



CALIFORNIA STATE SCIENCE FAIR 2015 PROJECT SUMMARY

Name(s) Charley Bi; Austin Chang; Cooper Ge	Project Number S0506
Project Title How Bacterially Secreted Intrinsic Factor Affects Vitamin B12 Transfer	
Abstract Objectives/Goals To test the effectiveness of recombinant gastric intrinsic factor secreted by genetically modified E. Coli, compared to normal human gastric intrinsic factor, in the ability to transfer vitamin B12 across a Caco-2 cell membrane in a transwell setup. Methods/Materials Caco-2 cells, Corning (c) transwell inserts, genetically modified E. Coli K12, E. Coli K12, Vitamin B12, gastric intrinsic factor Caco-2 cells were seeded into 24 transwell inserts and allowed to differentiate into a monolayer over 3 weeks. Then, a solution was introduced into the apical side of the insert for each testing group. Our negative control had neither E. Coli nor human gastric intrinsic factor (hgIF). Our positive control had no E. Coli and had hgIF. Our interference test had non-modified E. Coli with no hgIF. Finally, our experimental test group had our genetically modified E. Coli with no hgIF, but rather with recombinant gastric intrinsic factor. With four trials of each testing group, 30 minute timed cell-mediated transport of vitamin B12 from the apical side to the basolateral side of the insert was measured via ELISA and spectrophotometer. Results Plotting the samples, which were diluted 1:5 and 1:40, against the standard curve revealed that all the samples were below the limit of quantification. Conclusions/Discussion Because very little vitamin B12 was measured transferring through the cell monolayer, there is evidence to support the claim that our cell monolayer was healthy and effectively blocked passive diffusion of vitamin B12. In addition, if our Caco-2 cells were indeed healthy, there is evidence to support the claim that intestinal cells can remain healthy even in environments with bacteria or genetically modified bacteria.	
Summary Statement Our experiment tested whether or not bacterially produced gastric intrinsic factor would be an effective replacement for human gastric intrinsic factor that is missing from patients with pernicious anemia.	
Help Received Dr. Huff taught us proper lab technique and oversaw the purchase of equipment as well as the thawing, passaging, and seeding of the Caco-2 cells. Valley Christian High School allowed us to use the school's lab equipment and also assisted with funding the project.	