Neuberger, A. (1948), *Advan. Protein Chem. 4*, 297.
Neurath, H. (1960), *Enzymes 4*, 11.
Neurath, H., and Schwert, G. W. (1950), *Chem. Rev. 46*, 69.
Perlmann, G. E. (1966), *J. Biol. Chem. 241*, 153.
Photaki, I. (1963), *J. Am. Chem. Soc. 85*, 1123.
Rajagopalan, T. G., Stein, W. H., and Moore, S. (1966), *J. Biol. Chem. 241*, 4295.
Schwert, G. W., and Takenaka, Y. (1955), *Biochim. Biophys. Acta 16*, 570.
Silver, M. S., Denburg, J. L., and Steffens, J. J. (1965), *J. Am. Chem. Soc. 87*, 886.
Smith, E. L. (1948), *J. Biol. Chem. 175*, 39.
Stahmann, M. A., Fruton, J. S., and Bergmann, M. (1946), *J. Biol. Chem. 164*, 753.
Suwa, A. (1911), *Z. Physiol. Chem. 72*, 113.
Tang, J. (1963), *Nature 199*, 1094.
Toyoda, T., Suyama, T., and Kanao, S. (1963), *Yakugaku Zasshi 83*, 856.
Vallee, B. L. (1964), *Federation Proc. 23*, 8.
Winitz, M., Bloch-Frankenthal, L., Izumiya, N., Birnbaum, S. M., Baker, C. G., and Greenstein, J. P. (1956), *J. Am. Chem. Soc. 78*, 2423.

A Genetic Defect in Retention of Potassium by *Streptococcus faecalis**

F. M. Harold, Ruth L. Harold, J. R. Baarda, and A. Abrams

ABSTRACT: Cells of *Streptococcus faecalis* 9790 selectively concentrate K^+ and Rb^+ even when growing in a medium in which Na^+ is the predominant cation. Mutants were isolated which require high concentrations of K^+ or Rb^+ for growth at pH 6. The requirement is seen even if the growth medium contains Tris or triethanolamine in place of Na^+. The mutant phenotype results from a defect in the concentrative uptake of K^+ and Rb^+ and is defined by three experimental procedures. (i) Measurements of ^{86}Rb uptake reveal no clear difference between wild type and mutant with respect to the kinetic parameters of Rb^+ entry into the cells. (ii) Wild-type cells retain K^+ and Rb^+ while the mutants rapidly lose these cations to the medium by an energy-dependent exchange with external Na^+. (iii) Wild-type cells preloaded with Na^+ and H^+ can carry out energy-dependent net uptake of K^+ with concurrent extrusion of Na^+ and H^+; the mutants are severely deficient in this process. It is proposed that the uptake of cations by the wild type involves an energy-dependent transport system which mediates cation exchanges across the membrane. In the inward direction K^+ and Rb^+ are strongly selected over Na^+, while in the outward direction the relative affinities are reversed. In the mutants, selective transport of K^+ and Rb^+ *into* the cells is normal; the genetic defect involves primarily the relative affinities for cation transport *out* of the cells.

Current concepts of membrane transport in microorganisms derive to a large extent from studies on mutants defective in the uptake of specific metabolites against a concentration gradient. According to the permease model (Kepes and Cohen, 1962; Koch, 1964) the initial step in transport involves a stereospecific protein, the permease; this catalyzes association of the substrate with a carrier molecule, which then crosses the membrane. In the majority of transport mutants that have been analyzed kinetically the genetic lesion affects primarily the *entry* of the metabolite into the cells, and many of these mutants are thought to have a defective permease. Examples include mutants deficient in the uptake of monosaccharides (Rickenberg *et al.*, 1956; Wiesmeyer and Cohn, 1960; Hagihara *et al.*, 1963; Egan and Morse, 1966), amino acids (Kessel and Lubin, 1962, 1963; Ames, 1964), and inorganic anions (Pardee *et al.*, 1966; Harold *et al.*, 1965; Harold and Baarda, 1966).

A transport mutant of very different phenotype was isolated by Lubin and Ennis (1963). A mutant of *Escherichia coli* was found to have lost the ability to *retain* K^+ when suspended in potassium-free medium, while exchange of cellular K^+ with external K^+ was accelerated (Lubin and Ennis, 1963; Lubochinsky *et al.*, 1964, 1966). Very recently, Günther and Dorn (1966) demonstrated that the primary defect is an increased efflux of K^+ from the cells, and proposed that the mutation has altered the specificity of a carrier involved in the transport of cations out of the cells.

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The purpose of the present communication is to describe the isolation and characteristics of a mutant of *Streptococcus faecalis* which is defective in the concentrative uptake of K^+ and Rb^+ and, under certain conditions, requires high concentrations of these cations for growth. We shall present evidence that the primary defect concerns the specificity of cation exit from the cells, and shall attempt to relate this phenotype to current models of membrane transport.

Experimental Section

Organisms and Growth Media. S. faecalis strain 9790 and mutants derived from it were grown on the following media. (i) Medium NaM is a modification of the defined medium described earlier (Harold *et al.*, 1965). The following stock solutions were prepared; stock A $(NH_4)_2SO_4$, 50 mM; $MgSO_4$, 8 mM; $MnCl_2$, 1 mM; and stock D sodium maleate, 1.5 M, pH 7.0. One volume of each stock solution was diluted to make ten volumes of growth medium which was supplemented with sodium phosphate (10 mM) and with vitamins, sodium acetate, amino acids, purines, and pyrimidines. Various amounts of KCl were added as indicated below, and the initial pH was adjusted to 7.5. To grow a good yield of cells overnight, 10 mM KCl was employed routinely.

Because of the technical convenience of ^{86}Rb as an isotope of Rb^+, many experiments were carried out with organisms grown on medium NaM containing Rb^+ (10 mM, K & K Laboratories, purity of 99.9%) in place of K^+. No attempt was made to remove traces of K^+ which may have been present in any of the reagents used. This medium is referred to as NaM-Rb. (ii) Medium NaTY is the complex medium described by Zarlengo and Schultz (1966), containing/l. 10 g of Difco tryptone, 5 g of Difco yeast extract, 10 g of glucose, and 8.5 g of Na_2HPO_4. The final cation content is about 5 mM K^+ and 150 mM Na^+ and the pH is 7.5.

Growth experiments were performed at 37° and followed by turbidimetry at 600 mμ. Generally glucose (10–20 mg/ml) served as energy source and the pH was maintained by periodic additions of alkali. The bacteria were adapted to utilize arginine by overnight growth in medium NaTY supplemented with 6 mg/ml of arginine hydrochloride (Zarlengo and Schultz, 1966).

Isolation of Mutants. The ^{32}P-suicide method previously described (Harold *et al.*, 1965) was adapted to the isolation of mutants defective in K^+ uptake. Cells of *S. faecalis* 9790 were grown overnight in medium NaM, washed, and resuspended in 50 mM sodium phosphate buffer, at a density of about 10^8 cells/ml. The suspension was irradiated with ultraviolet light to kill 99–99.9% of the bacteria, diluted into fresh medium NaM, and grown overnight. The cells were then sedimented, washed twice with medium NaM (no P_i, 2 mM KCl), and resuspended in the same at a density of 10^8 cells/ml. Of this, 0.5 ml was placed in a dry tube containing 0.08 μmole of P_i and 250 μc of $^{32}P_i$ (carrier free), to give a final specific activity of 3100 μc/μmole. The culture was grown for 6 hr at 37° (pH 7.0). The cells were then sedimented and resuspended in 50 ml of medium NaM (no P_i) containing 20% glycerol. Aliquots were dispensed into small tubes and frozen at −70°. At intervals a tube was thawed to determine the viable count. After about 1 month all the survivors were plated out on medium NaM (10 mM KCl), and replicated onto plates of medium NaM containing low K (1 mM). Colonies that failed to grow on 1 mM K^+ were picked for further examination.

In some experiments the above procedure was modified in that a pH of 6 was maintained during growth on $^{32}P_i$ and on the low-K replication plates. In all, four experiments were carried out; mutants of $Cn^-{}_{K6}$ phenotype were obtained from each experiment and were identified as *S. faecalis* as described earlier (Harold *et al.*, 1965).

The experiments described below were performed with a single clone, strain 325B. Revertants were readily selected by inoculating individual colonies into medium NaM (1 mM K^+, pH 6), suggesting that the $Cn^-{}_{K6}$ phenotype results from mutation of a single gene.

Measurement of Cation Content. Samples of bacteria containing 1–3 mg dry weight were collected by filtration through Millipore filters, pore size 0.45 μ, and washed with water or 2 mM Mg^{2+} which did not cause loss of cations from the cells. The filters were extracted with 5% trichloroacetic acid (95°, 5 min) and the extracts were clarified by centrifugation. All operations were carried out in polyethylene tubes to avoid contamination by Na^+ extracted from glass. Potassium, sodium, and rubidium were measured by flame photometry in a Perkin-Elmer Model 52C.

The results are expressed as micromoles of cations per gram of cells (dry weight); the latter were calculated from the turbidity of the cell suspension. The water space of *S. faecalis* is approximately 2 ml/g dry weight of cells, and thus the cation content may also be expressed as moles per liter of cell water. We prefer to use micromoles per gram of cells because, as will be shown elsewhere, a large fraction of the cations appears to be associated with macromolecular phosphate compounds; the concentration of cations that is free in solution is thus unknown.

Measurement of ^{86}Rb Uptake and Efflux. Because of its technical convenience, ^{86}Rb was employed for studies on uptake and exchange rates. The bacteria were always grown overnight on medium NaM-Rb (containing Rb^+ or ^{86}Rb), harvested, and treated as described for the individual experiments. In many experiments, the cells were suspended in AD buffer, prepared by tenfold dilution of stock solutions A and D. Samples were collected by filtration and the ^{86}Rb content was determined on the filters or on extracts.

Steady-state flux rates for ^{86}Rb were calculated according to Slayman and Tatum (1965a). Provided that a true steady state obtains, and that the tracer is uniformly mixed with Rb^+ in both cells and medium

$$\ln\left(1 - \frac{X_B}{X_{B\infty}}\right) = -(k_{AB} + k_{BA})t \qquad (1)$$

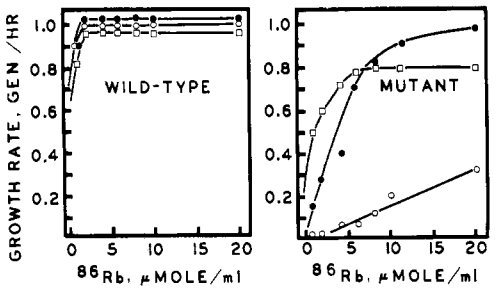

FIGURE 1: Growth rate (generations per hour) of wild type and mutant 325B in medium NaM as a function of the pH and of the extracellular ^{86}Rb level. Cells were grown on NaM-Rb overnight, washed with AD buffer, and resuspended in medium NaM supplemented with various amounts of ^{86}Rb. (○) pH 6, (●) pH 7, and (□) pH 8.

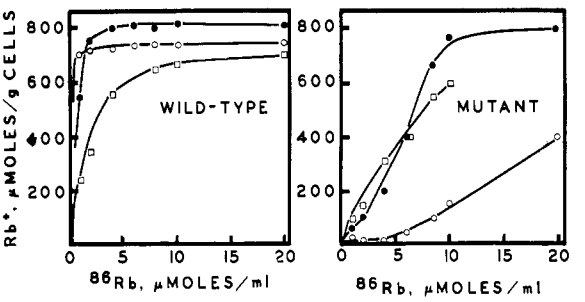

FIGURE 2: ^{86}Rb content of wild-type and mutant cells as a function of pH and the extracellular ^{86}Rb level. Samples of the cultures illustrated in Figure 1 were harvested after several hours of exponential growth and the ^{86}Rb content of the cells was determined. Same key as in Figure 1.

In this equation, X_B and $X_{B\infty}$ are the specific radioactivities of the cellular ^{86}Rb pool at time t and at equilibrium; k_{AB} is the rate constant for ^{86}Rb influx; and k_{BA} the rate constant for ^{86}Rb efflux. A plot of ln $(1 - (X_B/X_{B\infty})$ against time should give a straight line whose slope is $-(k_{AB} + k_{BA})$. From this value and the steady-state concentrations of Rb^+ in cells and medium, the rate constants for influx and efflux were calculated (Slayman and Tatum, 1965a).

Other Procedures. Protoplasts were prepared as described by Abrams (1959). Glycolysis was measured by titration of the lactic acid produced, using a Radiometer pH-Stat fitted with an electrode containing 1 M NaCl in place of KCl (Zarlengo and Schultz, 1966). Arginine metabolism was likewise monitored by titration of the ammonia produced.

Results

Growth of Wild Type and Cn^-_{K6} Mutants. In *S. faecalis*, as in many other bacteria (MacLeod and Snell, 1948; Lester, 1958), Rb^+ supports growth as well as does K^+. The minimal levels required for optimal growth rate and growth yield in medium NaM were about 2 mM for both cations. This relatively high K^+ requirement is due to the high Na^+ content of the medium, as will be shown below.

Wild type and mutant 325B were grown in medium NaM-Rb supplemented with various amounts of ^{86}Rb of known specific activity. Figure 1 shows the growth rates of the two strains as a function of pH and of the extracellular Rb^+ level. The growth rate of the wild type was essentially maximal at 1 mM Rb^+, and little affected by the pH over the range from 6 to 8. The mutant required elevated levels of Rb^+ for optimal growth, particularly at acid pH (100 mM Rb^+ at pH 6) but at pH 8 growth of the mutant was almost indistinguishable from that of the wild type. Analogous results were obtained in NaM supplemented with K^+.

Since NaM contains about 350 mM Na^+ which is known to inhibit K^+ uptake (MacLeod and Snell, 1948; Abrams, 1960), growth experiments were also conducted in modified media containing Tris or triethanolamine in place of Na^+. In these media 0.2 mM K^+ supported maximal growth of the wild type, regardless of pH. Mutant 325B required 5–10 mM K^+ for optimal growth at pH 6 and 1 mM K^+ at pH 7.3. Expression of the Cn^-_{K6} phenotype thus does not require the presence of Na^+.

Relationship between Growth Rate and Rb^+ Content. After several generations of exponential growth, samples of cells from the cultures illustrated in Figure 1 were counted, and the cellular Rb^+ content was calculated from the specific activity of the extracellular ^{86}Rb. The assumption that complete exchange was achieved is warranted by the experiments discussed below. It is clear from the results shown in Figure 2 that wild type and mutant differ strikingly with respect to intracellular Rb^+ content, particularly at pH 6.

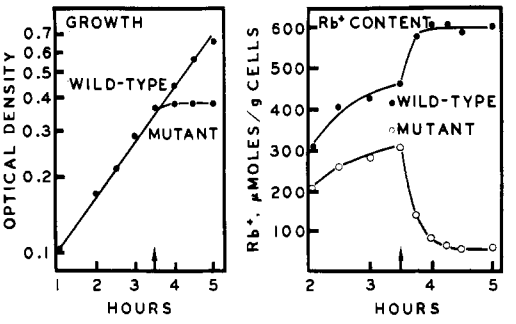

FIGURE 3: Effect of pH shift upon growth and Rb^+ content of wild type and mutant 325B. Both organisms were grown at pH 8 in medium NaM-Rb containing 3 mM ^{86}Rb. After 3.5 hr (arrow) the pH was lowered to 6 by addition of HCl. At intervals samples were withdrawn from both cultures and the ^{86}Rb content of the cells was determined.

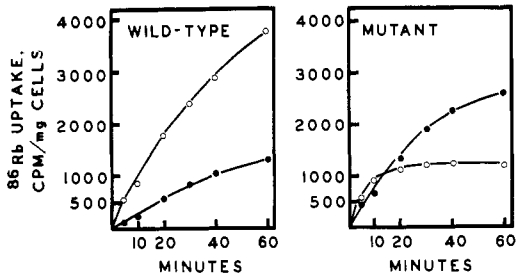

FIGURE 4: Steady-state exchange of ^{86}Rb in wild type and mutant 325B at pH 6 (O) and 7.5 (●). Cells were suspended in AD buffer, 37°, at a density of 1 mg/ml, and the suspensions were supplemented with glucose and RbCl (5 mM); after 30 min, ^{86}Rb was added. Initial specific activities normalized to 10,000 cpm/μmole.

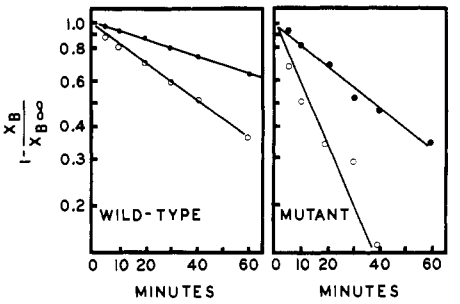

FIGURE 5: Semilogarithmic plot of the specific activities of the cellular ^{86}Rb pools in wild type and mutant 325B. Data from Figure 4, plotted according to eq 1. (O) pH 6.0 and (●) pH 7.5.

The implication that mutant 325B is defective in the uptake of Rb^+ against an apparent concentration gradient, is confirmed by the experiment illustrated in Figure 3. Wild type and mutant were grown on medium NaM-Rb containing 3 mM ^{86}Rb at pH 8; after 3.5 hr the pH was lowered to 6. The wild type was little affected by this procedure. Growth continued at the same rate and the ^{86}Rb content of the cells was increased somewhat. In the mutant, however, the ^{86}Rb level dropped abruptly, with concomitant cessation of growth. Similar results were obtained with five other isolates of independent origin. Revertants were found to have recovered the wild phenotype with respect to growth pattern and Rb^+ content.

Steady-State Exchange of ^{86}Rb in Mutant and Wild Type. From the results shown in Figure 3 it is clear that in mutant 325B at pH 6 there is a drastic effect on the rate of Rb^+ influx, Rb^+ efflux, or on both. In order to distinguish among these possibilities, the exchange of ^{86}Rb was studied under steady-state conditions. Cells of wild type and mutant were grown in medium NaM-Rb, washed, and resuspended in AD buffer containing glucose and 5 mM RbCl at pH 6 and 7.5. The suspensions were incubated for 30 min at 37° to establish a steady state, and carrier-free ^{86}Rb was then added. In all suspensions the Rb^+ contents remained reasonably constant for 120 min, but the absolute levels were very different (Table I).

The time course of ^{86}Rb uptake by the two strains at pH 6 and 7.5 is illustrated in Figure 4. It is evident that at pH 6, the *initial* rate of ^{86}Rb uptake by the mutant is the same as that of the wild type while at pH 7.5 it is somewhat higher. A semilogarithmic plot of the turnover of the cellular Rb^+ pools is shown in Figure 5. In both strains exchange was exponential, suggesting that most of the Rb^+ was present as a single pool. From these data, the rate constants for Rb^+ influx and efflux were calculated as described in the Experimental Section. The results are shown in Table I. It is evident that the rate contants for influx are nearly the same but that for efflux is considerably higher in the mutant than in the wild type. The effect is most pronounced at pH 6, but detectable even at pH 7.5.

If the low steady-state level of Rb^+ in mutant 325B at pH 6 (Figure 3, Table I) reflects primarily an increased rate of efflux, wild type and mutant should behave identically under conditions that minimize efflux. This is the case if cells are suspended in Tris-maleate buffer, for reasons which will be explained below. Indeed, when cells of wild type and mutant were incubated in Tris-maleate buffer at pH 6 with glucose and 0.1 mM ^{86}Rb the internal Rb^+ content of both cell types remained constant and almost identical exchange curves were obtained.

In both AD and Tris buffers, and regardless of the pH, uptake of ^{86}Rb was strictly dependent upon a source of metabolic energy. This was usually glucose, but arginine could be utilized by adapted cells.

Kinetic Parameters of ^{86}Rb Uptake. To fortify the conclusion that the genetic defect in mutant 325B is not primarily in the entry of Rb^+ into the cells, the apparent dissociation constants (K_m) and maximal rates (V_{max}) of this process were measured both in the presence and in the absence of Na^+. Initial rates

TABLE I: Cellular Rb^+ Levels and Rate Constants for ^{86}Rb Flux in the Steady State.[a]

Organism	pH	Steady-State Rb^+ Level (μmole/g)	Rate Constant (min^{-1}) Influx	Efflux
Wild type	6	600	1.5×10^{-3}	14×10^{-3}
	7.5	450	0.6×10^{-3}	7×10^{-3}
Mutant 325B	6	170	1.5×10^{-3}	51×10^{-3}
	7.5	420	1.3×10^{-3}	16×10^{-3}

[a] Cells of wild type and mutant 325B were incubated in AD buffer with glucose and 5 mM Rb^+. For details see text and Figures 4 and 5.

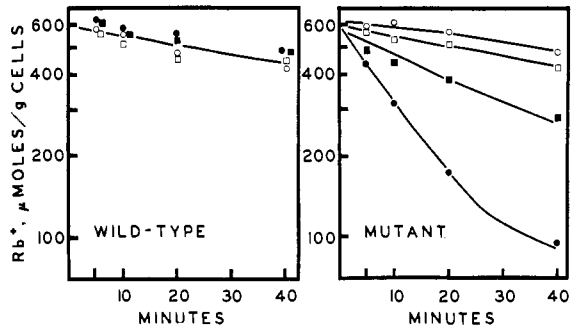

FIGURE 6: Efflux of ^{86}Rb from wild type and mutant 325B. Cells of both strains were grown on NaM-Rb supplemented with ^{86}Rb and harvested during the exponential phase. The cells were resuspended (0.6 mg/ml) in AD buffer at pH 6 and 7.5, and incubated at 37° with and without glucose. Samples of the cells were filtered, counted, and the Rb^+ content was calculated from the ^{86}Rb content of the cells. (○) pH 6, (●) pH 6 glucose, (□) pH 7.5, and (■) pH 7.5 glucose.

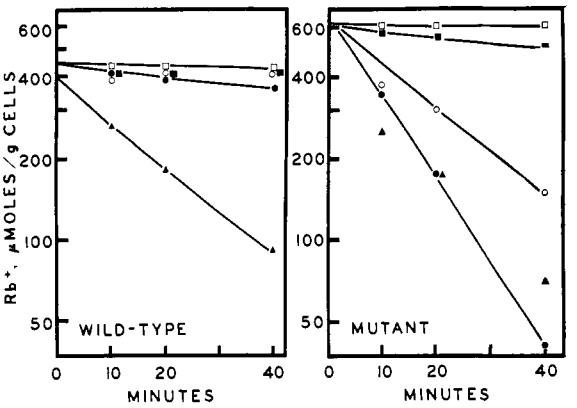

FIGURE 7: Displacement of ^{86}Rb from cells of wild type and mutant 325B by various cations. Cells were grown on medium NaM-Rb supplemented with ^{86}Rb and harvested during the exponential phase. The washed cells were resuspended in water at 37°. Glucose and cations (0.2 N cation, maleate, pH 6) were added at 0 min, and samples of the cells were filtered at intervals. (○) Li^+, (●) Na^+, (▲) Rb^+, (□) Mg^{2+}, and (■) $Tris^+$.

of ^{86}Rb uptake followed saturation kinetics and could be analyzed by the procedure of Lineweaver and Burk.

From the results presented in Table II it appears that the K_m for the mutant at pH 6 (in AD buffer) was twice that of the wild type. In agreement with earlier work (Abrams, 1960), Na^+ competitively inhibited the uptake of ^{86}Rb, and appeared to be a more potent inhibitor in the mutant than in the wild type. However, the rapid efflux of ^{86}Rb from 325B in the presence of Na^+, which will be documented below, would tend to produce misleadingly high values for the apparent K_m for ^{86}Rb entry in these cells; the significance of a twofold increase in K_m is thus uncertain. With this reservation we conclude only that the genetic lesion has not resulted in a drastic impairment of ^{86}Rb entry as measured by ^{86}Rb–Rb^+ exchange.

TABLE II: Kinetic Parameters of ^{86}Rb–Rb^+ Exchange in Wild Type and Mutant 325B at pH 6.[a]

Buffer (pH 6)	K_m (mM)		V_{max} (μmoles/g min)	
	Wild Type	325B	Wild Type	325B
Tris-maleate (0.1 N)	0.3	0.5	35	50
Tris-maleate plus 0.2 N NaCl	2	5	32	50
AD buffer	4	8	65	40

[a] Cells of wild type and mutant were harvested during the exponential phase of growth on medium NaM-Rb. The cells were washed and resuspended in AD or 0.1 M Tris-maleate buffer (pH 6) with glucose. After a brief incubation at 37°, various amounts of ^{86}Rb were added, and samples were filtered after 1–3 min. Where necessary, the specific activity of the extracellular ^{86}Rb was corrected for dilution of the radioactivity by efflux of unlabeled Rb^+ from the cells.

Efflux of Rb^+ and K^+ from Wild Type and Mutant. Since the experiments described above revealed no striking defect in entry of ^{86}Rb into mutant 325B, the loss of Rb^+ from the cells was examined. Wild type and mutant were grown for several hours in NaM-Rb containing ^{86}Rb of known specific activity. The cells were then harvested and incubated in AD buffer (no Rb^+), with and without glucose at pH 6 and 7.5. The results are shown in Figure 6. Loss of ^{86}Rb from cells of the wild type was slow, and not significantly affected by the presence of glucose. Reabsorption of ^{86}Rb does not appear to make a major contribution to the capacity of wild-type cells to retain ^{86}Rb. By contrast, in the mutant glucose markedly accelerated the efflux of ^{86}Rb from the cells, particularly at pH 6. The initial loss of ^{86}Rb was approximately exponential.

Essentially the same results were obtained with cells preloaded with K^+ rather than with ^{86}Rb. In the absence of an energy source, both wild type and mutant gradually lost K^+ with a half-time of about 30 min at 37°. In the presence of glucose, K^+ efflux from the mutant was sharply increased (half-time 5 min at pH 6, 37°). Electrical neutrality was preserved by cation exchange. K^+ or Rb^+ lost were stoichiometrically replaced by Na^+.

It was of interest to compare various cations with respect to their ability to displace ^{86}Rb. As shown in Figure 7, 200 mM Na^+, Li^+, K^+, and Rb^+ rapidly displaced ^{86}Rb from cells of mutant 325B, while Mg^{2+}

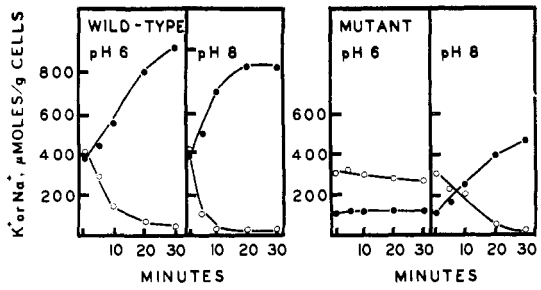

FIGURE 8: Net uptake of K^+ in exchange for internal cations by wild type and mutant 325B. Both strains were grown overnight on medium NaTY. The cells were washed with water and resuspended in water (1 mg/ml) at room temperature. Glucose was added, and pH 6 or 8 maintained by use of a pH-Stat. At 0 min, KCl was added to 2 mM. (●) K^+ and (○) Na^+.

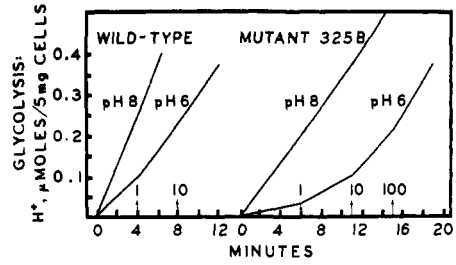

FIGURE 10: Glycolysis by wild type and mutant 325B. Both strains were grown on medium NaTY, and then allowed to accumulate K^+. Final levels of K^+ and Na^+ were wild type, 630 and 40 μmoles/g of cells, respectively; mutant 325B, 590 and 60 μmoles/g of cells. The cells were resuspended in water at 1 mg/ml, supplemented with glucose, and allowed to glycolyze at room temperature on a pH-Stat at pH 6 or 8. At arrows, K^+ was added to the final concentrations (micromoles per milliliter) shown. Production of H^+ was calculated from the rate of consumption of NaOH.

and Tris did not. Only K^+ and Rb^+ displaced ^{86}Rb from the wild type. The rate of ^{86}Rb displacement from mutant 325B by Na^+ attained one-half its maximal value at 0.05 N Na^+.

Analogous experiments were also carried out with protoplasts of wild type and mutant. In the presence of 200 mM Na^+ at pH 6, ^{86}Rb was rapidly lost from protoplasts of mutant 325B but was retained by those of the wild type. The cell wall thus appears to make no contribution to the expression of the $Cn^-{}_{K6}$ phenotype.

Net Uptake of K^+ in Exchange for Na^+. In a recent paper, Zarlengo and Schultz (1966) showed that cells of *S. faecalis* grown overnight on medium NaTy are depleted of K^+ but contain large amounts of Na^+ and H^+. In the presence of glucose such cells take up K^+ from low external concentrations with concomitant extrusion of Na^+ and H^+.

Net uptake of K^+ by wild type and mutant are compared in Figure 8. In order to minimize complications resulting from displacement of K^+ (Figure 7) the cells were suspended in water or in Tris buffer, which do not cause displacement. The wild type rapidly absorbed K^+ and extruded Na^+, both at pH 6 and 7.5. In the mutant both processes were severely inhibited, particularly at pH 6. It should be stressed that no external Na^+ was added in these experiments. Analogous experiments were carried out with arginine, rather than glucose, as energy source; once again, net K^+ uptake by the mutant was severely impaired.

Passive Uptake of K^+ by Wild Type and Mutant 325B. According to Zarlengo and Schultz (1966), net uptake of K^+ from dilute solution was strictly dependent upon a source of metabolic energy; this finding was confirmed in the present study. However, when high concentrations of K^+ (50–200 mM) were presented to the cells, considerable uptake occurred in exchange for internal Na^+ and perhaps H^+ even in the *absence* of an energy source. In some experiments, iodoacetate (1 mM) or sodium azide (10 mM) was added to suppress residual metabolism of endogenous substrates; these inhibitors did not prevent the cation exchange, which thus appears to be truly independent of energy generation. Passive uptake of K^+ by mutant 325B was somewhat more extensive than by the wild type (Figure 9).

Rates of Glycolysis in Wild Type and Mutant. In the course of these studies it was consistently observed that glycolysis by wild-type cells at pH 6 was considerably more rapid than that by $Cn^-{}_{K6}$ mutants. At pH 8, however, the rates were essentially the same.

An example of this effect is shown in Figure 10. Cells of wild type and mutant were loaded with K^+ by incubation with glucose and 10 mM K^+. The cells were washed and resuspended in water; glycolysis was followed on a pH-Stat at both pH 6 and 8. The wild type glycolyzed rapidly regardless of pH. In the mutant, glycolysis was inhibited at pH 6 but could be stimulated by addition of K^+ (or even by high concentrations of

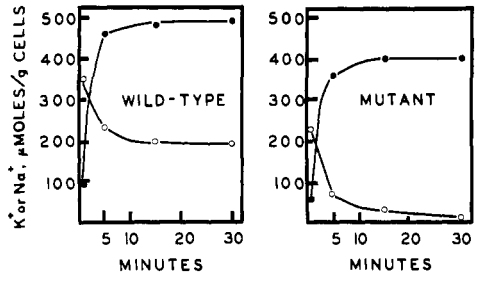

FIGURE 9: Passive uptake of K^+ by wild type and mutant 325B. Both strains were grown overnight on medium NaTY. The cells were washed with water and resuspended in water at 1 mg/ml. A pH-Stat was used to maintain the pH at 6, room temperature. At 0 min, KCl was added to 50 mM. Same key as Figure 8.

Na^+). It should be stressed that this effect is not due to lack of internal K^+ in the mutant cells, as both wild-type and mutant organisms contained large and equal amounts of K^+.

Dependence of the rate of glycolysis on external cations was even more pronounced when K^+-depleted cells were employed. For example, wild-type cells harvested after overnight growth on medium NaTY required small amounts of K^+ (1 mM) for optimal glycolysis at pH 6; in mutant 325B, larger amounts of K^+ were required, both at pH 6 (up to 100 mM) and 8 (5 mM). The reduced rate of glycolysis by the mutants may contribute to their inability to carry out net K^+ uptake (Figure 8), but is not the primary reason since stimulation of glycolysis (*e.g.*, by addition of NH_4^+; Zarlengo and Abrams, 1963) did not stimulate K^+ uptake.

Discussion

S. faecalis, like other microorganisms, selectively concentrates K^+ or Rb^+ during growth, even in a medium in which Na^+ is the predominant cation. Uptake of cations from the medium must be balanced electrically, either by concurrent uptake of anions or by the extrusion of cations from the cell. It is the latter process of cation exchange that concerns us here.

Cells rich in Rb^+ rapidly take up exogenous ^{86}Rb by exchange for Rb^+ from the intracellular pool ("autologous" exchange). This process is strictly energy dependent, obeys saturation kinetics, and is competitively inhibited by Na^+ but not by Tris. Presumably it reflects the operation of an energy-requiring, carrier-mediated transport system which strongly prefers Rb^+ to Na^+. Autologous exchange of K^+ was not studied, but is well known from other microbial species (Rothstein, 1959; Epstein and Schultz, 1966; Slayman and Tatum, 1965a).

Cation exchange can also bring about *net* uptake of K^+. Zarlengo and Schultz (1966) showed that cells of *S. faecalis* which are relatively depleted of K^+ but contain high concentrations of Na^+ and H^+ exhibit selective net uptake of K^+ if provided with an energy source. Electroneutrality is preserved by concurrent extrusion of Na^+ and H^+ ("heterologous" exchange). Similar exchanges occur in mammalian cells (reviewed by Judah and Ahmed, 1964), yeast (Rothstein, 1959), and *E. coli* (Schultz *et al.*, 1963).

In the hope of gaining insight into the molecular basis of K^+ transport, mutants deficient in this process have been isolated in several laboratories. Those reported to date fall into two distinct classes. Damadian (1966; Damadian and Trout, 1966) and Slayman and Tatum (1965b) have described mutants in which the defect appears to reside in the entry of K^+ into the cells and is reflected in an increased K_m for K^+ uptake. A very different phenotype was first described by Lubin and Ennis (1963). A mutant of *E. coli* is defective in *retention* of K^+ but rapidly exchanges external for internal K^+ (Lubochinsky *et al.*, 1964, 1966). In a paper that came to our attention after the present work had been submitted for publication, Günther and Dorn (1966) demonstrated increased efflux of K^+ from this mutant and proposed that the mutation altered the specificity of a carrier involved in the transport of cations out of the cells. The mutants of *S. faecalis* described here resemble the efflux mutants of *E. coli* and are designated Cn^-_{K6} in accordance with the suggestion of Lubochinsky *et al.* (1964).

Mutants of the phenotype Cn^-_{K6} require elevated levels of K^+ or Rb^+ for growth in medium NaM at pH 6 due to a defect in cation transport, but are almost normal at pH 8 (Figures 1–3). Expression of the phenotype is independent of the presence of Na^+ in the medium. The phenotype of these mutants at pH 6 is defined by three experimental procedures. (1) Measurements of ^{86}Rb–Rb^+ exchange in the steady state indicate that the kinetic parameters of Rb^+ entry are very similar in wild type and mutant, and point to rapid exit of Rb^+ from the cells as the primary site of the defect (Figures 4 and 5). (2) Cells of the mutant loaded with Rb^+ or K^+ rapidly lose these cations to the medium by energy-dependent heterologous exchange with external Na^+ or Li^+. These cations do not displace Rb^+ and K^+ from the wild type (Figures 6 and 7). (3) Cells of the mutant preloaded with Na^+ and H^+ are severely deficient in energy-dependent net uptake of K^+ by heterologous exchange for the internal cations (Figure 8). Passive cation exchanges are approximately normal (Figure 9).

It is evident that Cn^-_{K6} mutants, unlike the majority of transport mutants reported in the literature, are not defective in the entry reaction. The primary defect would rather appear to reside in the *exit* of cations from the cells.

Models for cation exchange (for recent references see Judah and Ahmed, 1964; Skou, 1965; Jardetzky, 1966) invoke carrier molecules which undergo movement or allosteric rearrangement by interaction with adenosine triphosphate. Appropriate cation-binding sites face one side of the membrane in one configuration and the opposite side in the other. Transport inward prefers K^+ and Rb^+ to Na^+, whereas in the outward direction Na^+ and H^+ are the preferred substrates. Thus, for example, cells loaded with Na^+ could carry out net uptake of ^{86}Rb by heterologous exchange, whereas cells loaded with Rb^+ would exhibit autologous exchange.

In terms of this generalized model, the Cn^-_{K6} phenotype can be explained by the assumption that in these mutants *a binding site involved in the transport of monovalent cations outwards has high affinity for K^+ and Rb^+*. The binding sites for entry and for exit are probably in some sense distinct since the mutation appears to alter the characteristics of exit more than those of entry. Such a defective transport system could still carry out ^{86}Rb–Rb^+ exchange, but would be deficient in the net uptake of K^+ and Rb^+ by heterologous exchange for Na^+ and H^+. The displacement of K^+ and Rb^+ from the mutant by Na^+ can be explained as follows. Na^+ has significant affinity for the entry site and will be transported into the cell. In the wild

type, the binding site for exit has high Na^+ affinity and will, therefore, pump Na^+ back out. In the mutant, high K^+ affinity for exit would result in transport of K^+ from the inside to the outer surface of the cell, there to be competitively displaced by Na^+. (Tris does not displace K^+, yet has been found to enter the cells. A possible reason for the failure of Tris to displace K^+ is that it has virtually no affinity for the K^+ binding site at the outer surface.) In principle this hypothesis is identical with that proposed by Günther and Dorn (1966) to explain their findings with *E. coli*.

The reason for the pH dependence of the $Cn^-{}_{K6}$ phenotype is unknown. Marked effects of the pH upon the selectivity of the cation-transport system in yeast have been described by Armstrong and Rothstein (1964) and the interaction of the transport system with H^+ may well have been modified by the mutation. Alternatively, distinct carriers may mediate cation transport at acid and alkaline pH, respectively; this, in fact, is the case for phosphate transport in *S. faecalis* (Harold and Baarda, 1966). The impairment of glycolysis in $Cn^-{}_{K6}$ mutants also remains to be clarified. We have confirmed the observation of Zarlengo and Schultz (1966) that low concentrations of K^+ or Rb^+ stimulate glycolysis by the wild type; the mutants required higher concentrations of K^+, as much as 100 mM K^+ at pH 6 (Figure 10). This effect was observed even in mutant cells loaded with K^+ and is thus not a consequence of K^+ depletion. It remains to be seen whether these findings can be explained by the suggestion (Zarlengo and Schultz, 1966; Whittam and Ager, 1965) that net K^+ uptake accelerates the hydrolysis of adenosine triphosphate which, in turn, stimulates glycolysis.

References

Abrams, A. (1959), *J. Biol. Chem. 234*, 383.

Abrams, A. (1960), *J. Biol. Chem. 235*, 1281.

Ames, G. F. (1964), *Arch. Biochem. Biophys. 104*, 1.

Armstrong, W. McD., and Rothstein, A. (1964), *J. Gen. Physiol. 48*, 61.

Damadian, R. (1966), U. S. A. F. School of Aerospace Medicine Report SAM-TR-66-19.

Damadian, R., and Trout, D. L. (1966), *Federation Proc. 25*, 632.

Egan, J. B., and Morse, M. L. (1966), *Biochim. Biophys. Acta 112*, 63.

Epstein, W., and Schultz, S. G. (1966), *J. Gen. Physiol. 49*, 469.

Günther, T., and Dorn, F. (1966), *Z. Naturforsch. 21b*, 1082.

Hagihara, H., Wilson, T. H., and Lin, E. C. C. (1963), *Biochim. Biophys. Acta 78*, 505.

Harold, F. M., and Baarda, J. R. (1966), *J. Bacteriol. 91*, 2257.

Harold, F. M., Harold, R. L., and Abrams, A. (1965), *J. Biol. Chem. 240*, 3145.

Jardetzky, O. (1966), *Nature 211*, 969.

Judah, J. D., and Ahmed, K. (1964), *Biol. Rev. 39*, 160.

Kepes, A., and Cohen, G. N. (1962), *in* The Bacteria, Vol. IV, Stanier, R. Y., and Gunsalus, I. C., Ed., New York, N. Y., Academic, p 179.

Kessel, D., and Lubin, M. (1962), *Biochim. Biophys. Acta 57*, 32.

Kessel, D., and Lubin, M. (1963), *Biochim. Biophys. Acta 71*, 656.

Koch, A. L. (1964), *Biochim. Biophys. Acta 79*, 177.

Lester, J. (1958), *J. Bacteriol. 75*, 426.

Lubin, M., and Ennis, H. L. (1963), *Biochim. Biophys. Acta 80*, 614.

Lubochinsky, B., Meury, J., and Stolkowski, J. (1964), *Compt. Rend. C. R. Acad. Sci. (Paris) 258*, 5106.

Lubochinsky, B., Meury, J., and Stolkowski, J. (1966), *Bull. Soc. Chim. Biol. 48*, 873.

MacLeod, R. A., and Snell, E. E. (1948), *J. Biol. Chem. 176*, 39.

Pardee, A. B., Prestidge, L. S., Whipple, M. B., and Dreyfuss, J. (1966), *J. Biol. Chem. 241*, 3962.

Rickenberg, H. V., Cohen, G. N., Buttin, G., and Monod, J. (1956), *Ann. Inst. Pasteur 91*, 829.

Rothstein, A. (1959), *Bacteriol. Rev. 23*, 175.

Schultz, S. G., Epstein, W., and Solomon, A. K. (1963), *J. Gen. Physiol. 47*, 329.

Skou, J. C. (1965), *Physiol. Rev. 45*, 596.

Slayman, C. W., and Tatum, E. L. (1965a), *Biochim. Biophys. Acta 102*, 149.

Slayman, C. W., and Tatum, E. L. (1965b), *Biochim. Biophys. Acta 109*, 184.

Whittam, R., and Ager, M. E. (1965), *Biochem. J. 97*, 214.

Wiesmeyer, H., and Cohn, M. (1960), *Biochim. Biophys. Acta 39*, 440.

Zarlengo, M. H., and Abrams, A. (1963), *Biochim. Biophys. Acta 71*, 65.

Zarlengo, M. H., and Schultz, S. G. (1966), *Biochim. Biophys. Acta 126*, 308.