Gastrointestinal Lab Report

Introduction:

The gastrointestinal system is an important organ system that helps to maintain hemostasis because it allows the body to digest, absorb, and excrete wastes. The gastrointestinal system breaks down food into its constituents, converts food into useable components, and provides energy to the body. Humans ingest food through the mouth, and teeth mechanically break down foodstuffs by chewing. (Sherwood, 2010, 590). Then, saliva in the mouth starts a simple carbohydrate digestion and food travels to the esophagus, stomach, small intestine, and large intestine. In the gastrointestinal system, there are four major processes, which are motility, secretion, digestion and absorption. (Sherwood, 2010, 582).

Once the food travels from the esophagus to the stomach, the stomach secretes hydrochloric acid and other enzymes, and begins protein digestion (Sherwood, 2010, 595). Then, the smooth muscle contracts and food starts moving and mixing; this is called motility. There are two mechanisms called segmentation and peristalsis that allow food mixing and moving in the smooth muscle. Segmentation is a ringlike contraction which allows the slow progress of moving chyme in both directions; therefore, it can increase time and nutrient absorption in the small intestine (Sherwood, 2010, 616). Unlike segmentation, peristalsis involves a one-way motion that allows food to move forward because of the contraction and shortening of the circular muscle (Al-Shboul, 2013, 7). In other words, it allows food to move from a higher to a lower pressure and moves food in the caudal direction. Thus, it can also refer to longitude contraction.

Furthermore, the extrinsic autonomic nervous system, the intrinsic enteric nervous system, and other hormones regulate motility in the system (Sherwood, 2010, 593). In this lab, it allowed for observation of the effects of the autonomic nervous system on the enteric nervous
system, and how these can affect the intestinal motility. First, the sympathetic system and the parasympathetic system act on smooth muscle cells and exert changes in the intestinal contraction. Then, the pacemaker cells of the smooth muscle—interstitial cells of Cajal (ICC), generate an electrical slow wave rhythm which has the potential to reach the threshold. After that, smooth muscles cells can contract due to action potential propagation in the muscle cells and form cross-bridges (Al-Shboul, 2013,7).

This lab experiment was to observe the motility of a rabbit’s intestinal segment under controlled conditions and compare the effects by adding epinephrine, methacholine, adenosine-5’-diphosphate and Ca$^{2+}$ free ringer-tyrode solutions. It was hypothesized that there would be a decrease in tension, but no changes in frequency in the presence of epinephrine would occur (Salak, 2001, 367). For methacholine, it was expected to see an increase in tension and no change in frequency. For adenosine-5’-diphosphate (ADP), it was expected to see an increase in tension, but no changes in frequency. On the last Ca$^{2+}$ free ringer-tyrode trial, it was expected to see a decrease in tension and no change in frequency.

**Materials and Methods**

In this experiment, a two centimeter-long intestinal segment of a rabbit was used. The piece of intestine was lowered in the cuvette that was in the thermocycler, with 38 degree Celsius normal ringer-tyrode solution. After that, intestinal activity was observed after the application of epinephrine, methacholine, adenosine-5’-diphosphate, and Ca$^{2+}$ free ringer-tyrode solution. The BioPac system was used to record and analyze the activity of the rabbit’s intestine, which hook to the transducer. During the experiment, two loops were tied on the intestine instead of three loops, which was suggested in the lab manual. Another variation from the lab manual was
recording the activity for six to seven minutes instead of ten minutes after the different solutions were added to the intestine in the cuvette. Also, due to an error, there was only one drop of methacholine added to the intestine, but not the following 3 additional drops after the 1 drop methacholine recording data. More details of procedures can be found in experiment 8 of the NPB 101L Systemic Physiology Lab Manual (Bautista, 2009, 75-82).

**Results:**

Figure 1 showed a constant rate in tension when the intestine wave lowered into the normal ringer solution.

![Figure 1](image)

Figure 1. Scatter graph shows the contractile changes when a 2 cm segment of rabbit’s intestine was lowered into a Ca2+ Ringer-Tyrode cuvette that was placed in a 38 degree Celsius thermocycler. The transducer was hooked to the 2 cm intestine and data were recorded by the Biopac software. The “P-P” tool was used to calculate the tension from five consecutive wave cycles and these numbers were averaged by a scientific calculator at that point in time. On the graph, it shows a constant rate during the trial.

Figure 2 showed a constant rate in frequency when the intestine wave lowered into the normal ringer solution.

![Figure 2](image)

Figure 2 shows the frequency of changes when a 2 cm segment of rabbit’s intestine was lowered into a Ca2+ Ringer-Tyrode cuvette which was placed in a 38 degree Celsius thermocycler. The intestine was hooked to the transducer and connected to the BioPac. Then, the intestine activity was shown on the computer. The “freq” tool was used to calculate the frequency from five
consecutive wave cycles, and these numbers were average by a scientific calculator at that point in time. On the graph, it shows a constant frequency during the trial.

Figure 3 showed the 2 cm intestine activity of a baseline tension and the tension when epinephrine applied to the intestine. The epinephrine was added at 82 seconds. The graph shows there was a decrease from 2.43 to 0.34g from 50 to 100 seconds. After that, the tension was increasing overall in the trial from 0.34 to 1.26g at 150-400 seconds.

Figure 3. Scatter graph shows the contractile changes of a 2 cm segment of rabbit’s intestine baseline tension and the intestine tension when the 3 drops of epinephrine added to the intestine at 82 seconds. The intestine was lowered into a filled solution Ca2+ Ringer-Tyrode cuvette which was placed in a 38 degree Celsius thermocycler. Data were record by the Biopac via the transducer hooked to the intestine. The “P-P” tool was used to calculate the tension from five consecutive wave cycles and these numbers were average by a scientific calculator at that point in time. On the graph, there was a decrease from 2.43 to 0.34g from 50 to 100 seconds. The tension of 0.34g was lower than the baseline tension readings. After that, the tension was increasing overtime during the trial starting at 150 seconds.

Figure 4 showed the 2 cm rabbit’s intestine activity of a normal frequency and the epinephrine frequency. Both of the frequency showed a constant rate in this trial.
Figure 4. A 2 cm segment of rabbit’s intestine frequency and the intestine’s frequency which added with 3 drops of epinephrine is shown above. The epinephrine added at 82 seconds in this trial. The “freq” tool was used in Biopac to covert the raw data to the data points, and the transducer was hooked to the intestine which allowed Biopac software record the activity of the intestine. The 2 cm segment of rabbit’s intestine was placed in a filled solution Ca2+ Ringer-Tyrode cuvette, and the cuvette was placed in a 38 degree Celsius thermocycler. In this experiment, the epinephrine frequency stayed in a constant rate and the reading points were similar as the frequency (normal ringer).

Figure 5. In the graph below, tension increased from 1.52 to 3.31g at 0-300 seconds. Then tension started getting stable readings at 300-400 seconds. 1-drop of methacholine was added to the intestine at 400 seconds. There was a decrease tension from 3.34 to 0.34 at 400-500 seconds. Then there was an increase afterwards, from 0.34 to 0.71g at 500-600 seconds in this trial.

Figure 6. The graph shows the frequency of a 2 cm rabbit’s intestine activity was at a constant rate when 1 drop of methacholine was added to the intestine.
Figure 6. A 2 cm segment of rabbit’s intestine was used and the transducer was hooked to the intestine. Then, data were recorded by the Biopac. The intestine was lowered into filled solution Ca2+ Ringer-Tyrode cuvette in a 38 degree Celsius thermocycler. The “freq” tool was used in Biopac to calculate the data points from five consecutive waves. Then, these numbers were averaged by a scientific calculator at that point in time. The 1-drop of methacholine was added to the intestine at 400 seconds. Overall, there was no any changed frequency in the intestine.

Figure 7. There was a decrease tension from 3.32 to 0.24g at 500 to 600 seconds when the ADP was applied to the intestine at 500 seconds. After that, an increase tension is shown below from 0.24 to 2.85g at 600 to 1000 seconds.

Figure 8. The graph shows the frequency of a 2 cm rabbit’s intestine activity was at a constant rate when 10 drops of ADP was added to the intestine at 500 seconds in this trial.
Figure 8. The frequency of a 2 cm segment of rabbit’s intestine activity was recorded by the BioPac via the transducer which was hooked to the intestine. The rabbit’s intestine was lowered into a filled solution Ca2+ Ringer-Tyrode cuvette in a 38 degree Celsius thermocycler. The “freq” tool was used to analyze the raw data to data points, and was calculated from five consecutive waves and averaged by a scientific calculator at that point in time. At 500 seconds, ADP was added to the intestine. The rabbit’s intestine did not show any changed after the administration of ADP. The frequency readings were constant overall.

Figure 9. At 350 seconds, the rabbit’s intestine was moved from a normal ringer solution to a calcium free ringer solution. There was a decrease tension from 3.48 to 0.61 g at 300 to 400 seconds. Thus, a decreasing tension was shown between 400 to 700 seconds. At 700 seconds, the intestine was moved to a normal calcium ringer cuvette. Moreover, there was also an increase of tension at 700 to 1000 seconds from 0.35 to 2.01 g.
calculator at that point in time. In addition, the intestine was lowered into a Ca2+ Ringer-Tyrode cuvette in a 38 degree Celsius thermocycler during the experiment.

Figure 10. The graph shows the intestine frequency was constant overall from 0.28 to 0.32 g at 0-1000 seconds during this experiment.

<table>
<thead>
<tr>
<th>Changes in Intestine Before and After applied Ca2+free Ringer solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency(Hz)</td>
</tr>
<tr>
<td>Time(seconds)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency(Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28</td>
</tr>
<tr>
<td>0.27</td>
</tr>
<tr>
<td>0.24</td>
</tr>
<tr>
<td>0.28</td>
</tr>
<tr>
<td>0.29</td>
</tr>
<tr>
<td>0.31</td>
</tr>
<tr>
<td>0.31</td>
</tr>
<tr>
<td>0.28</td>
</tr>
<tr>
<td>0.31</td>
</tr>
<tr>
<td>0.29</td>
</tr>
</tbody>
</table>

Figure 10 shows the frequency of changes when a 2 cm segment of rabbit’s intestine was lowered into a Ca2+ Ringer-Tyrode cuvette in a 38 degree Celsius thermocycler. Then, the transducer was hooked to the rabbit’s intestine, and connected to the BioPac. After that, the “freq” tool was used to covert raw data to data points. These numbers were found from five consecutive wave cycles, and were averaged by a scientific calculator at that point in time. On the graph, it shows a constant frequency during the trial when the intestine move to a calcium free ringer cuvette from a normal ringer cuvette.

**Discussion:**

The gastrointestinal tract is controlled by the autonomic nervous system (ANS) and their unique system, enteric nervous system (ENS). The ENS contains the submucosal plexus and the myenteric plexus, which are located in the submucosa and in the muscularis externa between the
longitudinal and the circular muscles. Both of the systems play an important role to regulate the GI activity (Sherwood, 2010, 588.)

Besides that, the GI is composed of four different layers. First, serosa is the most outer layer of the digestive tract that connects to the abdominal cavity, and secretes a lubricate fluid which helps prevent abrasion between the digestive system and the body. (Sherwood, 2010, 587). Second, the main layer of the intestine’s smooth muscles—muscularis externa which is composed of an inner circular layer and outer longitude layer. First, the inner circular muscles contract which causes a decrease in diameter of the tract while the outer longitude muscles contract which causes a shortening of the digestive tract, creating segmentation and peristalsis. The third layer of connective tissue, submucosa, provides a distensibilty and elasticity. Moreover, it lies on large blood vessels, lymph vessels and branches out into the inner and outer layer of the digestive tract. Next, the fourth and most inner layer is the mucosa. This layer is composed of three different layers: the mucous membrane, the lamina propria, and the muscularis mucosa. This layer is composed of many layers that help with greater surface area of absorption in the lumen. (Sherwood, 2010, 586).

In this lab, the intestinal activity was measured while applying the normal Ca2+ ringer-tyrode’s solution, epinephrine, methacholine, adenosine-5’-diphosphate (ADP), and Ca2+-free ringer-tyrode’s solution to the intestine. In general, action potentials propagate down during normal intestine activity, which results in contractions along the digestive tract. This causes the luminal contents to travel down the tract. Within this process, segmentation played a role which helped in breaking down and mixing the contents (Sherwood, 2010, 616). In addition, slow waves of the basic electrical rhythm also needed to propagate down to the GI tract, then reach the threshold to signal a series of contractions. Once the threshold was reached, the voltage calcium
gated channel opened; then the influx of calcium flowed inside the cell. This is called the depolarization state. After that, hyperpolarization occurred when the voltage calcium gated channel was closed and the efflux of potassium started to flow out of the muscle cell (Sherwood, 2010, 96) In the normal Ca2+ ringer-tyrode trial, the concentration of calcium was critical for muscle contraction. As seen in Figure 1 and 2, the intestinal activity showed a very constant contraction of both tension and frequency in the presence of calcium in the solution.

After the epinephrine was applied to the intestine, tension decreased, while frequency stayed constant, which is shown in Figure 3 and 4. This result was hypothesized and expected. When administering epinephrine, the intestine relaxed leading to a decrease in tension. Moreover, the decrease in tension was correlated with the stimulation of the sympathetic nervous system (SNS). This is because SNS inhibited digestion when the GI system released epinephrine. When epinephrine bound to the beta-2 receptors in the smooth muscle cells, the associated G-protein cascade was activated. Then, the adenylyl cyclase activity increased and caused an increase of cyclic AMP, which activated protein kinase A, deactivating the myosin light chain kinase (MLCK) and inhibiting the calcium channels (Guan, 1995, 492). So, the smooth muscle cells’ tension was decreased due to the concentration of the calcium.

On the contrary, with the addition of methacholine (Mch), there was a decrease in tension, which is shown in Figure 5. This result was not expected and could be explained by the rabbit’s intestine not being thoroughly rinsed before adding methacholine into the cuvette. On the other hand, a constant rate of frequency was expected (Figure 6). Unlike epinephrine, which was released by the stimulation of the SNS, methacholine was released by stimulation of the parasympathetic nervous system (PNS). Once methacholine was released, it bound to the muscarinic receptor and activated the phospholipase C via the G-protein cascade. After that, the
inositol tri-phosphate (IP3) and diacylglycerol (DAG) were formed and released, which in turn led to an increase intracellular calcium by the IP3 receptors (Ca2+Ch) opening (Sherwood, 2010, 126). Eventually, there were more calcium-calmodulin bindings to the myosin light chain kinase (MLCK), which promoted more cross bridging cycling and increased tension. However, in this trial, the activity of the intestine showed a decreased tension when methacholine was applied to the intestine.

In general, Frederick Bernheim’s study results were consistent with the experimental data that was observed and recorded in this lab. The results showed that the effect of methacholine caused a contraction of the intestine whereas epinephrine caused a relaxation of the intestine. (Bernheim, 1934, 59). In other words, epinephrine and methacholine also demonstrated the activity of the SNS or PNS which caused an increase or a decrease in the gut activity due to the activation or inhibition of the calcium channel, which then affected the cross-bridging promotion. Furthermore, it also explained that the concentration of calcium played a critical role in causing a series of pathways, and resulting the formation of cross bridging in the smooth muscle cells. Then, these muscle cells were determined the frequency of the slow waves, and thus the rate of the segmentation and peristalsis, allowing the greater absorption of nutrients in the digestive tract (Sherwood, 2010, 616).

In the third trial, ADP was added to the intestine and being used to mimic the effects of ATP on the intestine motility; then, caused an inhibitory result, which is shown in Figure 7. It was hypothesized and expected a decrease in tension, but a constant rate in frequency (Figure 8). During the process, the neurotransmitters were released to activate the purinergic pathway in order to inhibit the gut motility, which caused a decrease in amplitude and tension—a relaxation in the gut motility (Westfall, Todorov, 2002, 441). First, ADP bound to the 2-gamma purinergic
receptor, and activated the phospholipase C beta to convert PIP2 into DAG and IP3. Eventually, IP3 and DAG could cause an influx of calcium ions. Then, later of the series mechanism was same as epinephrine that mentioned before. At a result, the smooth muscles cells began to depolarize, and caused a decrease in contraction amplitude that was observed.

On the last trial, Ca2+ free ringer-tyrode solution was administered to the intestinal segment. Again, as mentioned before, calcium played a critical role which led to a series of event and which to determine the frequency and the rate of segmentation and peristalsis (Ponti, 1989, 133). Therefore, in Figure 9 shown there was a decrease in tension when the intestine was exposed to the Ca2+ free ringer-tyrode solution. The rabbit’s intestine frequency remained constant, which is shown in Figure 10. This result was expected. When the intestine segment exposed to the calcium free ringer-tyrode solution, the only calcium available to the cell was the remaining intracellular calcium. Without the extracellular supply of calcium from the normal Ca2+ ringer-tyrode solution, this is impossible allow muscle to maintain a constant rate of contraction due to the lack of calcium, and its inability to form the cross bridges for muscle contractions (Ward, 2000, 355).

To conclude, this lab experiment showed the intestine motility could be affected by various environments. Stimulation of the sympathetic activity will cause the motility decreases, while stimulation of the parasympathetic activity will cause the motility increases. During the experiment, the administration of epinephrine and ADP both showed the decrease tension of smooth muscle to the intestine. On the other hand, methacholine showed the opposite effect—increased tension of smooth muscle to the intestine. Lastly, all of these effects is to maintain the homeostasis circulate in the body.
References:


Bernheim Frederick. Interaction of acetylcholine and epinephrine on the isolated small intestines of various animals. Journal of Pharmacology and Experimental Therapeutics 1934; 51:59-67.


Appendices:

Figure 13. The normal gut activity of a 2 cm segment of Rabbit’s intestine was shown. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine. The intestine was placed in a cuvette in a 38 degree Celsius thermocycler.

Figure 11. Raw data of a 2 cm segment of Rabbit’s intestine was shown. At 350 seconds, the intestine was moved from the Ca2+ ringer tyrode cuvette to the Ca2+ free ringer cuvette which was placed in a 38 degree Celsius thermocycler. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine.

Figure 12. Raw data of a 2 cm segment of Rabbit’s intestine was shown. At 700 seconds, the intestine was moved from the Ca2+ free ringer-tyrode cuvette to the Ca2+ ringer tyrode cuvette which was placed in a 38 degree Celsius thermocycler. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine.
Figure 13. Raw data of a 2 cm segment of Rabbit’s intestine was shown. At 80 seconds, the intestine in the Ca2+ ringer tyrode cuvette was added with 3 drops of epinephrine to the intestine, which was placed in a 38 degree Celsius thermocycler. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine.

Figure 14. Raw data of a 2 cm segment of Rabbit’s intestine was shown. At 400 seconds, the intestine in the Ca2+ ringer tyrode cuvette was added with 1 drops of MCh to the intestine, which was placed in a 38 degree Celsius thermocycler. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine.

Figure 15. Raw data of a 2 cm segment of Rabbit’s intestine was shown. At 500 seconds, the intestine in the Ca2+ ringer tyrode cuvette was added with 10 drops of ADP to the intestine, which was placed in a 38 degree Celsius thermocycler. The BioPac was used and record the raw data via the transducer, which was hooked to the intestine.
**Calculations:**

Average Value: \( \frac{x_1 + x_2 + \ldots}{n} \), where \( x \) are values and \( n \) is the number of values.

\[
(0.265 + 0.247 + 0.270 + 0.228 + 0.292) / 5 \\
= 1.30 / 5 \\
= \textbf{0.26 Hz}
\]