Testing the capacity of biological buffer systems to resist changes in pH

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Personal Engagement and Exploration

Research Question: What is the capacity of plant and animal tissue to buffer against changes in pH?

The purpose of this investigation is to compare the pH buffering capacities of various tissues and substances. I am interested in this phenomenon because our bodies are compartmentalized with different compartments having different pH levels. For example, from a recent article in the journal Nature Materials (Khutoryanskiy 2015), I have learned that our mouths have a slightly basic pH of just above 7, while our stomachs have a very acidic pH of nearly 1. When our food leaves our stomach, the pH of the food/water solution rises quickly to around 6, and then reaches around 7.4 by the end of the small intestine. At the same time, our blood pH remains relatively stable at around 7.35-7.40.

I wonder then if organisms, and therefore their tissues and cells, are constantly working to maintain homeostasis of their internal environments. I have learned that blood itself is a tissue and that excess CO_2 in blood, as a product of metabolism, reacts with water in the blood to form carbonic acid. The carbonic acid releases hydrogen ions (H^+) into the blood, potentially lowering the pH of the blood and causing a dangerous condition called acidosis. Furthermore, other tissues, like the ones surrounding the digestive compartments mentioned above, must regulate pH to provide optimum working environments for enzymes, pH sensitive molecules, and products of metabolism may compromise pH homeostasis. Yet, organisms rarely die of acidosis and therefore must have adequate systems in their tissues for buffering against changes in pH (i.e. buffering capacity).

The animal liver is an organ that performs several metabolic processes, including building and breaking down glycogen and removing toxins from the blood. A potato is also an organ of the potato plant and is in charge of converting sugars to starches and storing those starches for later use. Thus, in this investigation I will test the hypothesis that animal liver, being an organ in charge of a lot of metabolic processes, has the ability to resist changes in pH. I will also test the hypothesis that a plant tissue, like potato, that doesn't perform much metabolism does not have much of an ability to resist pH changes.

Hypothesis: Plant and animal tissues can resist changes in pH, but animal tissues are better at it.

Prediction: Slowly adding drops of 0.1 M HCl and 0.1 M NaOH to potato puree and chicken liver puree will not produce much a change in pH at first (compared to distilled water), but the potato tissue buffering system will begin to fail with fewer drops of acid or base than the chicken liver buffering system.

Independent Variable: Volume (mL) of 0.1 M HCl or 0.1 M NaOH added

Dependent Variable: pH of water or tissue solution (puree)

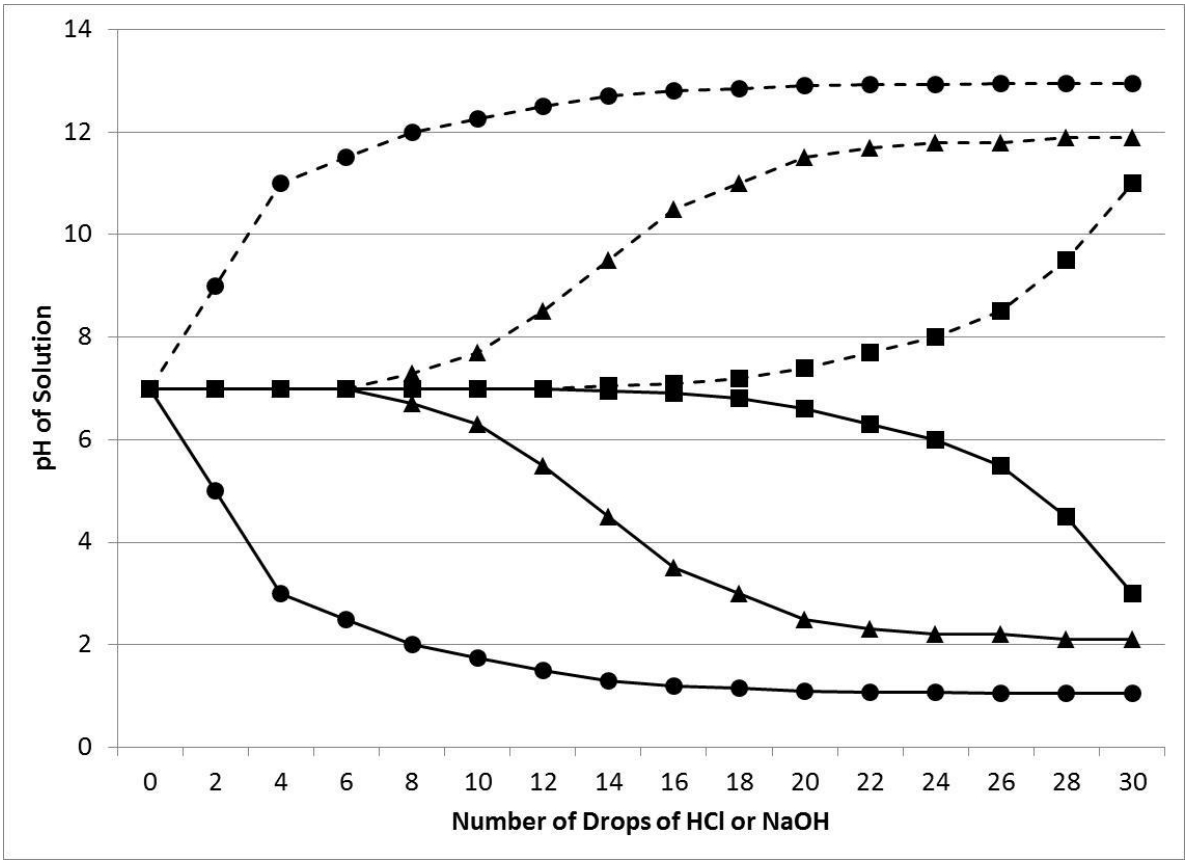

Figure 1. Graph of Predicted Results. Circles are distilled water, triangles are potato, and squares are liver. Dashed lines are with added NaOH and solid lines are with added HCl.

Controlled Variables: Temperature, concentration of tissue solution, volume of solution tested, water for making each solution.

Discussion for how variables listed above will be controlled: Temperature of test solutions will be recorded with a lab thermometer before acid or base are added and at the end of each experimental protocol. The mass of plant or animal material to volume of water ratio will remain constant (100 g tissue/1,000 mL water). All tests will be done on 25 mL of tissue solution. All tissues will be made with distilled water and distilled water will be used as the control against which the living tissues will be compared.

Materials:

- Safety goggles
- 2, 50-100 mL beakers
- 0.1 M HCl bottle, with pipette
- 0.1 M NaOH bottle, with pipette
- pH paper (pH 1-12 Hydrion)
- Forceps
- 25 mL graduated cylinder
- Water (pH 7)
- Liver and potato tissue
- Other plant tissues; Viele Lake water
- Mortar and pestle

Procedure A, Control:

1. Pour 25 mL of distilled water into each of two small beakers (50-100 mL).
2. Record the initial pH of both water samples with pH paper (1-12 Hydrion Paper, Micro Essential Laboratory, Inc.). The pH paper is expensive and thus will be used in small, square pieces (0.4 mm^2) (tear a small, square piece and use forceps to hold it in the liquid).
3. *For each independent variable, place paper squares on a white paper towel once they have been dipped.* To one sample, add 0.1 M (molar; moles HCL per Liter of water) HCl a drop at a time and record the pH after each drop until 10 drops have been added. Thereafter, record the pH after every 5 drops until 20 more drops have been added (total of 30 drops).
4. Repeat steps 1-3 with the other water sample, using 0.1 M NaOH instead.
5. WASH and dry the beakers.

Procedure B: Testing the buffering capacity of living tissue. This procedure will be used for testing potato (*Solanum tuberosum*) tissue puree and chicken (*Gallus domesticus*) liver puree.

1. To prepare each puree, blend 50 g of tissue in 1,000 mL distilled water. Strain any leftover pieces through cheese cloth.
2. Pour 25 mL of dilute bird (chicken; *Gallus domesticus*) liver puree into each of two 50-100 mL beakers. Record the initial pH. Add 0.1 M HCl one drop at a time and record the pH for each drop. From 11 to 30 drops, record the pH after each set of 5 drops. Do the same with the other sample and 0.1 M NaOH.
3. Repeat steps 1 & 2 for a total of 10 replicates.
4. Wash and dry the beakers and repeat the same procedures with fresh potato puree.

Statistical Analysis:

The mean, standard deviation, and 95% confidence intervals (95% CI) was calculated for each acid and base running volume total. The 95% CI for each replicate mean was used to determine at which drop volume the effect of acid or base addition may have become significant (i.e. the tissue loses its ability to buffer).

Data Analysis

Tables and Figures

Table 1. Change in pH for distilled water (control) with added 0.1 M NaOH or HCl. Numbers above columns are number of drops of NaOH or HCl. pH was recorded with 0.4 mm^2 pieces of 1-12 Hydrion pH paper.

	pH at Each Drop Benchmark (±0.25)														
Treat.	0	1	2	3	4	5	6	7	8	9	10	15	20	25	30
NaOH	7.0	8.0	8.0	8.0	8.5	8.5	8.5	8.5	9.0	9.0	10.0	10.5	11.0	11.5	11.5
HCl	7.0	5.5	5.5	5.5	5.5	5.0	5.0	5.0	4.5	4.5	3.0	2.5	2.5	2.0	2.0

Table 2. Change in pH for liver puree with added 0.1 M NaOH. Numbers above columns are number of drops of NaOH or HCl. pH was recorded with 0.4 mm^2 pieces of 1-12 Hydrion pH paper. Descriptive statistics are mean, standard deviation (s) and 95% confidence interval (95% CI).

	pH of liver at Each NaOH Drop Benchmark (±0.25)														
Replicate	0	1	2	3	4	5	6	7	8	9	10	15	20	25	30
1	7.0	7.0	7.0	7.0	7.0	7.5	7.5	7.5	7.5	8.0	8.0	8.5	9.0	10.0	11.0
2	7.0	7.0	7.0	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	10.0	10.0	11.0
3	7.0	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.0	8.5	9.0	10.0	11.0	11.0	11.5
4	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
5	7.0	7.0	7.0	7.0	7.0	7.5	7.5	7.5	7.5	8.0	8.0	8.5	9.0	10.0	11.0
6	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.5	7.5	7.5	7.5	8.0	8.0	8.5	10.0
7	7.0	7.0	7.0	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	10.0	10.0	11.0
8	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
9	7.0	7.0	7.0	7.0	7.0	7.5	7.5	7.5	7.5	8.0	8.0	8.5	9.0	10.0	11.0
10	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.5	7.5	7.5	7.5	8.0	8.0	8.5	10.0
Mean	7.1	7.2	7.2	7.3	7.3	7.5	7.6	7.8	7.9	8.2	8.5	8.9	9.6	10.1	11.0
s	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.9	0.8	1.2	1.0	0.6
95% CI	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.6	0.5	0.7	0.7	0.3

Table 3. Change in pH for liver puree with added 0.1 M HCl. Numbers above columns are number of drops of NaOH or HCl. pH was recorded with 0.4 mm^2 pieces of 1-12 Hydrion pH paper. Descriptive statistics are mean, standard deviation (s) and 95% confidence interval (95% CI).

	pH of liver at Each HCl Drop Benchmark (±0.25)														
Replicate	0	1	2	3	4	5	6	7	8	9	10	15	20	25	30
1	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	5.0	4.5	4.0
2	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	4.0	4.0
3	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
4	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.5	6.0	5.0	4.5	4.0
5	7.5	7.5	7.5	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.0	6.0	5.0	4.5
6	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	5.0	4.5	4.0
7	7.5	7.5	7.5	7.5	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.0	5.5	5.0	4.5
8	7.0	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	4.0
9	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	5.0	4.5	4.0
10	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.5	6.0	5.0	4.5	4.0
Mean	7.1	7.1	7.1	7.0	6.9	6.9	6.6	6.6	6.5	6.4	6.2	5.9	5.3	4.6	4.1
s	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3
95% CI	0	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2

Table 4. Change in pH for potato puree with added 0.1 M NaOH. Numbers above columns are number of drops of NaOH or HCl. pH was recorded with 0.4 mm^2 pieces of 1-12 Hydrion pH paper. Descriptive statistics are mean, standard deviation (s) and 95% confidence interval (95% CI).

	pH of potato at Each NaOH Drop Benchmark (±0.25)														
Replicate	0	1	2	3	4	5	6	7	8	9	10	15	20	25	30
1	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
2	7.5	7.5	8.0	8.0	8.0	8.5	8.5	9.0	9.0	10.0	10.5	10.5	11.0	11.5	11.5
3	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
4	7.0	7.0	7.5	7.5	7.5	7.5	7.5	8.5	8.5	8.5	8.5	9.5	11.0	11.0	11.5
5	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
6	8.0	8.0	8.0	8.5	8.5	8.5	9.0	9.0	10.0	10.0	10.0	10.5	11.0	11.5	11.5
7	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
8	7.0	7.0	7.5	7.5	7.5	7.5	7.5	8.5	8.5	8.5	8.5	9.5	11.0	11.0	11.5
9	7.5	7.5	7.5	7.5	7.5	8.0	8.0	8.5	8.5	9.0	10.0	10.0	11.0	11.5	11.5
10	7.5	7.5	8.0	8.0	8.0	8.5	8.5	9.0	9.0	10.0	10.5	10.5	11.0	11.5	11.5
Mean	7.0	7.5	7.7	7.7	7.7	8.1	8.1	8.7	8.8	9.2	9.8	10.1	11.0	11.4	11.5
s	0	0.3	0.2	0.3	0.3	0.4	0.5	0.2	0.5	0.6	0.7	0.4	0.0	0.2	0.0
95% CI	0	0.2	0.1	0.2	0.2	0.2	0.3	0.1	0.3	0.4	0.4	0.2	0.0	0.1	0.0

Table 5. Change in pH for potato puree with added 0.1 M HCl. Numbers above columns are number of drops of NaOH or HCl. pH was recorded with 0.4 mm^2 pieces of 1-12 Hydrion pH paper. Descriptive statistics are mean, standard deviation (s) and 95% confidence interval (95% CI).

	pH of Potato at Each HCl Drop Benchmark (±0.25)														
Replicate	0	1	2	3	4	5	6	7	8	9	10	15	20	25	30
1	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
2	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.5	5.0	4.5	4.0	3.5	3.0
3	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
4	7.5	7.5	7.5	7.0	7.0	7.0	6.5	6.5	6.5	6.0	6.0	5.0	4.5	4.0	3.0
5	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.0	6.0	5.0	4.0	3.0	2.5
6	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
7	7.5	7.0	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.5	6.5	5.0	4.5	4.0	3.0
8	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
9	7.0	7.0	7.0	7.0	7.0	7.0	6.5	6.5	6.0	6.0	6.0	5.0	4.5	4.5	3.5
10	7.0	7.0	7.0	6.5	6.5	6.5	6.5	6.0	6.0	6.0	6.0	5.0	5.0	4.5	3.5
Mean	7.1	7.0	7.0	6.7	6.7	6.6	6.5	6.2	6.2	6.0	6.0	5.0	4.7	4.2	3.3
s	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.4	0.2	0.4	0.5	0.4
95% CI	0	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.3	0.2

Sample calculation for 95% CI for each replicate mean within each drop volume benchmark (2.26 is the critical *t*-value for 9 degrees of freedom at an alpha of 0.05):

$$95\%\ CI = \frac{(2.26)(s)}{\sqrt{n}}$$

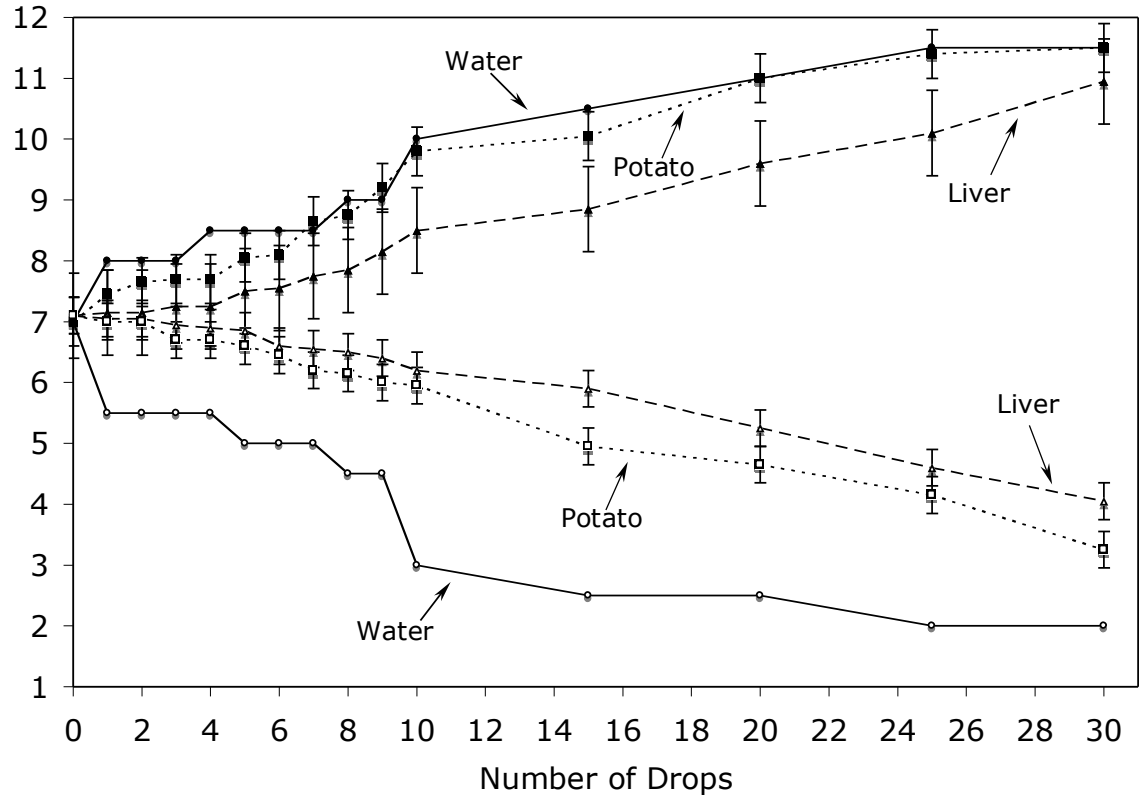

Figure 2. Change in pH (Y-axis) for distilled water (circles and solid line), liver (triangles and dashed line), and potato (squares and dotted line) as a function of number of drops 0.1 M NaOH (closed circles) and 0.1 M HCl (open circles). Error bars are the largest 95% confidence interval calculated within each tissue-drop number combination (liver: 0.7 and 0.3 for NaOH and HCl respectively; potato: 0.4 and 0.3 for NaOH and HCl respectively).

Results

Distilled water showed no capacity for buffering ability. Distilled water immediately changed in pH with the first drop of added NaOH and HCl and continued to increase to a maximum of 11.5 with added NaOH and a minimum of 2.0 with added HCl (Table 1, Fig. 2). The pH of liver puree and potato puree also increased with added NaOH and decreased with added HCl (Tables 2-5, Fig. 2). Both liver and potato puree buffered better than water against added HCl according to 95% CIs (Fig. 2). Liver, but not potato, puree appeared to buffer better than water against added NaOH according to 95% CIs (Fig. 2).

Evaluation

This study sought the capacity of plant and animal tissue to buffer against changes in pH? I tested the hypothesis that plant and animal tissues can resist changes in pH, but animal tissues are better at it. I predicted that slowly adding drops of 0.1 M HCl and 0.1 M NaOH to potato puree and chicken liver puree would not produce much a change in pH at first (compared to

distilled water), but the potato tissue buffering system would begin to fail sooner than the chicken liver buffering system. I found support for the hypothesis with the liver tissue puree. The liver puree buffered against a change in its initial pH of 7 for the first four drops of 0.1 M HCl and 0.1 M NaOH. Beginning with five drops of each solution, the liver puree began to lose its buffering capacity. However, compared to distilled water, the liver puree differed with the addition of either NaOH or HCl. The 95% CIs in Figure 2 show this well, as they never reach the water coordinates at any number of drops. I also found support for the hypothesis with potato puree, but only with the acid (HCl) treatment. Similar to the liver result, potato puree buffered against a change from its initial pH of 7 for the first four added drops of HCl. Beginning with the fifth drop of HCl, the potato buffering system appeared to begin to fail. However, compared to distilled water, the potato puree differed notably with the addition of HCl. The 95% CIs in Figure 2 show this well, as they never reach the water coordinates at any number of drops of HCl. For the NaOH treatment, the potato puree only seems to differ from water at one, three, and four drops, according to the 95% CIs. Thus, potato puree appears to not buffer against added NaOH any better than distilled water. For both the liver and potato means, the largest 95% CIs were used to increase confidence at any number of drops of NaOH or HCl.

In buffering capacity studies, the Slyke is used as the unit to indicate the buffering capacity of a tissue. A Slyke is the amount of acid or base in μmol required to change the pH of a tissue puree one entire pH unit (Sorokin 1964). In this study, drops of 0.1 M NaOH or HCl were added to a tissue (liver or potato) puree solution. Each drop from the pipettes used in this study had a volume of 0.05 mL (±0.005). Therefore, in each drop, there were roughly 0.000005 moles (0.05 mL x 1.0 L/1,000 mL x 0.1 mol/L) of hydrogen or hydroxide ion. In other words, each drop added 5.0 μmol of hydrogen or hydroxide ion to the tissue purees. That said the Slyke for adding NaOH or HCl to the liver puree in this study is 45 μmol (9 drops x 5.0 μmol per drop) and 75 μmol (15 drops x 5.0 μmol per drop), respectively. The Slyke for adding NaOH or HCl to the potato puree in this study is 25 μmol (5 drops x 5.0 μmol per drop) and 45 μmol (9 drops x 5.0 μmol per drop), respectively. Given these calculations, the Slyke values for liver tissue in this study are higher than the Slyke values for potato tissue. Therefore the buffering capacity in this study for liver is greater than that of potato. This result is not surprising, considering that animals, being heterotrophs are more biologically active than autotrophic plants and may produce more waste products that require a more efficient buffering system and higher buffering capacity.

The major source of error in this study comes from the method for making the puree solutions. A major assumption in the design of this study was that 50 g of liver tissue in 1.0 L of water would produce a comparable solution to 50 g of potato tissue in 1.0 L of water to test buffering capacity. It is possible that these masses and are arbitrary and the resulting purees are incomparable to each other. While perhaps not comparable to each other, the purees as prepared and their buffering capacities are comparable to distilled water and the results meaningful. A second source of error comes from the method used to measure pH. I used pH paper and a colorimetric guide to determine pH. The most precise I could measure the pH was in 0.5 increments (±0.25). This lack of precision makes it difficult to confidently determine significant differences in buffering capacity as the first few drops of NaOH or HCl are added. However, differences in pH become great enough with more drops in all treatments except for potato plus NaOH that precision loses its importance. A third source of error comes from the titration method for the NaOH and HCl. Pipettes with 1.0 mL capacity and large drop size were used. The drop volume of 0.05 mL can vary by as much as 0.025 mL. This variable can increase the

variance within the pH replicates as the number of drops increases in each treatment. A fourth source of error is simply the ability to generalize about the buffering capacities of animal tissues compared to plant tissues because only one animal tissue type and one plant tissue type was used in the investigation.

It is possible to mitigate the effects of these error sources if the study were to be repeated. First, I could calculate the dry masses of given masses of fresh liver and potato tissues and then control for actual mass of tissue. I could also research how specifically previous studies have determined the buffering capacities of living tissue. For example, a brief search to prepare for this investigation turned up a large literature for the capacity of muscle tissue to buffer against lactic acid production during intense exercise. Second, I could use a more precise pH meter that measures to the nearest 0.05. This device could solve the problem of guessing whether the color of the pH paper was indicating a pH of 8.5 or 9.0, for example. Third, I could use a graduated burette with smaller drops to increase the precision of the volume of NaOH and HCl added to the water, liver puree, and potato puree. I could use these more precise volumes to calculate Slyke values and compare the buffering capacities of liver tissue and potato tissue to each other. Fourth, I could expand the study to include more animal tissue sources like muscle and kidney, two organs upon which vertebrates depend to buffer against changes in pH. I could also test additional plant tissue sources. Leaves, for example, are active sites of both catabolic and anabolic reactions and are likely to need the capacity to buffer against metabolic products that influence extracellular pH.

Cited References

Khutoryanskiy, V. V. (2015). Supramolecular materials: Longer and safer gastric residence. Nature Materials 14:963–964

Sorokin, C. (1964). Van Slyke's buffer values for cell secretions. Protoplasma **60**:79-85.