It is believed that there are several charged amino acid residues in membrane-spanning α-helices of the lactose carrier of *Escherichia coli*. Evidence has previously been presented for two different salt bridges in membrane-spanning regions of the lactose carrier. One of these involves an interaction between Asp-237 and Lys-358; another involves interaction between Asp-240 and Lys-319. Additional studies of Lys-319 suggest that it may interact with Glu-269 as well as Asp-240. A cell containing the *LacY* gene with the mutation Lys-319 → Asn failed to ferment melibiose and after several days melibiose-positive mutants arose on indicator plates. These revertants showed second site mutations which replaced Asp-240 by neutral amino acids (Val or Gly). In addition, a second site mutation showed Glu-269 changed to Asn. Cells containing the mutation Lys-319 → Leu also failed to ferment melibiose and melibiose-positive revertants showed Asp-240 → Ala and Asp-240 → Tyr as well as Tyr-236 → Phe and His-322 → Arg. Second site revertants were also sought from the mutant Glu-269 → Asn which grew poorly on melibiose minimal plates. Melibiose-positive revertants included the double mutant Gln-269/Asn-319. All of the Glu-269 → Asn mutants were extremely defective in transport. It was concluded that Lys-319 interacts with Glu-269 and Asp-240 probably as salt bridges.

The presence of uncompensated charged amino acid residues in membrane-spanning α-helices of membrane proteins would appear to be energetically very unfavorable. In spite of this fact many membrane proteins show charged residues in the hydrophobic region of the molecule. Honig and Hubbel (1) have shown that while 10 kcal/mol are required to insert a charged residue into a hydrophobic region, a salt bridge may be neutralized by a neighboring residue of opposite charge. Salt bridges could potentially have at least two different functions in membrane transport proteins. The first role might be a structural one in binding together α-helices which require a particular orientation for proper function or for proper insertion into the membrane. A second possible role would be a direct interaction of the charged residues in substrate binding and in the energy coupling mechanism. In bacteriorhodopsin Arg-85 forms a salt bridge with the protonated Schiff base, Asp-96 probably salt bridges to Arg-227, and Asp-212 may interact with Arg-82 (2-4). The three aspartic acid residues at positions 85, 96, and 212 are apparently involved in the proton pathway across the membrane (2-4). A second example of a salt bridge in a proton translocating protein is the ion pair Glu-219 and His-245 in the α-subunit of the F$_{1}$-F$_{0}$-ATPase (5).

It has been postulated that salt bridges are involved in the mechanism of several ion transport carriers although the specific amino acid residues involved have not been identified. These proteins include: the uncoupling carrier protein of mitochondria (6), the anion carrier of the erythrocyte (7), the Na$^+$K$^+$ ATPase (8), the voltage gated Na$^+$ channel (9), and the Na$^+$ channel of toad bladder epithelium (10).

Recently evidence has been presented for the existence of a salt bridge between Asp-237 and Lys-358 in the lactose carrier of *Escherichia coli* (11, 12). A mutant with Lys-358 substituted by Tar failed to grow on melibiose. Eleven melibiose-positive revertants were isolated and each of them showed a change of Asp-237 to a neutral alanine amino acid (Asn, Tyr, or Gly). In the reverse type of experiment, a mutant with Asp-237 substituted by Asn failed to grow on melibiose and second site melibiose-positive revertant appeared with Lys-358 substituted by Gln. Sahin-Toth et al. (12) have shown that substitution of Ala or Cys for either Asp-237 or Lys-358 leads to loss of activity while the double mutant Asp-237 → Cys/Lys-358 → Cys has activity. Apparently a single neutral substitution for either charged residue is detrimental because this leaves an uncompensated charge while double neutral replacements give partial activity. Additional support for a salt bridge comes from experiments in which the two charges were reversed (Lys-237/Asp-358). Under these conditions substantial transport activity was obtained (12).

It is of interest to compare the amino acid sequences of the raffinose carrier of *E. coli* (13), the sucrose carrier of *E. coli* (14), and the lactose carrier of *Klebsiella* (15) with that of the lactose carrier of *E. coli*. All of the charged amino acids in membrane spanning helices are conserved in the case of RafB, *LacY* of *Klebsiella*, and the *LacY* of *E. coli*. In the case of the proton-sucrose cotransport carrier most of the charged amino acids are present with the notable exception of Asp-237 and Lys-358 both of which are substituted by neutral amino acids. From all of these observations it appears that although Asp-237 and Lys-358 are salt bridged in the lactose carrier of *E. coli*, these residues are probably not essential for proton-sugar cotransport.

Evidence for a second salt bridge in the lactose carrier, between Asp-240 and Lys-319 has recently been presented.
The EcoRI insert (containing the lacY gene) was in the opposite orientation from pTE18. pTE18C gave 80% of the activity of pTE18. Melibiose studies were in DW2(pcn).

**Strains**
- DW2R
- DW2(pcn)
- DW2/F′PZY′
- HB101

**Plasmids**
- pTE18
- pTE18C
- pN319
- pL319
- pQ269
- pN319/V240
- pN319/G240
- pN319/R268
- pL319/A240
- pL319/Y240
- pL319/P236
- pL319/R232
- pQ269/N319
- pQ269/M315
- pQ269/M265
- pQ269/V268
- pBR322

**TABLE I**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Lactose Melibiose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW2R</td>
<td>lacI′ ΔZ Y melA Δ8 strA recA tnlO (21)</td>
</tr>
<tr>
<td>DW2(pcn)</td>
<td>lacI′ ΔZ Y melA Δ8 strA pcn (21)</td>
</tr>
<tr>
<td>DW2/F′PZY′</td>
<td>lacI′ ΔZ Y melA Δ8 strA/F′PZY′ (21)</td>
</tr>
<tr>
<td>HB101</td>
<td>lacI′ Y (21)</td>
</tr>
</tbody>
</table>

**Genotype of E. coli strains and plasmids**

<table>
<thead>
<tr>
<th>Source</th>
<th>Genotype (chromosome/F-factor/plasmid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>King and Wilson (17)</td>
<td>lacI′ ΔZ Y melA Δ8 strA recA tnlO (21)</td>
</tr>
<tr>
<td>From Lopilato et al. (18)</td>
<td>lacI′ ΔZ Y melA Δ8 strA/F′PZY′ (21)</td>
</tr>
<tr>
<td>Boyer and Roulland-Dussoix (20)</td>
<td>lacI′ Y (21)</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL PROCEDURES**

**Materials**—Lactose, melibiose, and TMG were purchased from Sigma. IPTG was from Boehringer Mannheim. [methyl-14C]TMG was obtained from Du Pont-New England Nuclear, and [glucose-1-14C]lactose was from Amersham Corp. [3H]melibiose was a generous gift of Dr. G. Le blanc (Department de Biologie du CEA, Villefranche sur Mer, France). Radiolabeled sugars were further purified by paper chromatography on Whatman No. 3MM chromatography paper using a mixed solvent phase of three parts of 1-propanol to one part of water. Reagents for DNA manipulation and sequencing were from New England Biolabs. All other chemicals were of reagent grade.

**Bacterial Strains**—The genotypes of the bacterial strains and plasmids are detailed in Table I. The lacY gene was carried in plasmid pTE18 from Teather et al. (21). The EcoRI insert containing lacO′P′ΔZ Y was in pBR322. LB medium was employed in most experiments, and M63 medium (23) containing 1% trypton was used in the immunoblotting experiment. Ampicillin (0.1 mg/ml) and tetracycline (0.01 mg/ml) were always added to the medium. IPTG (0.5 mM) was used to induce the lactose operon. The plasmid pN319 was kindly provided by Dr. Brooker (University of Minnesota, St. Paul, MN).

**Oligonucleotide-directed Mutagenesis**—Mutagenesis was carried out according to the method of Eckstein et al. (24) using single stranded M13 DNA with a lacY insert. Conversion of Glu-269 to Gln was obtained from Du Pont-New England Nuclear, and [glucose-l-14C]lactose was from Amersham Corp. [3H]melibiose was a generous gift of Dr. G. Le blanc (Department de Biologie du CEA, Villefranche sur Mer, France). Radiolabeled sugars were further purified by paper chromatography on Whatman No. 3MM chromatography paper using a mixed solvent phase of three parts of 1-propanol to one part of water. Reagents for DNA manipulation and sequencing were from New England Biolabs. All other chemicals were of reagent grade.

**TABLE III**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Percent of pTE18</th>
<th>Strains</th>
<th>Percent of pTE18C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBR322</td>
<td>0</td>
<td>L319</td>
<td>40</td>
</tr>
<tr>
<td>N319</td>
<td>94</td>
<td>L319/P236</td>
<td>26</td>
</tr>
<tr>
<td>N319/R268</td>
<td>76</td>
<td>L319/A240</td>
<td>48</td>
</tr>
<tr>
<td>N319/G240</td>
<td>82</td>
<td>L319/R322</td>
<td>43</td>
</tr>
<tr>
<td>N310/V240</td>
<td>82</td>
<td>L310/Y240</td>
<td>43</td>
</tr>
<tr>
<td>Q299</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q299/V268</td>
<td>85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q299/N319</td>
<td>84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q299/M265</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q299/M315</td>
<td>61</td>
<td></td>
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</tbody>
</table>

*The EcoRI insert (containing the lacY gene) was in the opposite orientation from pTE18. pTE18C gave 80% of the activity of pTE18.*

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*The abbreviations used are: TMG, thiomethylgalactoside; IPTG, isopropyl-1-thio-β-D-galactopyranoside.
Salt Bridges in the Lactose Carrier

Lactose Downhill Transport

![Graph showing lactose downhill transport](image)

Lactose Accumulation

![Graph showing lactose accumulation](image)

Melibiose Accumulation

![Graph showing melibiose accumulation](image)

TMG Accumulation

![Graph showing TMG accumulation](image)

Fig. 1. Sugar transport by Asn-319 and its second site revertants. For downhill lactose transport and for TMG accumulation DW2/pZ-Y- containing the appropriate plasmid was exposed to 0.1 mM radioactive sugar. Samples were filtered at the times indicated, and radioactivity on the filter was counted. For lactose and melibiose accumulation DW2(pcn) containing a plasmid was exposed to 0.1 mM radioactive sugar. In each of the four experiments a cell containing pBR322 (no LacY insert) was included as a control and the sugar uptake was subtracted from each of the other curves.

CCCATTG (mutation in lowercase bold). The mutation in LacY gene was confirmed by complete sequencing by the method of Sanger et al. (25).

Subcloning.—The lactose carrier gene with a mutation of Lys-319 to Leu in pK319L (26), derived from pACYC184, was generously provided by Dr. R. H. Kaback (Howard Hughes Medical Institute, University of California, Los Angeles, CA). The LacY gene of pK319L was subcloned into EcoRI site of pBR322 using T4 DNA ligase. The ligated DNA containing L319 was transformed into HB101 and plated on lactose MacConkey plate plus ampicillin. Orientation of the fragment was determined by restriction analysis with AvoI. Cells containing the plasmid with each orientation of Leu-319 were used for selection of revertants.

Selection of Mutants.—DW2(pcn)/Gln-269 grows very slowly on melibiose minimal plates. Fast growing revertants of Gln-269 were obtained on 0.1% or 0.2% melibiose plates. DW2(pcn)/N319 and DW2(pcn)/L319 show very poor fermentation on melibiose MacConkey plates forming white colonies. Revertants of Asn-319 and Leu-319 forming red colonies on melibiose MacConkey plates were selected. Plasmids were isolated from these revertants and transformed back into DW2(pcn) to see if the phenotype was transferred. Transfer of phenotype indicated the mutation was on the plasmid. The plasmids from the transformed cells were isolated and purified. The mutations on these plasmids were identified by complete sequencing.

Sugar Transport Assays.—Overnight-grown cells in LB medium containing appropriate antibiotics were diluted into the same medium containing IPTG (1 mM). After three to four doublings at 37 °C the cells were harvested by centrifugation and washed twice with medium 63. The cell suspension (2 × 10⁹ cells/ml) in the same buffer was incubated at 25 °C with radioactive sugar. Samples (0.2 ml) were removed at 0.5-, 1-, 2-, and 10-min points after the incubation started and vacuum filtered through 0.65-μm pore-size filters (Sartorius). External sugar was removed with 4 ml of wash of the buffer. The radioactivity on the filter was counted in 4 ml of Liquiscint (National Diagnostics).

Measurement of Proton Transport.—Sugar-induced proton uptake was measured according to the method of West (27) as modified by
RESULTS

Lys-319 → Asn and Its Revertants—To test for lactose fermentation the Lac Y gene on a plasmid was placed into a cell containing the Lac Z gene (DW2/ F′TZY−). Cells with the Lac Y gene with Asn-319 (DW2/ F′TZY−/N319) showed fermentation on 30 mM lactose MacConkey plates indicated by bright red colonies (Table II). Attempts to isolate revertants on minimal plates with low concentrations of lactose were not successful due to the frequency of the I° to I′ mutation which made cells more lactose-positive. For studies of melibiose fermentation strain DW2 was used. DW2 containing a normal Lac Y gene on a plasmid is melibiose-positive because the lactose carrier transports this sugar which induces α-galactosidase. The normal melibiose carrier is absent in DW2 due to a deletion in the melB gene. The pcn mutation reduces the plasmid copy number which prevents excessive proton-sugar uptake. DW2(pcN)/ N319 failed to ferment melibiose and showed white colonies on 30 mM melibiose MacConkey plates. After 2–3 days of incubation of the melibiose MacConkey plates, small red colonies appeared on the white lawn of DW2(pcN)/N319. The DNA of some of these revertants was isolated and used to transform DW2(pcN). In the cases where the phenotype was not transferred the mutation was presumed to be located on the chromosome, and the cells were discarded. If the phenotype was transferred it was assumed that the mutation was on the plasmid. Complete sequencing of the Lac Y gene indicated that in 8 out of 13 revertants the Asn-319 had reverted to the wild-type sequence giving the original Lys-319. The remaining five revertants were divided into two groups. One group had a second mutation neutralizing the charge of Asp-240 either to valine (Asn-319/Val-240) or glycine (Asn-319/ Gly-240). The second group showed a second site mutation at or adjacent to the position of Glu-269; one with Glu-269 substituted by glutamine (Asn-319/Glu-269) and the other two showing Gly-268 to arginine (Asn-319/Arg-268). The latter mutation probably had the effect of neutralizing the negative charge of the adjacent Glu-269. These results suggest that Lys-319 can salt bridge to both Glu-269 and Asp-240.

Immunoochemical assay showed excellent expression of Asn-319 and its second site revertants in the membrane ranging from 76 to 94% of the wild type (Table III). Sugar transport assays were carried out with lactose, melibiose, and thimerothylgalactoside, each at 0.1 mM concentration (Fig. 1; see Fig. 5 for Asn-319/Gln-269, which is same as Gln-269/Asn-319). Asn-319 showed considerable downhill lactose transport activity (30% of normal), but was completely defective in accumulation of all sugars tested. Revertants Asn-319/Gly-240 and Asn-319/Val-240 restored downhill lactose entry to the level of that of the wild type (Fig. 1), and sugar accumulation was also significant (Asn-319/Gly-240 accumulated sugars 10–30% of normal and Asn-319/Val-240 showed 23–52% of normal). In sharp contrast, Asn-319/Gln-269 showed no activity in either lactose downhill assay or sugar accumulation (Fig. 5). Asn-319/Arg-268 also showed similar properties. It showed no sugar accumulation and only 7% of normal downhill lactose entry. All of the second site revertants showed strong fermentation of melibiose on MacConkey plates (Table II).

Sugar-induced proton uptake was measured with 10 mM lactose and 1 mM melibiose. Upon the addition of anaerobic sugar solution to anaerobic cells proton uptake via a sugar-coupled mechanism was indicated by alkalinization of the medium. Asn-319 showed no melibiose-induced proton uptake (Fig. 2). Asn-319/Gln-269 was an exceptional revertant that showed no proton uptake with lactose, and very little (15% of normal) with melibiose (Fig. 6). All the revertants in this group (Asn-319) showed considerable proton uptake in response to addition of melibiose (30–65% of normal). Most of the mutants in this group had measurable lactose-induced proton uptake activity (10–30% of normal); Asn-319/Gln-269 showed none.

Lys-319 → Leu and Its Revertants— pLeu319 was obtained from Dr. R. H. Kaback in vector pUC184 and was subcloned into pBR322. Because of an EcoRI site at both ends of the DNA insert, the product was a mixture of two orientations, one of these was the same orientation as in the pTE18 and four of the opposite orientation. Second site revertants were selected from Leu-319 of both orientations. Leu-319 plasmid in DW2/F′TZY− gave cells that failed to ferment lactose on MacConkey plates and grew as white colonies. DW2(pcN)/L319 was white on melibiose MacConkey plates. In addition they grew slowly on melibiose minimal plates. Nine red clones of DW2(pcN)/L319 were isolated from melibiose MacConkey plates. In addition three fast growing colonies of the same cell were isolated from melibiose minimal plates. Out of the 12 second site revertants 10 showed a neutral amino acid substitution for Asp-240 (either alanine or tyrosine). Four of the mutants (Leu-319/ Ala-240) were of the same orientation as pTE18 and four of the opposite orientation. Two mutants (Leu-319/Tyr-240) were of each orientation. Another had a second site mutation substituting phenylalanine for tyrosine at position 236. An additional mutant showed H322 substituted by arginine (Leu-319/Arg-322).

The expression of the mutants of this group (Leu-319) in the membrane was a little lower (26–48% of normal) than...
Salt Bridges in the Lactose Carrier

FIG. 3. Sugar transport by Leu-319 and its second site revertants. See legend for Fig. 1 for details. The curves for Leu-319/Tyr-240 and Leu-319/Ala-240 were identical.

FIG. 4. Sugar-induced proton uptake by Leu-319 and its second site revertants. See legend for Fig. 2 for details.

...and Leu-319/Tyr-240 showed much better lactose transport (67% and 47%, respectively). Accumulation activities were shown only by the Ala-240 and Tyr-240 second site revertants (25-30% normal for lactose and melibiose). Results of sugar transport by this group of mutants were similar to those by the Asn-319 group of mutants in that only the neutral substitutions of Asp-240 gained transport activity.

The mutant Leu-319 did not take up protons on addition of lactose or melibiose (Fig. 4). With lactose only Leu-319/Ala-240 and Leu-319/Tyr-240 showed some proton uptake. All mutants except Leu-319 showed significant melibiose-induced proton uptake.

Glu-269 → Gin and Its Revertants—The lactose carrier mutant Gin-269 was constructed by site directed mutagenesis. Fermentation studies were carried out with this lacY mutation on a plasmid. DW2/P'TY'/Q269 was found to be a bright red clone on a lactose MacConkey plate. DW2(pcn)/Q269 was red on a melibiose MacConkey plate. However, DW2(pcn)/Q269 grew very slowly on melibiose (0.2%) minimal plates...
forming small colonies in 48 h. Fast growing revertants were selected on these minimal plates. The mutations were identified by completely sequencing the LacY gene. Out of 16 revertants, 11 showed a wild-type sequence in which Gln-269 went back to normal Glu-269. Three of the remaining five revertants showed a second site mutation at or near K319: one showed substitution of Lys-319 by asparagine (Gln-269/Asn-319), another showed conversion of Val-315 to methionine (Gln-269/Met-315), and in the other revertant Thr-265 was substituted by methionine (Gln-269/Met-265). The other two revertants showed Gly-268 to valine (Gln-269/Val-268). The expression of these mutants in the membrane as judged by the immunochemical assay was good, ranging from 57 to 85% of normal (Table III).

None of the mutants in this group (Gln-269) showed either downhill lactose entry or accumulation of the three sugars (Fig. 5). Fermentation of lactose and melibiose was tested on MacConkey plates with sugar concentrations of 5 mM and 30 mM (Table II). All of the mutants showed positive fermentation of these sugars at 30 mM. Since the lactose entry rate is very low at 0.1 mM (Fig. 5) but greatly improved at 5 mM and 30 mM very poor affinity for lactose is indicated.

Sugar-induced proton uptake was measured (Fig. 6). The striking result was that the mutants of this group showed no proton uptake with lactose (Gln-269/Val-268, Gln-269/Asn-
Fig. 7. Model of the lactose carrier showing the relative positions of the second site mutations to the parental mutations. A, the postulated positions of Lys-319, Asp-240, and Glu-269; B, the positions of second site mutations of Asn-319; C, the positions of second site mutations of Leu-319; D, the positions of second site mutations of Gln-269.

Fig. 8. A postulated mechanism for proton-sugar cotransport involving Lys-319, Glu-269, and Asp-240. A, the sugar in the binding pocket of the carrier with the salt bridge (between Glu-269 and Lys-319) preventing sugar transport. Protons are drawn into the pocket by an inwardly directed proton motive force. B, the protonation of Glu-269 with opening of the gate. Asp-240 facilitates this process by attracting Lys-319 and forming a salt bridge. The sugar then passes through the membrane into the cytoplasm. C, the loss of the proton from Glu-269 and reestablishment of the original salt bridge with Lys-319.

319, or Gln-269/Met-315) or very little uptake (Gln-269/Met-265 showed 10% of normal). TMG-induced proton transport by Gln-269 and Gln-269/Asn-319 was zero (data not shown). All mutants, however, showed some melibiose-induced proton uptake (10%-20% for Gln-269, Gln-269/Val268, and Gln-269/Asn-319 and 30%-40% for Gln-269/Met-315 and Gln-269/Met-265. Since the entry of lactose by Gln-269 at high concentrations (5–30 mM) on MacConkey plates was positive (Table II) but no proton uptake was observed, lactose entry must occur at these concentrations in the absence of proton cotransport. These results strongly indicate that Glu-269 plays a critical role in proton translocation and its coupling with sugar transport.

DISCUSSION

The analysis of second site revertants of a mutant with a defective function is frequently very useful. The two regions of the protein affected by the mutations are clearly related in function and frequently the two altered amino acids residues are close to one another in the three-dimensional structure. Close proximity of the 2 residues is particularly likely when a mutant that neutralizes a charged residue gives rise to a second site mutation involving a residue of opposite charge (a potential salt bridge). In this study second site revertants have been isolated from cells containing a lactose carrier with
a charged amino acid (either Lys-319 or Glu-269) substituted by a neutral amino acid.

Replacement of Lys-319 by asparagine abolished sugar-proton cotransport. No accumulation of lactose, melibiose, or TMG was observed and sugar induced proton uptake was greatly reduced. DW2(pcn)/N319 failed to ferment melibiose on MacConkey plates (white clones) and after several days red mutant clones appeared on the white background. When the LacY DNA of the plasmid from the revertants was sequenced the initial mutation (Lys-319 → Asn) was retained and an additional second site mutation appeared. Two substitutions of Asp-240 by neutral amino acids (valine and glycine) were observed as second site mutations (see Fig. 7). Another revertant showed a neutral substitution (glutamine) for Glu-269. An additional second site mutation observed was in residue Gly-268 which was replaced by arginine. This addition of a positive residue next to Glu-269 would tend to neutralize its negative charge and thus be functionally similar to the mutation which changed Glu-269 to a neutral amino acid. The second site revertants involving Asp-240 (Asn-319/Gly-240 and Asn-319/Val-240) regained partial activity in accumulation of sugars. On the other hand, revertants involving Glu-269 (Asn-319/Glu-269) or Gly-268 (Asn-319/Arg-268) failed to accumulate sugar. It was concluded that Lys-319 interacts functionally with both Asp-240 and Glu-269, the interaction with Glu-269 being more critical than that with Asp-240.

A second neutral substitution for Lys-319 was studied (Lys-319→Leu). In agreement with Persson et al. (26) no lactose accumulation or lactose-induced proton entry was found with this mutation. In addition this mutant failed to accumulate melibiose or thiomethylgalactoside and showed no fermentation on melibiose MacConkey plates. These properties are similar to those found by Sahin-Toth et al. (12) for two other neutral substitutions for Lys-319 (Cys-319 and Ala-319). By selection for melibiose positive clones four different second site revertants of Leu-319 were found (Fig. 7). Two of these involved neutral substitutions at Asp-240 (alanine and tyrosine). These regained about 25% of normal accumulation of lactose and melibiose and a significant sugar stimulated proton uptake. It is interesting to note that the single mutation of Tyr-236 substituted by phenylalanine had previously been studied (30) and was found to alter sugar specificity, increasing the carrier’s recognition for maltose. This suggests that the changes are neutralized (or partially neutralized) by salt bridging between positive and negative residues. In bacteriorhodopsin Asp-85 (and possibly also Asp-212) salt bridges to the protonated Schiff base is also involved in proton transport. Cain and Simoni (5) suggested that in the α-subunit of the F0-F1-ATPase of E. coli Glu-219 and His-245 form a salt bridge and are involved in proton transport. As in the case of these two proton pumps where glutamic or aspartic acid residues are involved in salt bridges and in proton pumping it is possible that the carboxyl groups of either Asp-240 or Glu-269 may be involved in proton translocation in the lactose carrier. A possible role of the charged residues in proton and sugar translocation is indicated in Fig. 8. In this model the close opposition of Glu-269 and Lys-319 forms a sugar binding pocket. When protons are driven into this pocket by a proton motive force Glu-269 is protonated. The loss of the negative charge then releases the Lys-319 which is then attracted by Asp-240, thus opening the channel. Allosteric changes lower the affinity of the binding site for sugar which is then released into the cytoplasm of the cell. The subsequent release of the proton to the cytoplasm results in the characteristic stoichiometry of one proton per one sugar molecule. When Glu-269 is deprotonated it again attracts the Lys-319 and the cycle is completed. The key role of Glu-269 (compared with Asp-240) in this model is consistent with the fact that second site revertants involving Glu-269 are more severely defective than those involving Asp-240.

Acknowledgments—We thank Dr. Ronald Kaback for the mutant plasmid pLeu319 and Dr. Robert Brocker for the plasmid pAsn319. We also express our appreciation to Dr. Gerard Leblanc for the [3H] melibiose.

REFERENCES
Salt Bridges in the Lactose Carrier