Antacid Effects on Microbial Flora

Warren Austin
Grade 10
Pittsburgh Central Catholic High School
Microbial Flora of the Body

- A mixture of organisms at any anatomical site
- In contact with surface tissues and colonize:
  - skin
  - conjunctiva
  - nose
  - pharynx
  - mouth
  - lower GI

- Areas provide
  - moisture
  - nourishment, temperature, and stable pH
Common Microbial Flora

- **Eukaryotic-fungi, protist**

- **Prokaryotic-bacteria**
  - Gram positive or Gram negative
    - Staphylococcus epidermidis
    - Streptococcus salivarius
    - Neisseria meningitidis
    - Enterobacteriae
    - Pseudomonas aeruginosa
    - Lactobacillus
    - Mycobacteria
# Gram Negative and Gram Positive Bacteria

<table>
<thead>
<tr>
<th>Gram -</th>
<th>Gram +</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Contains a cell wall that is a thin layer of lipopolysaccharide (lipids and carbohydrates)</td>
<td></td>
</tr>
<tr>
<td>• Adds protection</td>
<td></td>
</tr>
<tr>
<td>• Protects from antibiotics</td>
<td></td>
</tr>
<tr>
<td>• Escherichia coli is an example</td>
<td></td>
</tr>
<tr>
<td>• Most pathogenic</td>
<td></td>
</tr>
<tr>
<td>• Contains a simple cell wall</td>
<td></td>
</tr>
<tr>
<td>• Antibiotics work against the formation of the cell wall</td>
<td></td>
</tr>
<tr>
<td>• Staphylococcus epidermidis is an example</td>
<td></td>
</tr>
</tbody>
</table>
Escherichia coli

- Belongs to Enterobacteriaceae group
- Rod-shaped body, covered with pili for mobility
- Gram negative facultative anaerobe
- Useful model that can double in twenty minutes
- Found in soil and water but most common in the intestines
- Consumed by humans when eat or drink
- Most is harmless and an important part of healthy intestinal tract
- Source of Vitamin B12 and K
Relevance of Project

- Microbial flora are present throughout the body
- Some are harmless and some can be dangerous
- It is appears that micro flora plays an overall role in human health
- Humans consume various types of products everyday
- What effect do these consumables have on microbial flora?
Antacids

- Used to relieve
  - acid indigestion
  - stomach ailments
  - heartburn
- Short duration of action
- Most appropriate for gastric discomfort for a short period of time

- Two methods of action:
  - chemical neutralization of gastric acid, most notably sodium bicarbonate
  - adsorption of the acid (non-absorbable antacids), such as calcium and magnesium salts
- More effective in liquid formulations
Antacids

Medication enters stomach

Acid stomach contents

Neutralised stomach contents
Common Antacids and Ingredients

- Pepto Bismol
- Tums
- Heartburn OTC
- Nexium
- Aluminum hydroxide
- Calcium carbonate
- Sodium bicarbonate
Bismuth Subsalicylate
(Pepto Bismol)

- Low salt
- Small molecule
- Antidiarrheal
- Antacid
- Antibiotic

- Treats nausea, heartburn, indigestion, upset stomach, and “Traveler’s Diarrhea”
Bismuth Subsalicylate (Pepto Bismol) Cont.

- Displays anti-inflammatory action due to salicylic acid
- Stops the removal of fluids that are released into the digestive system
- Slows the growth of bacteria
- Stimulates absorption of fluid and electrolytes across the intestinal wall
- Inhibits the synthesis of lipids
- Small amounts of heavy metals become toxic microbes
- pH of 7
Ingredients of Pepto Bismol

- Benzoic acid
- Magnesium aluminum silicate
- Methylcellulose
- Red 22
- Red 28
- Saccharin sodium
- Salicylic acid
- Sodium salicylate
- Sorbic acid
- Water
Common Side Effects:

- Black tongue
- Black stool
- Constipation
- Diarrhea
- Nausea
Past Studies

- Bismuth subsalicylate was able to bind large species of bacteria and effectively kill them.
- Caused continual decrease in cell survivorship.
- Intracellular ATP decreased rapidly and destroyed membrane.
- Conclusion: A pH lower than 7 was more effective than 7 but still effectively effaced gastrointestinal infections.
Does Pepto Bismol significantly effect Escherichia coli survivorship?

Determine if Pepto Bismol reduces the survivorship of Escherichia coli in various concentrations
Hypotheses

- **Null Hypothesis**: Pepto Bismol will not significantly alter the survivorship of *Escherichia coli*

- **Alternative Hypothesis**: Pepto Bismol will significantly reduce the survivorship of *Escherichia coli*
Materials

- Micropipettes
- Sterile pipette tips
- **Pepto Bismol (Stock Concentration of 10%)**
- **Escherichia coli (DH5-Alpha)**
- Sterile Dilution Fluid (100mM KH2PO4, 100mM K2HPO4, 10mM MgSO4, 1mM NaCl)
- **LB Agar Plates**
- **LB Media** (0.5% yeast extract, 1% tryptone, 1% sodium chloride)
- Spreader
- Labeling Tape
- Marker
- Vortex
- Test tube rack
- Sidearm flask
- **Klett Spectrophotometer**
- Ethanol
- Bunsen Burners
- Matches
- Latex gloves
Procedure

- Escherichia coli was grown overnight in sterile LB media.
- A sample of this culture was added to LB media in a sidearm flask.
- The culture was incubated until a density of 50 Klett spectrophotometer units was reached. This represents a density of $10^8$ cells/ml.
- The culture was then diluted in a sterile dilution fluid to a density of $10^5$ cells/mL.
- Pepto Bismol was pipetted out of the bottle (assumed to be sterile because of a non-broken seal) and was diluted to concentrations of 0%, 0.1%, 1%, and 10% in sterile dilution fluid.
# Chart of Liquid Concentrations

<table>
<thead>
<tr>
<th>Contents</th>
<th>0% Concentration</th>
<th>0.1% Concentration</th>
<th>1% Concentration</th>
<th>10% Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Fluid</td>
<td>8.9mL</td>
<td>8.9mL</td>
<td>8.9mL</td>
<td>8.9mL</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.1mL</td>
<td>0.1mL</td>
<td>0.1mL</td>
<td>0.1mL</td>
</tr>
<tr>
<td>Additional Sterile Fluid</td>
<td>1.0mL</td>
<td>0.99mL</td>
<td>0.9mL</td>
<td>1.0mL</td>
</tr>
<tr>
<td>Pepto Bismol</td>
<td>0.0mL</td>
<td>0.01mL</td>
<td>0.1mL</td>
<td>0.0mL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>10mL</td>
<td>10mL</td>
<td>10mL</td>
<td>10mL</td>
</tr>
</tbody>
</table>
0.1 mL of the E. coli culture was added to 2 tubes for each concentration of Pepto Bismol. This created 8 tubes with a final volume of 10 mL in each tube, and a cell density of $10^3$ cells/mL.

After vortexing each of the 8 tubes to evenly suspend cells, 0.1 mL aliquots from each tube was spread evenly on 24 LB agar plates, three plates for each of the 8 tubes.

The plates were stacked in a protective sleeve and incubated for 2 days.

The resulting colonies on each plate were counted. Each colony is assumed to have risen from one cell.

The pH of the variable solutions were tested.
Pepto Bismol Effects on E. coli Survivorship

P value: 2.00E-06

Average Number of Surviving Colonies on Plates

<table>
<thead>
<tr>
<th>Concentration of Pepto Bismol</th>
<th>Number of Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>472.75</td>
</tr>
<tr>
<td>0.1%</td>
<td>354.2</td>
</tr>
<tr>
<td>1%</td>
<td>336.5</td>
</tr>
<tr>
<td>10%</td>
<td>308.2</td>
</tr>
</tbody>
</table>
## ANOVA: One Way

<table>
<thead>
<tr>
<th>Summary</th>
<th>Count</th>
<th>Sum</th>
<th>Average</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>4</td>
<td>1891</td>
<td>472.75</td>
<td>1400.92</td>
</tr>
<tr>
<td>0.10%</td>
<td>5</td>
<td>1771</td>
<td>354.2</td>
<td>1039.2</td>
</tr>
<tr>
<td>1%</td>
<td>4</td>
<td>1346</td>
<td>336.5</td>
<td>419.667</td>
</tr>
<tr>
<td>10%</td>
<td>5</td>
<td>1541</td>
<td>308.2</td>
<td>180.7</td>
</tr>
</tbody>
</table>

### ANOVA

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>Ftest</th>
<th>P-value</th>
<th>F-Critical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>66379.1</td>
<td>3</td>
<td>22126.4</td>
<td>29.9544</td>
<td>2.00E-06</td>
<td>3.34389</td>
</tr>
<tr>
<td>Within</td>
<td>10341.4</td>
<td>14</td>
<td>738.668</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>76720.5</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Dunnett’s Test

T Critical Value: 3.29
Alpha: .05

<table>
<thead>
<tr>
<th>Pepto Bismol Concentration</th>
<th>T-Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>6.542868241</td>
<td>Significant</td>
</tr>
<tr>
<td>1%</td>
<td>7.519745237</td>
<td>Significant</td>
</tr>
<tr>
<td>10%</td>
<td>9.081644614</td>
<td>Significant</td>
</tr>
</tbody>
</table>
Conclusions

• The null hypothesis which stated that Pepto Bismol will not significantly alter the survivorship of Escherichia coli was rejected for all of the concentrations for Pepto-Bismol.

• Evidence suggest that Pepto-Bismol significantly reduces the survivorship of Escherichia coli.
Limitations

• Synchronization when spreading plates was not exact

Extensions/Future Studies

• More than one species
• Higher and lower concentrations of variable
• Perform a liquid agar infusion
• Have more replicates
• Perform more than one experiment
• Test the growth rate of the flora
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