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Investigating Gentamicin Mechanism of Action with Fluorescence Microscopy: Relationships between Colony Growth, Antibiotic Accumulation, and Membrane Permeability[1] JILLIAN RANKIN, Emory University — Aminoglycosides constitute a powerful, broad-spectrum class of antibiotics. While aminoglycosides are known to exert their antimicrobial activity through ribosome targeting, the precise mechanism of action through which they produce cell death remains undefined. In particular, there is no clear scientific consensus regarding the role of membrane damage in initiating growth arrest. In this study, we probed aminoglycoside mechanism of action by using fluorescence microscopy to evaluate the relationships between membrane damage, growth arrest, and antibiotic accumulation in *Escherichia coli* microcolonies. A fluorophore-conjugated aminoglycoside, gentamicin-Texas Red, was used to assess antibiotic accumulation, while SYTOX Green, a stain impermeable to live cells, was used to assess membrane permeability. To further evaluate the role of membrane integrity in growth inhibition, treatment groups with variable degrees of induced membrane permeability were compared. We found that, in the context of gentamicin treatment, induced degrees of membrane permeability were associated with increased rates and extents of gentamicin accumulation; however, these outcomes were not reliable predictors of growth rate or the onset of growth inhibition. Furthermore, our analysis of the relationships between changes in membrane permeability, accumulation, and growth inhibition suggests that the onset of significant losses in membrane integrity are not required to initiate growth arrest.

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