mutations (46 to 47 min). In view of the results with Lac revertants, it was considered that Mal^+ reversions might occur in genetic regions known previously to be involved with maltose metabolism. Two genetic regions have been indicated (7): *malA* (66 min) which includes genetic information for amylomaltase and phosphorylase; *malB* (79 min) which includes genetic information for maltose permease and a gene regulating maltose metabolism.

The method for locating Mal reversions was by transduction, utilizing a mutation cotransducible with each of the Mal regions. For the *malA* region, *cysG* [26% cotransduction (11)] was selected; for *malB*, *metA* [20% cotransduction (7)] was chosen. A stock containing both *metA* and *cysG* was synthesized and into it was introduced a series of *ctr* mutations (all shown to be linked to *ctr* mutants previously characterized). These mutants served as recipients in transductions from Mal revertants with selection for either Cys^+ or Met^+, with the fraction of Mal^+ cotransductions being ascertained by streaking subsequently on maltose indicator agar.

Maltose reversions of *ctr1* and *ctr5*, selected because of differing physiological characteristics, were analyzed in some detail, by making transductions from them to several independent *ctr* mutants induced in the *cysG metA* tester strain. The results are given in Table 3 in which it is clear that: (i) maltose reversions of *ctr* mutants

TABLE 2. *Test crosses of Lac revertants*

Donor		F^- test, recipient 1	Progeny phenotype	F^- test, recipient 2	Progeny phenotype
Genotype	Phenotype				
1. *ctrA*/F'Lac$^+$[a]	Lac$^-$	*lacY lacZ*	Lac$^+$	*ctrA lacY lacZ*	Lac$^-$
2. Lac$^+$ reversion of no. 1[b]	Lac$^+$		Lac$^+$		Lac$^+$
3. Lac$^+$ reversion of no. 1[c]	Lac$^+$		Lac$^+$		Lac$^+$
4. Lac$^+$ reversion of no. 1[a]	Lac$^+$ Mal$^+$		Lac$^+$		Lac$^-$

[a] Parental culture. Test cross 1 shows that the F′ is Lac$^+$; test cross 2 produced no Lac$^+$, indicating that *ctrA* prevents lactose metabolism.

[b] Lac$^+$ reversion of no. 1, constitutive for β-galactoside uptake and β-galactoside synthesis. Test cross 1 shows the F′ to be Lac$^+$; test cross 2 shows that the reversion site is transferred by the F′.

[c] Lac$^+$ reversion of no. 1, nonconstitutive for uptake and β-galactoside synthesis. Test cross results as in no. 2.

[d] Lac$^+$ Mal$^+$ reversion of no. 1, nonconstitutive for β-galactosidase synthesis. Test cross 1 shows that the F′ is Lac$^+$; test cross 2 yielded no Lac$^+$, indicating that the site of the reversion is not transferable by the F′.

TABLE 3. *Genetic location of revertants selected on maltose*

Revertant donor	Selection	Recipient *ctr* mutants						
		1927	E	5-1	5-2	5-3	5-4	5-7
224[a]	MetA$^+$	31/126[b]	11/31	0/223	26/102	0/102	31/211	17/106
	CysG$^+$		0/33	0/34	0/98	0/101	0/204	0/106
220[c]	MetA$^+$					0/119	23/103	25/145
	CysG$^+$					0/65	0/96	0/117
230[d]	MetA$^+$					0/99	0/99	0/97
	CysG$^+$					0/96	0/95	0/124
233[e]	MetA$^+$					0/97	0/109	0/130
	CysG$^+$					0/45	0/92	0/133
2547A-8[f]	MetA$^+$			0/133	0/150	0/126	0/128	0/187
	CysG$^+$			0/188	0/226	0/168	0/127	0/101

[a] Revertant 224 was Mal$^+$, transported ^{14}C-maltose constitutively, but transported at greater velocity after prior growth in the presence of maltose.

[b] Number of Mal$^+$ transductants per total number of selected (Met$^+$, Cys$^+$) transductants. Reversions 224, 220, 230, and 233 are reversions of *ctr1*, whereas 2547-8 is a reversion of *ctr5*.

[c] Revertant 220 was Mal$^+$ Lac$^+$ (Lac$^+$ possibly secondarily) and was similar to 224 with regard to uptake.

[d] Revertant 230 was Mal$^+$ Lac$^+$ Glp$^+$, and its maltose transport was inducible by growth in maltose.

[e] Revertant 233 was Mal$^+$ Lac$^+$ Glp$^+$. Its maltose uptake was constitutive and was not increased by growth in maltose.

[f] Revertant 2547A-8 was Mal$^+$ Lac$^+$ and weakly positive on Glp and Fru. Its maltose uptake was inducible by growth in maltose.

may occur at a site distant from *ctr* region, at *malB*. (ii) A maltose reversion of a particular *ctr* mutation is not necessarily a reversion of another independent *ctr* mutation. Note that reversion 224 is a reversion of *ctr* mutants 1927, E, 5–2, 5–4, and 5–7, but not 5–1 and 5–3. (iii) The location of some maltose reversions is neither *malA* nor *malB*.

Regulatory characteristics of revertants. The parental strain in which *ctr* mutations were selected are inducible for the transport systems which are affected by *ctr* mutations. They are also inducible for the enzymes involved in the various initial metabolic steps required for further utilization.

Test of Lac$^+$ revertants for the ability to take up C^{14}-lactose or ^{14}C-isopropyl-β-D-thiogalactopyranoside, with and without prior exposure to galactosides, indicated that the revertants had become modified in many instances for their regulatory properties. Of more than 50 lactose revertants, about one-third transported galactosides without prior inductive exposure, one-third were modified in response to induction, and one-

third were not distinguished from wild type. An example of this behavior is given in Table 4. Representatives of the three general types of Lac reversions was located in the *lac* region by the procedures described in an earlier section.

Similarly, when revertants selected on maltose were tested, 8 of 16 were found constitutive for maltose transport, and 8 were not distinguished from the parental strains. Of the eight strains transporting maltose constitutively, five were induced to more rapid rates of transport by prior maltose exposure. Surprisingly, among those responding in a different manner with regard to uptake of ^{14}C-maltose were two revertants whose maltose transport was inducible by lactose. Two of three reversions constitutive for maltose uptake were shown by the procedures described in an earlier section to be located in the *malB* region; however the location of the other reversions remains unknown.

Two transductants derived from a constitutive strain (224) selected by linkage to *metA* were inducible for maltose uptake. This result shows that the Mal^+ revertant characteristic was *metA* linked, but the constitutive property was not.

Function of the state of the Lac operon on the Ctr phenotype. Strain MM6, a mutant previously shown to lack enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system, resembles to a slight extent the *ctr* mutants. Its phenotype on indicator agar plates with carbohydrates is different from that of *ctr* mutants. Comparison of cholate indicator agar, for which red colonies indicate carbohydrate utilization, and white to pale pink failure to utilize carbohydrates, gives the following phenotypes:

MM6	Glu^+	Gal^+	Fru^-	Mal^+	Mtl^-	Lac^+	Man^-	Suc^-	Glp^+	Ara^+
ctr	Glu^-	$Gal^\pm$	Fru^-	Mal^-	Mtl^-	Lac^-	Man^-	Suc^-	Glp^-	Ara^+

A portion of the difference in phenotypes can be explained on the basis of the fact that the MM6 mutation was induced in a strain constitutive for lactose utilization. It has been found that *ctr* mutations, induced in either repressor or operator constitutive mutants, are Lac^+ in phenotype. Of three *ctr* mutants induced in a repressor constitutive strain, three were Lac^+; a *ctr* mutant induced in an operator constitutive strain was Lac^+.

Utilizing this information to interpret the MM6 phenotype, it appears that MM6 differs from a *ctr* mutant by Glu^+ Mal^+ Glp^+, which resembles one or more reversion classes of the *ctr* mutants studied in this laboratory.

Reversion behavior of a point mutant deficient in enzyme I and HPr. Strain 911IVA containing *ctr4* was previously described (13) and the wild-type allele of this mutation shown to be cotransducible with *SupN*. Complementation studies (H. G. Morse et al., *manuscript in preparation*) of 911IVA with tester strains lacking enzyme I of HPr of the phosphotransferase system showed that it was not complemented by extracts of a strain containing HPr, and only slightly by a strain containing enzyme I. (This latter strain is also a leaky producer of HPr.) Strain 911IVA therefore appears to be doubly mutant, lacking both these factors. Strain 911IVA is, however, revertible and is probably a point mutant. A mutant similar to *ctr4* has also been isolated in another laboratory (V. N. Gershanovitch, *personal communication*). The reversion pattern of *ctr4* is shown in Table 5.

Genetic consideration of the Ctr region. Evidence has been presented (1) that a mutation producing a deficiency of both enzyme I and HPr of the phosphotransferase system is located at approximately 48 min on the *E. coli* chromosome map. The data giving this location is derived from interrupted bacterial conjugations and is probably identical to the region of *ctr* mutations (46 to 47 min). We have previously shown (13) that the mutation of *E. coli* strain MM6, a mutant defective in enzyme I synthesis, is closely linked to the *ctr* mutations, and that the wild-type allele of *ctr4*, a mutant lacking both HPr and enzyme I, was cotransducible with *SupN* at a frequency of 1.9%. A reciprocal experiment, transduction of *ctr5*, presumed linked to *SupN*, did not show cotransduction at the $<0.2\%$ level of detection.

We reexamined linkages in the 46 to 48 min region of the map and now find that *ctr5* is cotransducible with *SupN* (77 to 79%), and that the wild-type allele of *ctr4* is cotransducible at 81% frequency. The reason for the discrepancy between the previous and the present results is not known.

In addition, transductions to test for the location of the HPr mutation in the Fox and Wilson strain 1101 have been made. By using the wild-type strain and *ctr3* as donors, and the histidine requirement of strain 1101 as an internal control, the yield of Mtl^+ from the two donors was measured. Mutant *ctr3* gave a reduced yield of Mtl^+, equivalent to that expected if the site of *ctr3* was 63% cotransducible with the wild-type allele of the mutation in strain 1101. This close linkage places the *ctr* mutation closest to *SupN* at about 45.5 min. Bukhari (Ph.D. Dissertation, University of Colorado, 1970) has studied diaminopimelic acid (*dap*) mutations in *E. coli*. He finds *dapE* (succinyl-diaminopimelic acid-

TABLE 4. *Enzyme levels of the Ctr+ parent, Ctr− mutant and Ctr+ revertants*

Genotype	Induction	Z/B[a]	Y/B[b]
Ctr+	903	0.04	1
	903 + IPTG[c]	100.0	100
ctr1	903C	0.02	1
	903C + IPTG	16.0	16
Ctr− Lactose+ revertants	903C67	0.02	1
	903C67 + IPTG	24.0	41
	903C625	0.03	2
	903C625 + IPTG	68.0	130
	903C611	0.04	<1
	903C611 + IPTG	48.0	82
	903C61	1.8	4
	903C61 + IPTG	32.0	28
	903C62	4.2	4
	903C62 + IPTG	53.0	32
	903C63	58.0	40
	903C63 + IPTG	65.0	85
	903C617	75.0	40
	903C617 + IPTG	76.0	100

[a] Z/B = amount of β-galactosidase per unit cell mass with the Ctr+ cells fully induced set equal to 100.

[b] Y/B = amount of ^{14}C-lactose taken up per unit cell mass per minute with the Ctr+ cells induced set equal to 100.

[c] Amount of isopropyl-β-D-thiogalactopyranoside (IPTG) used for induction was 10^{-3} M. Growth was in the minimal medium described previously (12).

deacylase) at about 47.5 minutes and 9.8% cotransducible with *ctr2*. In some preliminary studies we have introduced a series of *ctr* mutants in a *dapE* strain and made transductions to them selecting for Dap+. Although there are some irregularities that must be explained, the results reveal that there are *ctr* mutational sites cotransduced <0.1% with *dapE* and sites that are cotransduced approximately 20% with *dapE*.

DISCUSSION

The results presented here and previously indicate that the region of the *E. coli* chromosome map between 45.5 and 47 min contains genetic information that affects the metabolism of a large number of substances. Certain observations, such as cotransduction frequencies, and the existence of a polar type mutant affecting both enzyme I and HPr formation suggest that the *ctr* region is large. Thus cotransduction frequencies of *ctr* mutations with adjacent genes vary from less than 1% to as much as 20%. The variation represents a large segment of deoxyribonucleic acid. Also fitting with this concept is the apparent target size of the *ctr* region. Nearly 100 mutants were isolated on glucose indicator agar, and at least 26 are *ctr* mutants (H. G. Morse et al., *manuscript in preparation*).

The normal function of the *ctr* region remains unknown, since we know only its mutant form, in which the accumulation of carbohydrates is affected. Enzyme I and HPr of the phosphoenolpyruvate-dependent transferase system are affected also, either singly or together, but it is not known whether the involvement is primary or secondary.

Mutations in the *ctr* region can be changed genetically by reversion for only part of the phenotype, as well as for the complete phenotype. The former mutations are located at distant sites: in the case of maltose utilization, at *malB*, 33 min away; and in the case of lactose utilization, in the *lac* region, 37 min away. The reversions to the complete wild type appears to be in the *ctr* region, but this has not been extensively investigated as yet. The effect of mutations in the *ctr* region also can be nullified at a distant site, as judged by the observation that *ctr* mutations induced in Lac constitutive strains, either *i* or *o* mutations, do not affect lactose metabolism.

Partial reversion of a *ctr* mutation may be specific to certain *ctr* mutations. In the study of maltose partial reversions, a reversion of one *ctr*, shown by transduction be to located at *malB* in a tester recipient *ctr*, was not a reversion of another *ctr* when transferred to it by transduction.

TABLE 5. *Characteristics of reversions of ctr4, a mutant deficient in enzyme I and HPr*

Selection	Revertant phenotypes
Lactose	Lac+ Glp+ Glu+ Lac+ Glu±
Fructose	Ctr+
Manitol	Ctr+
Melibiose	Ctr+ Mel± Lac+ Glp+ Glu+ Mel+ Lac+ Glp−
Glycerol	Glp+ Fru+ Glu± Glp+ Glu+ Lac+ Glu+ Fru± Glp+
Glucose	Ctr+ Glu+ Glu±

The partial reversions, both Lac and Mal, are characterized, approximately two-thirds in each case, by a high incidence of alteration in regulatory properties as compared with the wild-type inducible form.

A summary of the observations presented indicates that the genetic material in the *ctr* region codes for material that acts on distant genetic material, affecting its expression. The mechanism by which this effect is achieved is unknown, but at least two general possibilities exist. (i) The gene product of the *ctr* region is an enzyme-like protein, the function of which is necessary for the expression or regulation of expression, or both, of the affected functions; (ii) the gene product is a repressor-like substance which in the mutant form has "shut off" various chromosomal regions, in a manner analogous to the super-repressed form of the Lac repressor.

Under the first of these general models, enzyme I of the phosphotransferase system can be considered. There is no obvious connection between metabolism by the phosphotransferase system and the metabolism of maltose, lactose, glycerol, melibiose, and succinate. Since the partial phenotypic reversions studied still lack enzyme I activity, a further complication is introduced. A revertible mutant such as *ctr4*, which is deficient in both enzyme I and HPr, indicates that the structural genes involved are located close to one another and are subject to polar-like mutations. Perhaps the deficiency of enzyme I or enzyme I and HPr in *ctr* mutants is a secondary event; if not, a new function of the phosphotransferase system must then be sought, perhaps in the proper synthesis of cell walls and membranes.

If the *ctr* region coded for a repressor-like substance of importance in the regulation of cell metabolism, it might give the pattern of behavior seen with *ctr*. In its mutant form it has shut down the various genes affected, and escape from this "shut down" is achieved by changing the recognition sites or the regulatory properties, or both, of systems concerned. It could be predicted from such model that the Ctr^+/Ctr^- diploid would be minus in phenotype. Unfortunately it has been impossible to synthesize diploids in this region thus far, although considerable effort has been expended.

Recently, pleiotropic mutants resembling *ctr* mutants have been described (5), the biochemical defect of which has been found to be in adenyl cyclase. The phenotype of these mutants is reversed when high concentrations of 3′,5′ cyclic adenosine monophosphate (cAMP) are added to cultures. Although an extensive investigation has not been completed as yet, none of approximately 50 *ctr* mutants thus far examined in this laboratory responded to glucose utilization to added cAMP. One *ctr* mutant, *ctr1*, was examined (5) and found to have increased (3×) amounts of adenyl cyclase. If the steady-state level of cAMP in the cell is critical for cell regulation, a model for the behavior of *ctr* mutations based on the amount of cAMP present (a function of synthesis by adenyl cyclase and destruction by phosphodiesterase) might be possible. This model might also predict that certain diploids would have a minus phenotype.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-01345 from the National Institute of Allergy and Infectious Diseases. M. L. Morse is the recipient of Public Health Service Career Development Award 5-K03-GM-02819-10 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Bourd, G. I., et al. 1969. The characteristic of the transport mutants of *E. coli* with different lesions in Roseman's phosphotransferase system. Mol. Biol. (Russian) 3:256–266.
2. Egan, J. B., and M. L. Morse. 1965. Carbohydrate transport in *Staphylococcus aureus*. I. Genetic and biochemical analysis of a pleiotropic transport mutation. Biochim. Biophys. Acta 97:310–319.
3. Hanson, T. E., and R. L. Anderson. 1968. Phosphoenolpyruvate-dependent formation of D-fructose 1-phosphate by a four-component phosphotransferase system. Proc. Nat. Acad. Sci. U.S.A. 61:269–276.
4. Kundig, W., and S. Roseman. 1966. A phosphoenolpyruvate-hexose phosphotransferase system from *Escherichia coli*, p. 396–403. *In* W. A. Wood (ed.), Methods in enzymology, vol. 9. Academic Press Inc., New York.
5. Perlman, R. L., and I. Pastan. 1969. Pleiotropic deficiency of carbohydrate utilization in an adenyl cyclase deficient mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. 37:151–157.
6. Roseman, S. 1969. The transport of carbohydrates by a bacterial phosphotransferase system. J. Gen. Physiol. 54:138s–180s.
7. Schwartz, M. 1967. Location of the maltose A and B loci in the genetic map of *Escherichia coli*. J. Bacteriol. 92:1083–1089.
8. Simoni, R. D., et al. 1967. Genetic evidence for the role of a bacterial phosphotransferase system in sugar transport. Proc. Nat. Acad. Sci. U.S.A. 58:1963–1970.
9. Tanaka, S., D. G. Fraenkel, and E. C. C. Lin. 1967. The enzymatic lesion of strain MM-6, a pleiotropic carbohydrate-negative mutant of *Escherichia coli*. Biochem. Biophys. Res. Commun. 27:63–67.
10. Tanaka, S., S. A. Lerner, and E. C. C. Lin. 1967. Replacement of a phosphoenolpyruvate-dependent phosphotransferase by a nicotinamide adenine dinucleotide-linked dehydrogenase for the utilization of mannitol. J. Bacteriol. 93:642–648.
11. Taylor, Austin L., and Carol Dunham Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
12. Wang, R. J., and M. L. Morse. 1968. Carbohydrate accumulation and metabolism in *E. coli*. I. Description of pleiotropic mutants. J. Mol. Biol. 32:59–66.
13. Wang, R. J., H. G. Morse, and M. L. Morse. 1969. Carbohydrate accumulation and metabolism in *Escherichia coli*: the close linkage and chromosomal location of *ctr* mutations. J. Bacteriol. 98:605–610.