Transfer of a Gene for Sucrose Utilization into
Escherichia coli K12, and Consequent Failure of Expression
of Genes for D-Serine Utilization

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As the first stage in investigating the genetic basis of natural variation in Escherichia coli, the gene(s) conferring the ability to use sucrose as a carbon and energy source (given the symbol sac+) was transferred from a wild strain to K12, which does not use sucrose. The sac+ region was transferred by two different methods. On both occasions it took a chromosomal location at minute 50-5 on the linkage map, between aroC and supN, in the region of the dsd genes, which confer the ability to use D-serine as a carbon and energy source. When the sac+ region was present in the K12 chromosome the bacteria were unable to use D-serine as a carbon and energy source. In F'sac+/dsd+ diploids, the dsd+ genes were similarly not expressed. Strain K12(sac+) bacteria were sensitive to inhibition by D-serine; they mutated to D-serine resistance with much greater frequency than did a dsd mutant of K12. Such bacteria also mutated frequently to use raffinose. Strain K12(sac+) bacteria did not utilize sucrose when they carried a mutation affecting the phosphotransferase system.

INTRODUCTION

This report describes the first stage of an investigation into the genetic basis of the variation shown by wild strains of Escherichia coli. The character chosen for analysis was the ability to use sucrose as a carbon and energy source. The method envisaged was to introduce the chosen character from a wild strain into the laboratory strain K12 and to examine whether the introduced genes became integrated into the chromosome. For ease of manipulation it was necessary that the chosen character should be easily selectable, and that K12 should be naturally negative for the character. It was also necessary that the character should be specified by one gene or cluster: a more complex character might depend upon genes dispersed about the chromosome, and transfer and analysis of the character would then be difficult.

A consideration in the choice of the character was that about 50% of isolates of E. coli are sucrose-positive (Edwards & Ewing, 1972); Edwards & Ewing’s data relate to the proportion of isolates which ferment sucrose, but the ability to ferment a sugar is commonly correlated with the ability to use it as a carbon and energy source. Many characters of E. coli show a skewed distribution, in that the majority of isolates are positive or negative. When a small proportion of isolates are positive for a character there is probably a fair chance that the character may be conferred by a plasmid. For example, it is known that H2S production by some E. coli strains (Ørskov & Ørskov, 1973) and lactose fermentation by some Salmonella typhi strains (Baron et al., 1959) are due to plasmids. It seems less likely that a character for which 50% of strains are positive may be due to plasmids, and it is improbable that all variation in E. coli is caused by plasmids. In this investigation special importance was
attached to finding and studying a character showing natural variation in chromosomal genes. The supposition was that variation due to differences in chromosomal genes might reflect less ephemeral selective effects in nature than variation due to the presence and absence of plasmids. The present paper shows that ability to use sucrose is a chromosomal character. In a subsequent report it will be shown that linked genes are necessary for \( E. \) \( \) coli to use sucrose, but in the present report the genes involved will be referred to as the sucrose-'gene' and will be represented by the symbol \( \text{sac} \).

**METHODS**

**Media.** Glucose minimal medium was medium E of Vogel & Bonner (1956) supplemented with thiamin (5 \( \mu \)g ml\(^{-1} \)) and glucose (5 g l\(^{-1} \)). When necessary, glucose was replaced by other carbon and energy sources at the same concentration unless stated otherwise. D-Serine was from Sigma. Minimal medium was supplemented with growth factors (40 pg ml\(^{-1} \)) and streptomycin (200 \( \mu \)g ml\(^{-1} \)) as required. Complete medium contained (g l\(^{-1} \)): Tryptone (Oxoid), 10; yeast extract (Difco), 5; \( K_2 \)HPO\(_4\), 3; \( K_H \)PO\(_4\), 1; glucose, 5. \( T_2 \) nutrient broth contained (g l\(^{-1} \)): nutrient broth base (Difco), 8; NaCl, 4. Dehydrocholic acid/neutral red agar (Morse & Aire, 1958) contained (g l\(^{-1} \)): Tris (pH 7.9), 1-3; dehydrocholic acid, 1-5; yeast extract, 1; Proteose Peptone (Difco), 10; neutral red, 0.075. When required, media were solidified with Difco Bacto-agar (10 g l\(^{-1} \)), autoclaved separately (5 g in 100 ml water). Soft \( T_2 \) nutrient agar for overlays contained 6 g agar l\(^{-1} \), autoclaved in the medium.

**Transduction.** Phage P1kc was used. To prepare lysates, 0.03 ml 0-1 m-Ca(NO\(_3\))\(_2\) and 10\(^8\) phage (0-1 ml) were added to 2 ml overnight broth culture and 15 min were allowed for adsorption; then 4 ml molten soft agar was added and the mixture was poured over 75 ml \( T_2 \) nutrient agar in a Petri dish (10 \( \times \) 10 cm). Lysates were assayed on strain Ab1621. For transduction, 10\(^8\) phage (0-1 ml) and 0-03 ml 0-1 m-Ca(NO\(_3\))\(_2\) were added to 10\(^8\) bacteria in 2 ml overnight broth culture and 15 min were allowed for adsorption; the suspension was then washed and resuspended in 1 ml minimal medium and 0-1 ml volumes were plated on selective media. Transductants were purified by restreaking before testing for the presence of unselected donor markers.

**Conjugation.** Bacteria were grown in complete medium to a density of 5 \( \times \) 10\(^8\) ml\(^{-1} \); 0-5 ml of donor culture was then mixed with 9-5 ml of recipient culture, after plating control samples. In time-of-transfer experiments, the conjugating bacteria were separated by agitating for 30 or 60 s by the method of Low & Wood (1965).

**Gene symbols and map distances.** These follow Bachmann et al. (1976). The strains used are shown in Table 1. Except in Table 1, genes are shown in their order on the linkage map, starting at min 0. By agreement with Dr Bachmann the gene for sucrose utilization has been given the symbol \( \text{sac}^+ \), and is shown in parentheses when reference is to \( \text{k12} \) strains, to indicate that the gene was introduced.

**Induction of mutations.** N-Methyl-N'-nitro-N-nitrosoguanidine (30 mg l\(^{-1} \)) was used by the method of Adelberg et al. (1965). For ultraviolet irradiation, a Camag Universal lamp, type T6-900, was used: 10\(^8\) bacteria were resuspended in 10 ml minimal medium in a glass Petri dish, irradiated for 60 s to kill about 95% of the bacteria and then incubated overnight in complete medium to allow expression of mutations. When appropriate, mutants were selected by the penicillin-cycloserine method of Ornston et al. (1969).

**Curing bacteria of plasmids.** The method of Salisbury et al. (1972) was used. To 100 ml nutrient broth, 1 g sodium dodecyl sulphate (SDS) was added; the solution was autoclaved, the pH value was adjusted to 7-6, and the solution was steamed for 1 h. Control solutions, without SDS, were prepared in the same manner. Overnight stationary-phase cultures in broth were diluted 100-fold and 0-5 ml volumes were added to 100 ml volumes of the SDS solution and control solution. The suspensions were incubated at 37 \( ^\circ \)C with shaking. When SDS was to be added to a growing culture, similarly prepared SDS solution (10%, w/v) was added to bring the final concentration to 1% (w/v).

**RESULTS**

**Transfer of the ability to use sucrose to strain \( \text{k12} \)**

Strain \( \text{k12} \) did not use sucrose as a carbon and energy source but many isolates from nature did. Of the strains used in the present experiments, only \( \text{k12} \) was sensitive to phage P1kc. At the time when the experiments were begun, the method of Goldberg et al. (1974) for selecting PI-sensitive variants of wild strains had not been published. The method used
Effects of introducing sucrose genes into K12 49

Table 1. Escherichia coli strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype or derivation</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB265</td>
<td>F− thiA his leu thr argG lacY rpsL mtl xyl malA ara gal λ− λ+</td>
<td>See Bachmann (1972)</td>
</tr>
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<td>AB1515</td>
<td>F− thiA mtl xyl mal trp purF lacY proC leu rpsL</td>
<td>E. A. Adelberg</td>
</tr>
<tr>
<td>AB1621</td>
<td>F− lacY thiA mtl xyl galK ara rpsL tsx λ+</td>
<td>Adelberg et al. (1965)</td>
</tr>
<tr>
<td>EM1600</td>
<td>F− thiA dsdO lacZ supE rpsL</td>
<td>McFall (1967a)</td>
</tr>
<tr>
<td>EM3000</td>
<td>F− thiA cysC purF argH xyl malA ara? gal tonA tsx rpsL supE? λ−</td>
<td>McFall (1967a)</td>
</tr>
<tr>
<td>EM3003</td>
<td>F− thiA argH aroC purF dsdA7 tsx</td>
<td>McFall (1967a)</td>
</tr>
<tr>
<td>JC5466</td>
<td>F− his trp rpsE recA56</td>
<td>R. A. Dixon</td>
</tr>
<tr>
<td>FF8040</td>
<td>F− proC ptsI rpsL</td>
<td>Epstein et al. (1970)</td>
</tr>
<tr>
<td>AB312</td>
<td>Hfr thiA thr leu rpsL</td>
<td>Taylor &amp; Adelberg</td>
</tr>
<tr>
<td>AB347</td>
<td>Hfr thiA thr leu aroC lacZ ara rpsL λ−</td>
<td>Huang &amp; Pittard (1967)</td>
</tr>
<tr>
<td>AT997</td>
<td>Hfr thiA rel dapC λ−</td>
<td>Bukhari &amp; Taylor (1971)</td>
</tr>
<tr>
<td>AB2547</td>
<td>Hfr ilvD argH purF xyl supN supE44</td>
<td>Eggertsson &amp; Adelberg (1965)</td>
</tr>
<tr>
<td>AB2575</td>
<td>Hfr ilv thiA tsx</td>
<td>E. A. Adelberg</td>
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<tr>
<td>GA121</td>
<td>Nalidixic acid-resistant mutant of AB162</td>
<td></td>
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<tr>
<td>GA122</td>
<td>sac+ transductant* of GA121</td>
<td></td>
</tr>
<tr>
<td>GA301</td>
<td>cys+ purF aroC transconjugant from conjugation between AB347 and EM3000</td>
<td></td>
</tr>
<tr>
<td>GA515</td>
<td>sac+ transductant* of AB1515</td>
<td></td>
</tr>
<tr>
<td>GA501–509</td>
<td>Sucrose-utilizing hybrids between GAE10 and GA121; see text</td>
<td></td>
</tr>
<tr>
<td>GA601–610</td>
<td>Strains of JC5466 carrying F’sac+ plasmids derived from a sac+ transductant* of AB312</td>
<td></td>
</tr>
<tr>
<td>GAE10</td>
<td>Sucrose-utilizing isolate from the River Kennet, obtained by A. T. Regnier; carries transmissible antibiotic resistance</td>
<td></td>
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</table>

* sac+ transductants were derived from the indicated parent using an R− derivative of GA501 as donor.

for transferring genes in the present experiments was to irradiate sucrose-positive strains which contained R factors and select for transfer of the sucrose character to K12. The method gave transfer of the character to K12, but only in one of several experiments. The events leading to transfer of the genes for sucrose utilization are not understood, but the final result proved to be the same when a different method was used, as described below. The same results were also obtained when the method of Goldberg et al. (1974) was used (unpublished results).

The wild strains chosen as donors carried transmissible R factors but were sensitive to inhibition by nalidixic acid and streptomycin. The recipient strain, GA121, was resistant to nalidixic acid and streptomycin. The successful procedure was as follows. Late exponential-phase bacteria of GAE10 and other wild strains were resuspended (5 × 10⁸ bacteria ml⁻¹) in minimal medium without glucose, exposed to ultraviolet radiation to kill 90%, held in the dark for 20 min, and then diluted 10-fold in complete medium (37 °C) and incubated. When the numbers of bacteria had reached about 5 × 10⁹ ml⁻¹, the cultures were mixed with equal volumes of culture of GA121 (5 × 10⁹ bacteria ml⁻¹) and incubated for 18 h. The mixtures of bacteria, and unmixed controls, were resuspended in minimal medium, plated on to minimal agar medium supplemented with sucrose (1%), nalidixic acid (25 mg l⁻¹) and streptomycin (500 mg l⁻¹) and incubated for 2 d. Ten experiments of this kind were made, using the same parental strains but different doses of radiation and different concentrations of antibiotics. Only one experiment, using strain GAE10, was successful, and gave nine transconjugants which were numbered GA501 to 509.

The characters of the transconjugants showed that they were GA121 bacteria which had acquired the ability to utilize sucrose and the ability to grow in the presence of certain antibiotics. The transconjugants resembled GA121 and differed from strain GAE10 in being (a) resistant to nalidixic acid (25 μg ml⁻¹) and streptomycin (200 μg ml⁻¹), (b) unable to
utilize xylose, galactose, raffinose, lactose, melibiose and arabinose, (c) inhibited by valine and (d) susceptible to lysis and lysogenization by phage P1. The transconjugants resembled GAE10, and differed from GA121, in growing on sucrose and in carrying one or more R factors which conferred resistance to tetracycline (25 µg ml⁻¹), kanamycin (30 µg ml⁻¹), neomycin (30 µg ml⁻¹), ampicillin (25 µg ml⁻¹) and chloramphenicol (25 µg ml⁻¹).

Tests for linkage between the resistance genes and the gene for sucrose utilization

The R factor in GAE10 did not transfer the gene for sucrose utilization on other occasions. It therefore seemed that a gene for sucrose utilization may have become associated with an R factor and been transferred with it to strain GA121. Tests were made to see whether loss of the R factor from a GAE10 × GA121 hybrid resulted in loss of the ability to use sucrose. Strain GA501 bacteria were treated with sodium dodecyl sulphate (SDS) as described by Salisbury et al. (1972). The treated suspension was plated on non-selective medium and 550 colonies were picked and tested; 211 were unchanged, 103 had lost only tetracycline resistance, 3 had lost tetracycline and chloramphenicol resistance, 113 had lost tetracycline, chloramphenicol and kanamycin resistance and 120 had lost resistance to the three antibiotics and also the ability to use sucrose. Untreated hybrid bacteria did not lose the characters. When GAE10 was treated with SDS in the same way, about 40% of the bacteria lost all the resistance characters but none lost the ability to use sucrose. The evidence suggests that a gene for sucrose utilization had become associated with the R factor. To test whether the sucrose character and the R factor were transmissible together in conjugation, strains GAE10 and GA501 were incubated with appropriate recipient bacteria of K12 and Salmonella typhimurium LT2 under conditions which allowed transfer of the R factor by conjugation, and the mixtures of bacteria were then plated on media which selected for recipients which had received resistance determinants. The transconjugants were tested by replica-plating, after restreaking, to see if they were able to use sucrose. In the first conjugations involving GA501 as donor, it was found that about 5% of transconjugants were sac⁺; in later experiments, the resistance determinants were still transferable but the sac⁺ gene was not transferable. In similar experiments with strain GAE10, the R factor was transferred to K12 but the sac⁺ gene was not. Tests were done to see whether the sucrose-utilization gene and the resistance genes were cotransducible from GA501; selection was made separately for transfer of resistance genes and of sac⁺. Over 1000 transductants which had received one or more resistance genes were tested but none were sac⁺. Nevertheless, the gene for sucrose utilization was separately transducible, although at a lower frequency (1 in 10⁷ phage) than the resistance genes (1 in 10⁵ to 10⁶ phage).

Strain K12 bacteria did not mutate to use sucrose

In the experiments described in this report, and in many other experiments, control bacteria of strains of K12 plated on sucrose minimal medium never mutated to use sucrose. In the experiment which achieved the transfer of sac⁺ from GAE10, the numbers of K12(sac⁺) bacteria which were obtained were very small and the possibility was considered that the sac⁺ bacteria may have been rare mutants of K12 which had occurred, by chance, in the mixed cultures and not in the controls. The possibility of mutation to use sucrose was considered seriously because Coetzee (1962) showed that some groups of Proteus characteristically mutate to become sucrose-utilizing. A large number and variety of tests were therefore made to see whether K12 bacteria mutated to use sucrose, including all the tests described by Coetzee (1962). The tests included incubation with deoxycholate and incubation for long periods in peptone broth containing sucrose. Mutants able to use sucrose were not obtained.

Other workers have indicated that K12 bacteria do not utilize sucrose, and K12 bacteria
Effects of introducing sucrose genes into K12

Table 2. Cotransduction frequencies in the sac+ region

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Allele generating selected phenotype</th>
<th>Transductants per 10^7 phage</th>
<th>No. scored</th>
<th>Unselected donor allele scored</th>
<th>Cotransduction frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA122</td>
<td>AB265 his</td>
<td>sac+</td>
<td>3</td>
<td>136</td>
<td>his+</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>his+</td>
<td>45</td>
<td>98</td>
<td>sac+</td>
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<tr>
<td>GA122</td>
<td>AB347 aroC</td>
<td>sac+</td>
<td>1</td>
<td>134</td>
<td>aro+</td>
<td>62</td>
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<tr>
<td></td>
<td></td>
<td>aro+</td>
<td>2</td>
<td>139</td>
<td>sac+</td>
<td>42</td>
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<td>AB352 purF</td>
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<td>sac+</td>
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<td>200</td>
<td>pur+</td>
<td>6</td>
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<tr>
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<td></td>
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<td>25</td>
<td>160</td>
<td>sac+</td>
<td>17</td>
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<td>84</td>
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<td>GA501</td>
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<td>29</td>
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<td>195</td>
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<td></td>
<td></td>
<td></td>
<td>15</td>
<td>235</td>
<td>aro+</td>
<td>28</td>
</tr>
</tbody>
</table>

have been used as recipients in experiments demonstrating that some plasmids carry genes for sucrose utilization (Le Minor et al., 1973; Smith & Parsell, 1975).

Mapping of the gene for sucrose utilization by conjugation

To map the sac+ gene, three Hfr strains were made sac+ by transduction from GA501. The Hfr(sac+) strains were used in time-of-transfer experiments with appropriate K12 recipients. The Hfr strains were AT997 (a derivative of KL16), AB2575 (of Hfr Hayes type) and AB312. Their sac+ derivatives transferred gene sac+ after 18, 55 and 65 min, respectively. The data indicated a map position for the gene in the min 45 to 50 region, clockwise to his at min 44.

Gene order was purF–aroC–(sac+)–supN

The sac+ gene, after introduction into K12, was tested for cotransduction with genes his, nalA, purF, aroC, dsdA and dapE (shown in their order on the linkage map, from min 44 to min 53, with certain genes omitted). The donor strains were GA501 and derivatives of GA121 and AB1515 which had been transduced to sac+ using GA501 as donor. Selection was made separately for sac+ and for the other wild-type gene under test, and transductants were tested for the presence of the unselected donor allele. The wild-type alleles of all the genes were transduced separately with satisfactory frequencies, except that the dsd+ gene was not transducible: the special problem of gene dsd is described below. The sac+ gene was cotransducible with only two of the genes, purF and aroC, with frequencies of about 20% and 60%, respectively, indicating that sac+ was close to aroC (Table 2).

The order of sac+ relative to purF and aroC was found by transduction. Phage grown on GA501 was used to transduce GA301 purF aroC. Selection was made for purF+, aroC+ and sac+ in all combinations. Table 3 shows that the gene order was purF aroC (sac+).

To check whether GA501 was typical, strains GA502 to 509 were also used as donors in
Table 3. Transductions to establish the relative position of gene sac+

The data show the distribution of donor alleles amongst transductants of each selected class. The numbers in parentheses are the frequencies of transductants per 10^7 phage. The sac+ gene is shown in its probable position in relation to the known sequence purF-aroC-supN. s. Selected allele; +, unselected donor allele received by transductants.

**Transduction:** donor GA501 purF+ aroC+ (sac+), recipient GA301 purF aroC

<table>
<thead>
<tr>
<th>No. scored for each selection</th>
<th>Donor alleles present</th>
<th>No. of transductants of each kind</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>purF+</td>
<td>aroC+</td>
</tr>
<tr>
<td>122</td>
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**Transduction:** donor GA501 purF+ (sac+) supN+, recipient A82547 purF supN ilv

<table>
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<th>Donor alleles present</th>
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<td>s</td>
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Transduction with GA301. Selection was made for transduction of sac+; each experiment gave at least one sac+ transductant per 10^7 phage. About 80 sac+ transductants were analysed from each transduction; the frequencies of cotransduction of purF and aroC were similar to those given by GA501. Summing the data for donors GA502 to 509, of 619 sac+ transductants, 93 were purF+ and 229 were aroC+.

The position of sac+ relative to supN (Eggertsson & Adelberg, 1965) was also determined. The donor was GA501 and the recipient was A82547 purF supN ilv. Selection was made for sac+ and purF+, separately and together, and the transductants were tested for unselected markers (Table 3). In the recipient the ilv allele was suppressed by supN and the bacteria did not require isoleucine and valine, except when they received supN+ by transduction. Table 3 shows that the gene order was purF (sac+) supN. Taken with the order purF aroC (sac+) established above, the data show that the order was purF aroC (sac+) supN.
Introduction of the sac+ region caused K12 bacteria to be unable to use D-serine

For much of the project it was thought that the sac+ region had replaced one or more of the dsd genes. Early in the project K12(sac+) bacteria were treated with mutagens and plated on media selecting for bacteria able to use D-serine, to see whether the dsd genes were present but inactive. Mutants able to use D-serine were not obtained. A later experiment showed that such mutants occurred with low frequency. Some were unable to use sucrose. This evidence suggests that the sac+ region did not displace the dsd genes.

Judging from the evidence of the preceding section, gene sac+ had taken a position close to genes dsdAOC in the otherwise barren region 1.5 min long between aroC and supN (McFall, 1967a, b; Bachmann et al., 1976). Gene dsdA codes for D-serine deaminase (D-serine dehydratase; EC 4.2.1.14) which enables K12 to use D-serine as a carbon and energy source, and genes dsdO and dsdC are regulatory genes (McFall, 1964a, b, 1975; Bloom et al., 1975). To determine the relative order of the sac+ and dsd genes, phage grown on GA501 was used to transduce EM3003 purF aroC dsdA7 and selection was made separately for aroC+, sac+ and dsdA+ transductants; aroC+ and sac+ transductants were obtained but dsd+ transductants were not. The frequencies of aroC+ and sac+ transductants validated the transduction procedure. The lack of dsd+ transductants was caused by donor GA501 which proved unable to use D-serine. The K12 strain from which it derived was able to use D-serine. To check that the dsd+ character was being scored correctly, dsd+ and dsd strains from Professor Elizabeth McFall were compared with the strains which had already been used. The validity of the scoring procedure was confirmed. Strain EM3003 was transducible to dsd+ by other dsd+ donors, confirming that the difficulty was caused by GA501.

As a preliminary to finding the order of genes sac+ and dsd+ by transduction, it was desirable to make GA501 dsd+ as well as sac+ and this was attempted by transducing gene dsd+ from AB1621 into GA501. Selection was made for transductants able to use D-serine; the dsd+ transductants were then tested for ability to use sucrose. None were able to use sucrose, showing that introduction of dsd+ caused loss of the ability to use sucrose. The converse was also true; many sac+ transductants of K12 were available from transductions involving GA501 as donor, and all were unable to use D-serine. The wild donor of the sac+ gene was also unable to use D-serine, as were the hybrids GA502 to 509.

In further transduction experiments involving GA501 as donor and K12 dsd+ strains as recipients, 500 sac+ transductants were tested, and all were unable to use D-serine. When the direction of transduction was reversed, and selection was made for transductants able to use D-serine, not one of 185 transductants was sac+.

In two experiments the frequency of cotransduction of aroC+ and dsd+ was measured. The recipient was EM3003 aroC dsdA7. One donor was a dsd+ transductant of GA501 and was unable to use sucrose. Of 675 dsd+ transductants, 30% were aroC+, and of 330 aroC+ transductants, 23% were dsd+. None were sac+. The results when the donor was AB1621 are in Table 2. McFall (1967a) reported cotransduction frequencies of 49% and 25% for aroC and dsd.

Phage grown on GA501 was also used to transduce strain EM1600 which is constitutive for the deaminase because of a mutation in the regulatory gene dsdO (McFall, 1973). Over 1000 sac+ transductants were tested for their dsd character; none were able to use D-serine.

Independent evidence that the locus occupied by gene sac+ in K12 was the locus it occupied in wild strain GAE10

It was possible that the position taken by gene sac+ in the K12 chromosome had no relation to the position it occupied in wild strain GAE10. For example sac+ may have entered a region of the K12 chromosome which had affinity for the R factor. The sac+ region was therefore transferred to K12 by a different method, using Hfr (or R') derivatives
of GAE10(R+). The Hfr (or R') strains were isolated from a trp met mutant of GAE10(R+) by the method of Taylor & Adelberg (1960). One of the putative Hfr or R'sac+ strains was then tested to see whether the sac+ gene mapped at the same position as in the GAE501 to 509 hybrids. In standard time-of-transfer experiments with K12 strains as recipients, it was found to have the gene-transfer properties of an Hfr strain, except that transfer was infrequent, about three sac+ colonies being obtained per 1000 donor bacteria. Gene sac+ was transferred by the donor after the same time interval as genes purF+ and aroC+, and the sac+ transconjugants had lost the ability to use D-serine as carbon and energy source. The donor was stable; it was tested after one year and had retained the ability to transfer the sac+ gene.

The transconjugant colonies from the experiment in which the GAE10 Hfr strains had been detected were also tested. They were all sac+, and unable to use D-serine, but otherwise retained the characters of AB1621, and were sensitive to phage P1. One was tested, as donor, in transduction experiments. The sac+ gene was cotransducible with aroC, and the sac+ transductants did not use D-serine.

**D-Serine deaminase activity was absent from K12(sac+) bacteria**

It seemed likely that the sac+ region in some way interfered with the formation or action of the enzyme D-serine deaminase (EC 4.2.1.14). The fact that K12(sac+) bacteria were inhibited by D-serine was evidence that the sac+ region did not affect uptake of D-serine. Appropriate K12 strains were assayed by the method of Pardee & Prestidge (1955) as modified by McFall (1964a). In one experiment, typical of several, bacteria were grown in complete medium to a density of 2 x 10^8 bacteria ml^-1 and resuspended in minimal medium without glucose but containing D-serine (150 μg ml^-1); samples were taken at the time of adding D-serine (time 0) and at 30 min intervals for 2 h, and assayed for D-serine deaminase. The constitutive mutant EM1600 had a specific activity of about 16 units from time 0 onwards. Strain GA121 showed inducible activity, rising from less than 0.5 units after 1 h to about 17 units after 1.5 h. Strain GA501(sac+) and a sac+ transductant of EM1600 showed no activity at any time, and the deaminase-less mutant EM3003 was also devoid of activity. The activity shown by the constitutive and inducible bacteria was of the order reported by McFall (1964a).

**The dsd+ genes were not expressed in K12 dsd+(F'sac+) partial diploids**

In considering how sac+ bacteria failed to use D-serine there seemed to be three possibilities: (a) the dsd+ region may have been replaced by the sac+ region; (b) the sac+ region may have become integrated into the dsd+ genes and inactivated them; (c) the dsd+ genes may have remained intact but the sac+ region may have produced a cytoplasmic product which blocked expression of the dsd genes. To test the third possibility, partial diploids were made by isolating F'sac+ plasmids and inserting them into K12 dsd+ bacteria.

F' plasmids carrying the sac+ region were selected from sac+ transductants of Hfr AB312, which transferred sac+ after about 70 min. The bacteria were treated with ultraviolet radiation to kill 90% and were used as donors in conjugation with JC5466 trp his recA as recipient. A recA recipient was used to prevent entry of sac+ into the recipient's chromosome; recA+ was 5 min distal to sac+ in the donor. A sample of conjugating bacteria was taken after 5 min and selection was made for sac+ recipients; the donor was counterselected by omitting threonine and leucine from the medium. Colonies occurred with a frequency of 1 per 10^4 donor bacteria. Ten colonies, denoted GA601 to 610, were tested to see if they were donors of sac+, with AB1621 serving as recipient. All behaved as if they contained F' plasmids, transferring the sac+ gene with high frequency within 5 min. The F' plasmids carried purF+ and aroC+, and four carried longer regions.

An F' carrying the shortest chromosomal insert was used for making partial diploids.
Donor GA601 trp his recA (F'purF+aroC+sac+) was incubated with AB1621, and selection was then made for sac+ bacteria which did not require tryptophan and histidine. Many were obtained: they were assumed to be the required partial diploids in which the dsd+ and sac+ regions were present on different replicons in the same bacteria. Sixty were purified and tested for ability to use D-serine and sucrose as carbon and energy sources. None were able to use D-serine, indicating that the sac+ region acted through the cytoplasm to make K12 bacteria unable to use D-serine. To test whether sac+ would block expression of the genes in a mutant which synthesized the deaminase constitutively, partial diploids were made in conjugation between GA601 recA his trp (F'purF+aroC+sac+) and EM1600 dsdA+ dsdO dsdC+, the recipient being constitutive for D-serine deaminase (McFall, 1973). Selection was made for sac+ bacteria which did not require histidine and tryptophan. Ninety-five partial diploids were restreaked and tested. All were able to use sucrose and unable to use D-serine. To check that the dsd+ region was still present, even though it was not achieving phenotypic expression, one of the partial diploids was used as donor in transduction with a dsdA mutant as recipient. The donor was EM1600 dsdO+ (F'sac+) and the recipient was EM3003 dsdA7. Selection was made for transductants able to use D-serine; they were obtained with normal frequency. One of the dsd+ transductants was tested for the presence of D-serine deaminase; the enzyme was present and constitutive, confirming that the genes dsdO+ and dsdA were present but not expressed in the partial diploids.

Strain K12(sac+) bacteria showed frequent mutation to resistance to D-serine and D-cycloserine

In E. coli B and K12, D-serine is detoxified by D-serine deaminase, an inducible enzyme (Pardee & Prestidge, 1955; McFall, 1964b). When K12 is exposed to D-serine, growth is at first strongly inhibited, but once sufficient deaminase has formed to detoxify D-serine, the amino acid serves as a source of carbon and energy (McFall, 1964a). Curtiss et al. (1965) isolated mutants of K12 which were resistant to D-cycloserine and unable to take up D-cycloserine, D-serine and D-alanine. Robbins & Oxender (1973) and Cosloy (1973) obtained evidence for a permease, called ‘dag’ permease, which transports D-serine, D-alanine and glycine.

Mutants which are defective in D-serine deaminase do not utilize D-serine as a carbon and energy source and are inhibited when plated on glucose minimal medium containing D-serine (500 mg l-1). We found that when K12 bacteria were made sac+ they were similarly inhibited by D-serine but they gave frequent mutants which were resistant to D-serine. Possibly the resistant mutants were impermeable to D-serine. They did not use D-serine as a source of carbon and energy. Frequent mutation to D-serine resistance was shown only by K12(sac+) strains. They gave about one D-serine resistant colony per 10⁸ bacteria plated, whereas strain EM3003 dsdA7 gave resistant colonies only when treated with a mutagen. Other K12 strains were not tested for mutation to D-serine resistance, because they possessed the dsd+ genes and were able to detoxify and utilize D-serine. Transduction tests, at first sight, suggested that the resistance mutation was cotransducible with sac+, but the evidence was invalid because the sac+ transductants mutated to D-serine resistance sufficiently frequently to score as resistant when the colonies were replicated.

The K12 dsd+(F'sac+) partial diploids described in the preceding section also showed frequent mutation to D-serine resistance.

Cosloy (1973) described a mutation which greatly impaired the ability of bacteria to accumulate D-serine, D-alanine and glycine, and conferred resistance to D-cycloserine at 30 mg l-1. This confirmed the conclusion of Kessel & Lubin (1965) and Wargel et al. (1971) that D-serine was transported by a system responsible for uptake of D-cycloserine. The gene was given the symbol dag. Cosloy (1973) and Robbins & Oxender (1973) mapped gene dag at min 94.
The D-serine resistant mutants arising in K12(sac+) strains were resistant to D-cycloserine. D-Cycloserine at 20 mg l\(^{-1}\) completely inhibited growth of K12 strains, whether sac+ or not, and resistant mutants were not observed. At 10 mg l\(^{-1}\), K12(sac+) derivatives gave resistant mutants, and other K12 strains did not; the frequency was about 1 per 10\(^8\) bacteria plated. Once isolated, the mutants were resistant to 20 mg l\(^{-1}\). They were also resistant to D-serine (500 \(\mu\)g ml\(^{-1}\)). Strain EM3003 dsdA7 was inhibited by cycloserine and did not mutate to resistance at the high frequency shown by K12(sac+). The evidence suggests that the mutations to D-serine resistance in K12(sac+) may have been in gene dag.

Strain K12(sac+) bacteria mutated to use raffinose

Strain K12 bacteria do not use raffinose and do not mutate to use it. Strain K12(sac+) bacteria mutated to use raffinose with a frequency of about 1 per 10\(^5\) bacteria plated. The mutations were independent of the mutations to D-serine resistance. They were not induced by the sac+ region and are not further considered here. Smith & Parsell (1975) reported that K12 bacteria which received plasmid-borne genes for sucrose utilization mutated to use raffinose.

Gene sac+ was cotransducible with gene ptsI+ and required ptsI+ for expression

Clockwise on the map, within 0·1 min of supN, are genes ptsH and ptsI of the phosphotransferase system for transport of carbohydrates (Roseman, 1969, 1972; Epstein et al., 1970). Since it seemed that sac+ might confer permeability to sucrose, the degree of linkage between sac+ and the pts genes was of interest because of the possibility of a functional relationship. Authentic ptsH and ptsI mutants were provided by Professor W. Epstein. Mutant RR8040 ptsI was chosen for transduction experiments because it was the most stable in our experiments. Ability and inability to grow on mannitol and sorbitol were taken as the criteria for distinguishing ptsI+ and ptsI transductants. Using GA501 as donor and RR8040 ptsI as recipient, selection was made separately for transductants able to grow on sucrose, sorbitol and mannitol. The ptsI mutant did not revert on control plates. Sorbitol-utilizing and mannitol-utilizing transductants were all able to use both hexitols and were scored as ptsI+. Of 881 ptsI+ transductants, 12% were sac+. About 2 ptsI+ transductants were obtained per 10\(^6\) phage, but selection for sac+ gave only about 3 transductants per 10\(^8\) phage, and all were pts+. This suggested that sac+ required ptsI+ for expression.

To test whether sac+ was expressed in pts mutants, new pts mutants of a sac+ transductant of EM3000 were isolated on dehydrocholic acid/neutral red agar (Morse & Alire, 1958) containing sorbitol and mannitol. Eight white mutant colonies were isolated; they were mannitol, sorbitol and sucrose negative. Six were stable. To test whether they were genotypically sac+, one was used as recipient in transduction with AB1621 as donor. Selection was made for mannitol-positive and sorbitol-positive transductants. Of 301 transductants tested, all grew on mannitol, sorbitol and sucrose, confirming that the recipient was genotypically sac+. Transductants which were pts+ but unable to use sucrose were expected but not obtained. Taken together, these data show that sac+ depended upon genes of the phosphotransferase system for expression. The cotransduction frequency of 11% was in agreement with the interval of about 1·3 min between dsd and ptsI shown on the linkage map.

DISCUSSION

The transfer of chromosomal genes for sucrose utilization has not been reported before, but some plasmids transmit the ability to use sucrose (Le Minor et al., 1973; Smith & Parsell, 1975; Wohlhieter et al., 1975). The symbol sac+ (Le Minor et al., 1973) seems to have priority, and is the symbol used for Bacillus subtilis (Lepesant et al., 1972). The term ‘sac+ region’ will be used when effects may be due not to sac+ but to accompanying DNA.
Effects of introducing sucrose genes into K12

The evidence of cotransduction points to sac+ having taken a chromosomical position in K12. The data are summarized in Fig. 1. The fact that it took, at least approximately, the same position by two methods of transfer indicates that the region has affinity for the dsd region and that the methods of transfer did not determine the position taken. The way in which the sac+ region first entered the K12 chromosome is not known. It may have entered by homologous pairing and even numbers of crossovers or it may have behaved as a transposable element (see below). Since K12 need not be devoid of all sac DNA, introduction of only a part of a gene or operon may have enabled K12 to use sucrose. Reversion of sac+ bacteria to use D-serine indicated that the D-serine genes were not replaced. Possibly sac+ may have replaced a part of the dsd+ genes, or become inserted into them. Closely related may be the question of how sac+ prevented utilization of D-serine by partial diploids. One possible explanation may be that a protein from the sac+ region interacted with dsd nucleic acid, e.g. to block transcription. Another possible explanation may be interaction of gene products; for example, protein subunits may have had mutual affinity and aggregated in such a way that an active dsd protein was not formed. It seems more than coincidence that sac+ mapped close to dsd and interacted with it. Mutation of K12(sac+) to use D-serine permits genetical analysis of the interaction. The test of significance of the interaction, in relation to the aims of the project, is whether sac+ and dsd+ genes from other wild strains interact.

Strain K12(sac+) bacteria showed increased mutation to resistance to D-serine and D-cycloserine compared with a K12 dsd mutant. The mutations may have been in gene dag. One possible explanation is that ordinary K12 bacteria may have two systems which transport both substances, and one system may have been rendered inactive on introduction of the sac+ region, thereby allowing mutations which inactivate the second system to be expressed as resistance mutations. Another possible explanation is that an insertion sequence or transposable element or mutator gene may have been introduced or generated.
The ability of K12(sac+) to use sucrose was dependent upon at least one gene of the phosphotransferase (PT) system. Sucrose is not reported to be transported by the PT system. Wang et al. (1969, 1970) observed that some ptsI mutants did not grow on compounds such as lactose which are not transported by a PT mechanism. Saier & Stiles (1975) suggest that a regulatory protein inhibits the transport of non-PT sugars, except when the protein is inactivated under appropriate conditions by a phosphorylation process involving the ptsI product, Enzyme I. The problem is discussed by Kornberg & Jones-Mortimer (1977).

When strain GA501 was first isolated, gene sac+ gave evidence of being plasmid-borne. This raises the question of whether sac+ may have acted as a transposable element (Hedges & Jacob, 1974; Cohen, 1976). A more general question is whether characters which are present in only a proportion of wild strains may be due to phage or other transposable elements. Transposability is now recognized to be of greater significance than when the present experiments were done. With regard to phage, tests were made to see whether supernatants of sac+ bacteria were able to transfer the sac+ gene without the addition of phage PI. The tests did not give positive results. With regard to the possibility of another kind of transposable element, transfer of the sac+ region was first achieved in only one of many experiments with several sac+ strains containing R factors. The evidence from conjugation experiments was that sac+ took one position on the K12 chromosome and remained in that position. Translocation, if it occurred at all, seems not to have been a frequent property of the region under the conditions of the experiments, unless the effect on dag was due to translocation. The possibility remains that sac+ may insert preferentially into the dsd region. Investigations concerned with this problem will be reported elsewhere.

The way in which the relationship between sac+ and dsd+ was discovered in this investigation illustrates the value of a detailed linkage map in the study of natural variation. The availability of simple and efficient methods of gene transfer, and of extensive and discontinuous variation in biochemical characters, may make E. coli a favourable subject for experiments in ecological genetics.

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