to the uptake of soluble protein antigens, antibodies present in the mucus layer may combine with antigens to form complexes and thereby prevent the molecules from reaching the surface of the enterocyte from whence uptake by pinocytosis is initiated (*2*). With respect to the uptake of preformed antigen-antibody complexes or those forming in the lumen of the intestine, mucus may serve as a mechanical barrier to the diffusion of the complexes, thereby again limiting the access of complexes to the surface of the enterocyte (*1*). Our experiments suggest an additional relation between immune complexes and mucus, that is, the ability of the former to stimulate release of mucus by the intact small intestine of the rat. Release of mucus may in turn serve to clear the surface of the gut of adherent immune complexes.

The mechanisms by which immune complexes stimulate the release of goblet cell mucus is still to be determined. Certain antigen-antibody complexes might interact with receptors on the surface of goblet cells and thereby stimulate release of mucus, or interaction of complexes with epithelial cells might stimulate such cells to release unknown mediators which in turn activate goblet cells. Alternatively, it is known that the first component of complement and possibly others are produced by the intestine (*16*), and that rat IgG-1 antibodies are capable of activating the complement sequence (*17*). Therefore, it is possible that components of the complement system might be involved in mediating the release of mucus.

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Circulation of H^+ and K^+ Across the Plasma Membrane Is Not Obligatory for Bacterial Growth

Abstract. Streptococcus faecalis *grows normally in the presence of gramicidin and other ionophores under conditions such that there is no gradient of* pH *or of electrical potential across the plasma membrane and that currents of* H^+, K^+, *and* Na^+ *are short-circuited. Growth requires a rich medium, a slightly alkaline* pH, *and a high concentration of external* K^+. *The proton circulation maintains the cytoplasmic* pH *and pools of ions and other metabolites but is not obligatory for biosynthetic functions including DNA replication, cell division, or assembly of the structural framework of the cell.*

It is now generally accepted that a central feature of energy metabolism in bacteria is the circulation of protons across the plasma membrane (*1*). Several major metabolic pathways, including the respiratory chain and the membrane-bound adenosine triphosphatase (E.C. 3.6.1.3) complex, mediate electrogenic transport of protons outward; the current loop is completed by an array of molecular devices that allow protons to return to the cytoplasm while performing useful work. Examples of proton-linked processes are oxidative and photosynthetic phosphorylation, transhydrogenation of pyridine nucleotides, motility, and the transport of many metabolites (*1*, *2*). In order to determine whether ion currents are involved in other essential functions, particularly in the construction and replication of the fabric of bacterial cells, we have examined the growth of *Streptococcus faecalis* in the presence of ionophores. This fermentative organism normally maintains a circulation of protons across the membrane by means of a proton-translocating adenosine triphosphatase (*2*, *3*); gramicidin and other ionophores were used to short-circuit the proton current and to dissipate gradients of K^+ and Na^+. The results confirm that the proton circulation is required to maintain cytoplasmic pools of ions and metabolites as well as a neutral *p*H. However, it is not obligatory for the synthesis of macromolecules and organelles (cell wall, membranes, nucleoid and other structural elements), for DNA replication, or for cell division.

The basic observation is illustrated in Fig. 1A. Growth of *Streptococcus faecalis* (*faecium*) (American Type Culture Collection 9790) in the standard complex medium NaTY (*4*) was completely blocked by gramicidin D, an ionophore that renders the cytoplasmic membrane permeable to K^+, Na^+, and H^+ (*5*, *6*). By contrast, in the medium designated KTY2XH (*4*) exponential growth continued at a rate not much less than that of control cells. This particular medium is buffered at *p*H 7.7 and contains 0.28*N* K^+ and less than 0.01*N* Na^+. Similar results were obtained with complex media containing as much as 1*N* K^+ and buffered with bicine, tricine, or Hepes (*7*) between *p*H 7.5 and 8.2. Other ionophores, including gramicidin A, valinomycin plus nigericin (1 μg/ml each), and the proton conductors tetrachlorosalicylanilide and carbonylcyanide *m*-chlorophenylhydrazone ($10^{-5}M$) also block growth in NaTY but not in media enriched with K^+ (data not shown).

Although the properties of the ionophores are well documented (*5*, *6*), it is important to establish that cells growing in the presence of gramicidin on medium KTY2XH maintain neither an electrical potential across the cytoplasmic membrane nor concentration gradients of H^+, K^+, or Na^+. With methods presently available (*8*) these measurements cannot be made directly on growing cells. We therefore compared cells that had been grown for at least four generations in KTY2XH in the absence or presence of gramicidin under the conditions of Fig. 1A; the cells were then washed and resuspended in buffer (0.14*M* K_2HPO_4, 0.05*M* Hepes, *p*H 7.5). Cells grown with gramicidin retain the antibiotic through

washing and resuspension. With glucose as an energy source, control cells generated a pH gradient of about 0.6 unit, with the interior alkaline, as measured by the distribution of ^{14}C-dimethyloxazolidinedione (*8*); in gramicidin-grown cells there was no detectable pH gradient. Attempts to measure the membrane potential on the basis of the accumulation of lipid-soluble cations or by fluorescence quenching (*8, 9*) were vitiated by the high concentration of external K^+; we therefore used the accumulation of ^{14}C-methyl-β-D-thiogalactoside as an index of the total proton motive force (*10*). In the presence of glucose, control cells achieved a concentration gradient of about 24 (average of three experiments), corresponding to a total proton motive force of at least −83 mv; there was no measurable accumulation by gramicidin-grown cells. In other experiments we found that gramicidin-grown cells exhibit neither glucose-stimulated uptake of threonine (*11*) nor valinomycin-induced quenching of dye fluorescence (*9*); these results support our expectation that gramicidin-grown cells are permeable to cations and cannot sustain a metabolic proton circulation. Donnan effects could still give rise to a membrane potential or pH gradients, but this should not be the case here as the external K^+ concentration (0.28*N* to 1*N*) brackets the cytoplasmic concentration (0.4*N* to 0.8*N*) (*3*). We conclude that the cells are indeed permeable to H^+, K^+, and Na^+ and that they are growing in the absence of a significant membrane potential or cation concentration gradients.

We now turn to the capacities and limitations of cells short-circuited by gramicidin. Exponential growth continued for at least 15 generations when portions of the culture were periodically diluted with fresh medium containing gramicidin; the doubling time was generally somewhat longer than that of control cells (on the average, 50 and 40 minutes, respectively). Cell division was essentially normal. After 15 doublings in the presence of gramicidin the viable count was 45 percent of that of a control culture at the same turbidity; the organisms tend to form somewhat longer chains in the presence of the antibiotic. Normal and gramicidin-grown cells did not differ markedly with respect to their adenosine triphosphate content, their rates of glycolysis and adenosine triphosphate turnover, or their ability to adapt to arginine fermentation with induction of the arginine dihydrolase (E.C. 3.5.3.6) pathway. *Streptococcus faecalis* (*faecium*) 9790 generates adenosine triphosphate entirely by substrate-level reactions

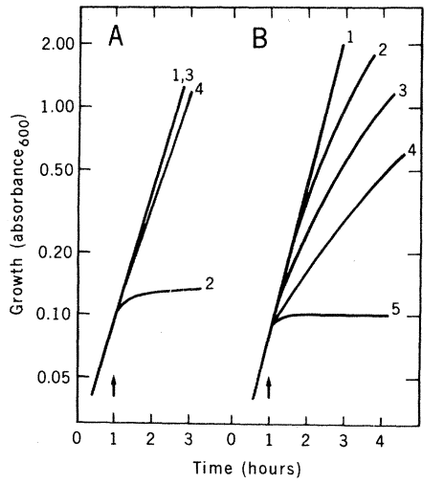

Fig. 1. Effect of gramicidin D on the growth of *Streptococcus faecalis*. (A) The role of K^+. Organisms were grown at 34°C in either the Na^+-rich medium NaTY (pH 7.5; curves 1 and 2) or in the K^+-rich medium KTY2XH (curves 3 and 4) at pH 7.7. The arrow indicates the addition of gramicidin D (1 μg/ml final, in ethanol) to tubes 2 and 4; control cultures received ethanol only. (B) The role of pH. Cultures were grown in KTY2XH at pH 7.7, 7.1, 6.6, or 6.0. Control cultures received ethanol at the arrow; all grew at the same rate (curve 1). Experimental cultures received gramicidin D (1 μg/ml final); growth was increasingly inhibited at the lower pH (curves 2 through 5). Growth was monitored on the basis of the turbidity at 600 nm.

rather than by phosphorylation linked to electron transport. Most important, electron micrographs revealed no obvious morphological abnormalities; thin sections of control and gramicidin-grown cells were indistinguishable at a magnification of × 100,000 (data not shown).

However, gramicidin restricts growth to a narrow range of conditions. Growth in KTY2XH required a pH above 7 (Fig. 1B), presumably because in the presence of gramicidin the cytoplasmic pH equals that of the medium. A very high concentration of K^+ was also required (at pH 7.8, the doubling time was approximately 50 minutes in 0.3*N* K^+ and 90 minutes in 0.1*N* K^+; the cells did not grow in 0.02*N* K^+), and growth was inhibited by Na^+ above 0.1*N*. By contrast, control cells grow well on 0.001*N* K^+, even in the presence of 0.3*N* Na^+, at a pH as low as 5. The concentrations of tryptone and yeast extract were not critical, but, when the experiments were repeated in defined medium (*12*), the limitations of short-circuited cells became apparent. In the standard medium (*12*), containing most of the amino acids at 1 to 2 m*M*, the cells grew almost as well in the presence of gramicidin as in its absence. However, when the amino acid concentrations were reduced to 0.1 m*M*, gramicidin severely inhibited both the rate and the extent of growth, by comparison with control cells. Presumably these stringent nutritional conditions require the cells to accumulate amino acids against the concentration gradient, a process that depends upon the proton circulation (*2, 6, 10, 11*).

The disabilities of gramicidin-grown cells are consistent with the known roles of the proton circulation in the transport of metabolites and the maintenance of the cytoplasmic pH (*1–3*). Under conditions that compensate for these disabilities, *S. faecalis* can grow normally in the presence of ionophores. The proton circulation is thus dispensable for the construction and replication of a bacterial cell. The finding of Kopecky *et al.* (*13*) that *Escherichia coli* "killed" by colicin K grow when supplied with excess K^+ and Mg^{2+} suggests that this conclusion may be generally applicable. By contrast, there is mounting evidence (*14*) that many eukaryotic microorganisms rely upon ion currents localized in space to guide their growth and development.

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