

PROJECT FINAL REPORT

GROUP NUMBER: W3

PROJECT TITLE: The Rate of Calcium Extraction From Chicken Bone

DATE SUBMITTED May 12, 2000

<b>Abstract</b>	<b>Objectives</b>	<b>Background</b>	<b>Theory</b>	<b>Methods</b>	<b>Results</b>	<b>Analysis</b>	<b>Conclusion</b>	<b>GRADE</b>
10	10	10	10	10	20	20	10	100 /100

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SUMMARY OF CONCLUSIONS

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The rate of calcium extraction from three chicken femurs was determined by varying surface areas of a whole bone filled with wax, a longitudinally halved bone, and a ground bone. Each bone was suspended in a beaker that was filled with 900mL of 1M HCl solution. Samples were taken from the beakers for a period of 4.5 hours at 15 minute intervals and then twice daily for four days afterwards. The samples were then analyzed by atomic absorption spectrophotometry. Results were analyzed by fitting a second order polynomial to the calcium released versus time graph. The results showed that the ground bone released the most calcium, 69.92% the estimated maximum calcium available, as opposed to 61.24% for the halved bone and 52.14% for the whole bone. Experimental data also revealed that the original hypothesis of the experiment was false - surface area was not the only determining factor in the rate of calcium extraction from a chicken femur. The halved bone had 10% greater surface area than the whole bone, but a calcium extraction rate that was 290% greater. It was hypothesized that the penetration distance of each bone, rather than the surface area, was the determining factor in the extraction rate. The experimental data fell within  $\pm 12\%$  of the values predicted by the equation  $R \propto (D^0 \cdot SA) / \delta'$ , where R is the rate of extraction,  $D^0$  is the diffusion constant, SA is the surface area, and  $\delta'$  is the average diffusion distance. This showed that the thickness of the bone, or effective diffusion distance of the HCl solution, is a significant in the calcium extraction rate of chicken femur bone. Further experimentation would be necessary to determine the exact relationship between the thickness of the bone, surface area, and the rate of calcium extraction.

**(THIS COULD BE A LITTLE SHORTER, LEAVING OUT SOME OF THE DETAIL AND PRESENTING THE MAJOR FINDINGS MORE CONCISELY)**

## OBJECTIVES

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The objective of this experiment was to determine the rate at which calcium was extracted from chicken femur samples with varying surface areas. Two specific goals were set for the accomplishment of this objective. The first goal was to compare the relationship between surface area and extraction rate using whole, halved and ground chicken femur samples. The second was to extract the greatest total amount of calcium possible from all samples and compare. By holding constant the HCl concentration, volume of HCl used, and temperature of sample; and by normalizing each bone by its respective mass, the surface area was the only varying element in the experiment. The concentration of the calcium ion extracted for each sample was plotted with respect to time in order to determine the rates. Additionally, the total calcium yield per unit mass of bone was also determined by observing the total calcium extracted from each sample and was normalized by the respective samples' masses (8.5 for the ground, 4.1 for the halved, 1.4 mg Ca/g bone\*min). The experiment calls for the use of atomic absorption spectrophotometry to determine the rate of calcium extraction from a chicken femur.

## BACKGROUND FOR PROPOSED PROJECT

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Bone is primarily composed of three components, collagen, protein polysaccharides, and the mineral phase. The mineral phase, the part of bone that contains the calcium, makes up 65% of the bone by mass.<sup>1</sup> The ideal component of the mineral phase is hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})^2$ . Other components of the mineral phase include calcium carbonate and calcium apatite<sup>2</sup>.

In order to determine the calcium concentration of bone, calcium ions must be released into solution. This is done by the method of acid precipitation, in which proteins are broken up, and calcium ions are released into solution<sup>3</sup>. The acid of choice in this experiment was 1M hydrochloric acid. 1M HCl was used because of its ability to extract most of the calcium from a chicken femur in one day.

After calcium is released into solution, the concentration of calcium can be determined by the use of atomic absorption spectrophotometry. Atomic absorption spectrophotometry is based on the principle that when atoms become excited, electrons undergo a transition from a higher energy level to a lower energy state. With the change of an increased energy level the absorption of a particular ion can be measured. The more ions are present in the solution, the higher the absorbency values.

## THEORY AND METHODS OF CALCULATION

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By determining the total concentration of calcium in the bone, we can make an estimation of how much calcium we expect to extract from bone. Knowing that 65% of bone is in mineral phase and that the ideal component of the mineral phase is hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})^2$ , we can make the preliminary calculation of the final concentration of bone expected.

$$\begin{aligned} \text{Percentage of Ca in } \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})^2 &= \text{molecular weight of Ca} / \text{molecular weight of total} & (1) \\ &= 400.78 / 1004.62284 = 0.3989 = \mathbf{39.89\%} \end{aligned}$$

$$\begin{aligned} \text{Percentage of Ca in bone} &= \text{percentage of calcium in mineral phase} \times \text{percentage of mineral phase in bone} \\ &= 0.3989 \times 0.65 = 0.2593 = \mathbf{25.93\% \text{ calcium in bone}} \end{aligned}$$

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<sup>1</sup> Dr. Paul Ducheyne, UPenn bioengineering faculty

<sup>2</sup> "bone" Encyclopædia Britannica Online.

<http://www.eb.com:180/bol/topic?eu=118814&sctn=2>

<sup>3</sup> Friel, J., Hahn, R., Lee, P., Patel, A. "Determination of Calcium Content of Breakfast Foods"

<http://bioeng.seas.upenn.edu/ugrad/projects/1999/be210s99w6r01.html>

Knowing this information, we can determine a preliminary dilution scheme as follows:

$$1 \text{ g bone (0.259 g Ca / 1 g bone) (1 / 0.900 L) = 0.288 g / L} \quad (2)$$

Rounding gives a concentration of approximately 300 mg / L or 300 ppm. As the upper limit of [Ca] absorbance limit is 5 ppm, approximately 1:60 dilution is required.

It must be noted, however, that the above calculation of calcium concentration is made based on the assumption that the bone is primarily composed of hydroxyapatite. It does not take into account the presence of calcium carbonate and calcium apatite, which are also present in bone, but in lesser amounts. Larger dilutions would be expected in order to account for the presence of these two compounds.

### Calculations

Within the atomic absorption cuvette, the concentration can be calculated as:

$$\text{Concentration (ppm) = Absorbance} \times 0.0694 \quad (3)$$

However, the concentration inside the cuvette is not an indicator of the concentration in the solution beginning the start of the experiment (**THIS IS AN AWKWARD STATEMENT**). This is so because at each sampling, 5 mL of solution is taken out. This means that there is some amount of calcium that was lost through sampling as well as a reduction in volume. Such losses must be taken into account and corrections must be made.

As a general rule,

$$\text{Concentration (ppm) = Amount of Calcium } (\mu\text{g}) / \text{Total volume of liquid (mL)} \quad (4)$$

We can thus use this method to calculate the absolute amount of calcium that is supposed to be present and divide that amount ( $\mu\text{g}$ ) into the total amount of liquid to begin with (900 mL) to get the total concentration in ppm.

$$\text{Absolute amount of calcium in total solution } (\mu\text{g}) = \text{Concentration} \times 900 \text{ mL} \quad (5)$$

The method of calculating the concentration at first sampling is thus as follows:

$$\text{Concentration of the first reading (ppm) = Absorbance} \times 0.0 \quad (6)$$

The calculations of additional readings are as follows:

$$\text{Concentration of second reading (ppm) = } [(\text{Concentration of first reading} \times 5 \text{ mL}) + (\text{Absorbance of Second Reading} \times 0.0694 \times 895 \text{ mL})] / 900 \text{ mL} \quad (7)$$

$$\text{Concentration of third reading (ppm) = } [(\text{Concentration of first reading} \times 5 \text{ mL}) + (\text{Absorbance of Second Reading} \times 0.0694 \times 5 \text{ mL}) + (\text{Absorbance of third reading} \times 0.0385 \times 890 \text{ mL})] / 900 \text{ mL} \quad (8)$$

And so on.

## MATERIALS, APPARATUS, AND METHODS

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### Materials

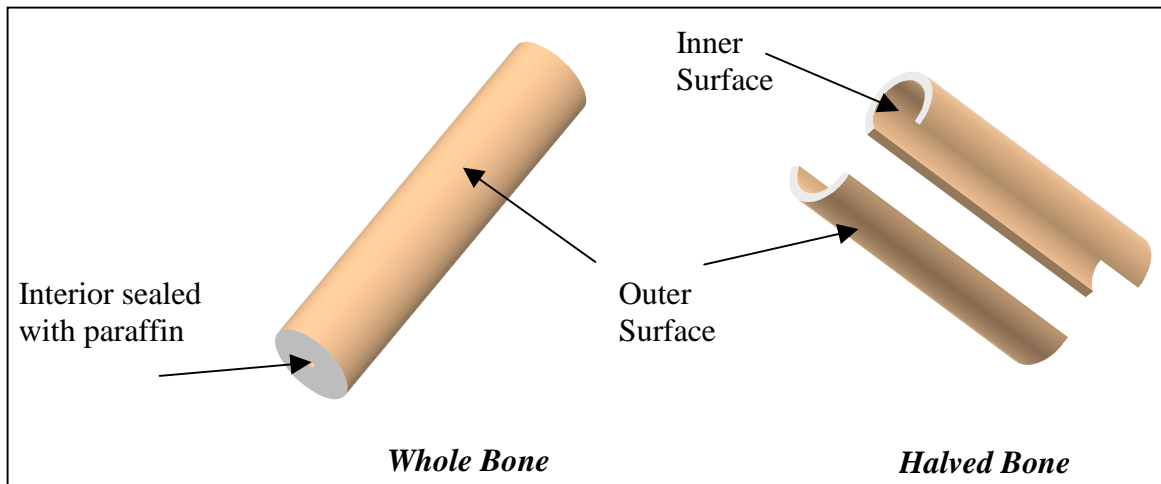
- Perkin-Elmer Model AA 4000 Atomic Absorption Spectrophotometer set at a wavelength of 422.7nm
- Standard solution of calcium (1000 ppm)
- 1M HCl solution
- 1000mL flasks, eyedroppers, 3 electromagnetic stirrers and stirring plate

- 5 whole chicken femurs
- 0.5 wt% lanthanum
- Scalpels and other cutting tools

### Methods

Three chicken femurs were prepared using the following method. First, the bones were stripped of meat and muscle using a scalpel. Secondly, both ends of the bones were sawed off and bone marrow was removed with Q-Tips. Following the first two steps, each bone underwent specific preparation. The whole bone's inner cavity was filled with wax in order to ensure that only the outer surface was exposed to the 1M HCl solution. The second bone was longitudinally halved in order for both the inner and the outer surface to be exposed to the 1M HCl solution. The whole and halved bones are illustrated in Figure 1. The ground bone was broken up into small pieces using a scalpel and other cutting tools that were in the laboratory. Finally, all bones were massed and measurements for length, diameter, and thickness were taken and recorded.

**Figure 1. Whole and Halved Bone Diagrams**



The above figure illustrates the final appearance of both the whole and the halved bone after preparation.

### Apparatus and Procedure

Following bone preparation, each bone was suspended in its respective beaker filled with 900mL of 1M HCl solution. After the bone was suspended in solution, 5mL samples were drawn from each of the three beakers using eyedroppers and were then deposited into 10mL conicals. 5mL samples were taken from each beaker at 15 minute intervals for a period of 4.5 hours, as well as twice daily (10:30am and 5:00pm) for a period of 4 days after initial suspension.

In order to measure the calcium levels in each sample the Perkin Elmer AA Spec was set to detect a wavelength of 422.7 nm. Lanthinum (0.5 wt%) was added to each sample in order to stabilize them. Once all the samples were prepared to their proper dilutions (meaning that they are between 3-5ppm so that they lie in the upper limits of the linear working range of the calcium concentration versus time graph), the machine was calibrated with the standard 1000ppm calcium stock solution and samples were tested immediately afterwards. Samples were tested in bulk. For example, once all the samples for the whole bone were taken, they were diluted and tested at the same time, without re-calibrating the spectrophotometer. There was no break in testing the samples among each of the three groups.

### RESULTS

In order to find the relationship between the rates of calcium extraction from the various bone types, an approximate maximum yield of calcium from the bones had to first be determined. Since 25.93 wt% of the

bone is typically calcium (subject to the limitations discussed in page 2 of the report), the approximate maximum calcium yield is 25.93% of the mass of each bone sample. Next, the amount of actual calcium extracted by the 1M HCl was determined by using the spectrophotometer to find the grams of calcium extracted into each solution. This amount of calcium is then normalized by the weight of the respective sample.

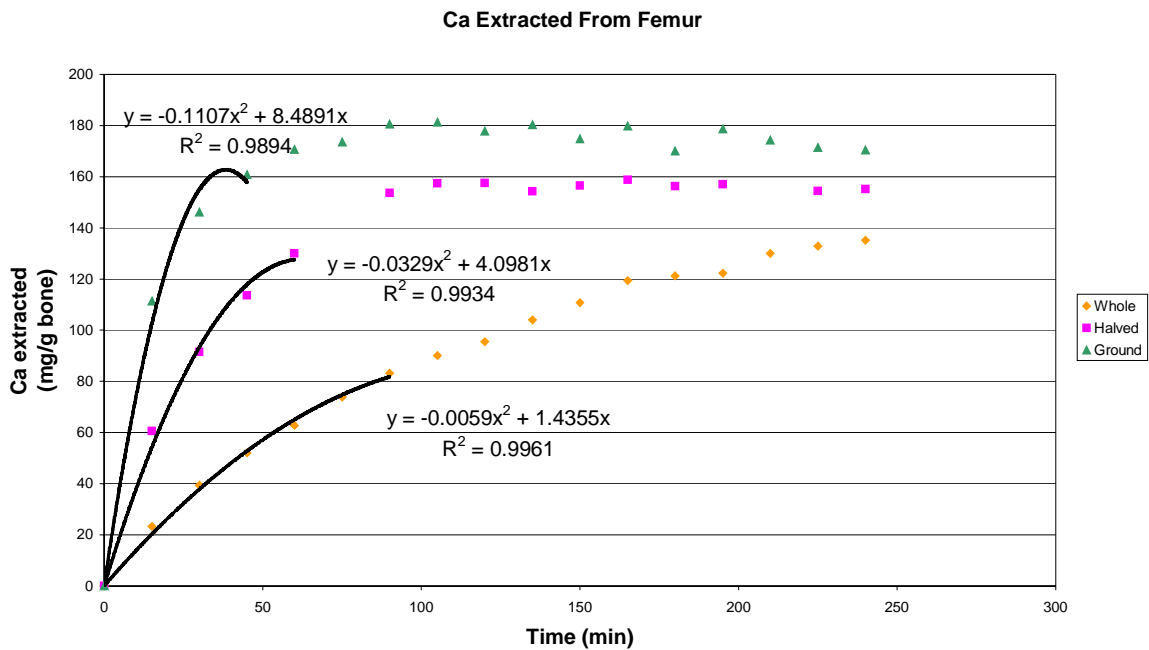
Table 1 shows that the ground bone released the most calcium as a percentage of the expected total (69.92%). This is significantly greater than the whole bone yield of 52.14% of the calculated maximum yield value. The halved bone gave a yield value of 61.24%, which is between that of the whole bone and the ground bone values. The bones did not yield the same amount of calcium per gram.

**Table1. Calcium Extracted: Experimental vs. Expected Values**

	Mass (g)	Max Extractable Ca (g)	Ca Extracted (g Ca/g bone)	Actual Amt. Ca Extra. (g)	% of Max Ca Extracted
Whole	2.480	0.6431	0.1352	0.3350	52.14
Halved	2.000	0.5186	0.1588	0.3176	61.24
Ground	2.446	0.6342	0.1813	0.4435	69.92

To better view the comparative rates of calcium extraction from the varying bone shapes, the calcium extracted from each bone per unit time is plotted, each curve representing a specific bone. Such a graph shows the change in the concentration with respect to time, which permits the comparison of the extraction rates and how they vary for whole bone, to halved bone, and to ground bone.

**Figure 2. Calcium Extracted From Chicken Femurs vs. Time<sup>4</sup>**



For each bone's geometry, a time period that modeled the initial growth period of Ca concentration/extraction was chosen. Second order polynomials that modeled these initial growth periods (during the first 90 minutes) were fitted to the data. Second order polynomials were chosen because the initial rates of extraction could be easily identified by taking the derivatives and setting times equal to 0. The calcium extraction rate should be greatest at first but then the rate of extraction should become less

<sup>4</sup> \*Standard deviations are not displayed with the data because only one sample was tested per group during the final data collection phase.

positive as time continues until it approaches steady state value. Higher polynomials have complicated derivatives and do a poor job of modeling these expected rates. Also, at the relatively short times we examined to determine the rates (less than 100 minutes), the  $x^3$  term of the third order equations is much smaller than its coefficient and thus is inconsequential and did not need to be included in order to determine an accurate result. According to the above graph, the ground bone has the highest initial extraction rate (8.5 mg Ca/g bone\*min – it is the steepest curve) and the highest total amount of calcium yield (about 180 mg. Ca/g bone – the highest steady state value). The halved bone has the second greatest rate of extraction, 4.1mg Ca/g bone\*min, followed by the rate of the whole bone, 1.4mg Ca/ g bone\*min.

To explore the relationship between surface area and the rate of calcium extraction the following table is constructed.

**Table 2 – Relationship Between Surface Area and Rate of Extraction**

	Surface Area (m <sup>2</sup> )	Ratio	Rate of Extraction (mg Ca/g bone) per min.	Ratio
<b>Whole Bone</b>	0.0020	1.0	1.4355	1.0
<b>Halved Bone</b>	0.0022	1.1	4.0981	2.9

This table examines the relationship between the surface areas of the bone samples versus their respective rate of calcium extraction (mg Ca/g bone) per minute. Because of the lack of precision involved in bone cutting, the surface area of the halved bone was only 10% greater than that of the whole bone. Only the halved bone and the whole bone were examined in this table, because the surface area of the ground bone could not be accurately determined. The original hypothesis predicted that if the halved bone surface area was 10% greater than the whole bone surface area, then the rate of extraction for the halved bone should be 10% greater as well. However, the results show that the experimentally determined ratio of the extraction rates of the two samples is 2.9:1, or 290% greater for the halved bone. This result suggests that the rate of calcium extraction from a chicken femur is not solely dependent upon surface area.

## **DISCUSSION AND ANALYSIS OF RESULTS**

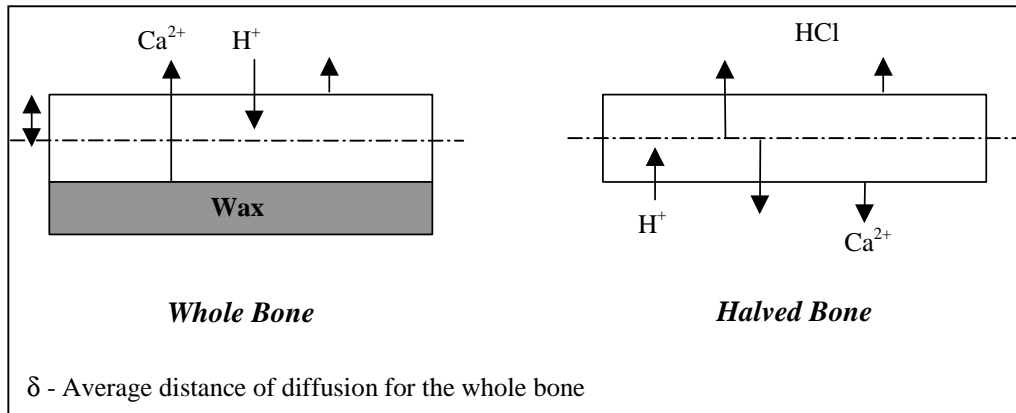
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The general patterns observed in the project were predicted in the initial project proposal. The ground bone had the fastest rate of calcium extraction (8.5 mg/g bone\*min, compared to rates of 4.1 and 1.4, respectively, for the halved and whole bones) and reached steady state in shorter time than the other bones.

However, as seen from Table 2, the surface area exposed to HCl is only 10% greater (0.0022 m<sup>2</sup>) for the halved bone than for the whole bone (0.0020 m<sup>2</sup>), while the rate for the halved bone is nearly 3 times as fast. This means that the original hypothesis - that the rate of extraction depends only on surface area exposed to the HCl - is wrong. Since all other variables had been held constant to examine the effect of surface area on the rates of calcium extraction, the only other difference between the whole and halved bone was the fact that both the inner and outer surface of the bone were exposed. The hypothesis that diffusion must affect the rates of calcium extraction was formulated to explain the different rates of extraction between the whole and halved bone. As seen in Figure 3, only the outer surface area of the whole bone is exposed to HCl (the area at the ends of the bones were discounted, since the thickness was less than 2.5% of the length of both the whole and halved bones). In contrast, both the inner and outer surfaces of the halved bone are exposed. Because of this, H<sup>+</sup> ions must penetrate all the way to the wax and back out in the whole bone to extract all of the calcium - a total distance of 4 $\delta$ . However, the early H<sup>+</sup> ions extract Ca<sup>2+</sup> directly from the surface of the bone. The average distance the H<sup>+</sup> ions must travel to complex Ca<sup>2+</sup> ions into solution is thus 2 $\delta$  [(0+4 $\delta$ )/2]. In the case of the halved bone, H<sup>+</sup> ions penetrate from both sides and meet in the middle of the bone - a distance of  $\delta$ . Traveling back out to release Ca<sup>2+</sup> ions into solution adds another distance of  $\delta$ . Therefore, the maximum penetration distance for the halved bone is 2 $\delta$ , and the average distance is  $\delta$ . If this hypothesis is true, given two bones with the same surface area and

thickness, if one is exposed to penetration from only one side and one is exposed to the solution on both sides, the rate of Ca extraction from the second bone will be twice as fast.

**Figure 3. – H<sup>+</sup> ion Diffusion Distance**



A more quantitative analysis of our chicken femurs was conducted using the relationship:

$$R \propto (D^{\circ} \cdot SA) / \delta'^5 \quad (9)$$

In words, the rate of extraction is proportional to a diffusion constant ( $D^{\circ}$  - same for both bones) and the surface area, and is inversely proportional to the average diffusion distance,  $\delta'$ .

**Table 3 - Diffusion Distance and Extraction Rates for Femurs**

	Thickness (mm)	Avg Diffusion Distance (mm)	Surface Area (m <sup>2</sup> )	Predicted Extraction Rate Ratio	Actual Extraction Rate Ratio
<b>Whole</b>	2.25	1.25	0.0020	1.00	1.00
<b>Halved</b>	1.97	0.985	0.0022	2.51	2.85

This hypothesis predicts the experimental rates within  $\pm 12\%$ , and accounts for the variation of the data from our original hypothesis. Further testing would have to be performed to confirm the validity of this hypothesis. However, a few important conclusions can be drawn based upon the data.

## CONCLUSIONS

1. The rate of calcium extraction from a chicken femur is not just a surface area effect.
2. The rate of extraction is significantly influenced by the relative (diffusion penetration) thickness of the bone.
3. Using the equation  $R \propto (D^{\circ} \cdot SA) / \delta'$ , where R is the rate of extraction,  $D^{\circ}$  is the diffusion constant, SA is the surface area, and  $\delta'$  is the average diffusion distance, the experimental data fall within  $\pm 12\%$  of the predicted values. This hypothesis requires further testing for confirmation.

<sup>5</sup> Diller, Kenneth. "Biotransport: Heat and Mass Transfer in Living Systems." New York, NY, 1998.

## REFERENCES

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### *PERSONAL REFERENCE*

1. Ducheyne, Paul: UPenn bioengineering faculty

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3. Friel, J., Hahn, R., Lee, P., Patel, A. "Determination of Calcium Content of Breakfast Foods"  
<http://bioeng.seas.upenn.edu/ugrad/projects/1999/be210s99w6r01.html>
4. Diller, Kenneth. "Biotransport: Heat and Mass Transfer in Living Systems." New York, NY, 1998.

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FORMAT AND DID A VERY THOROUGH JOB PRESENTING YOUR DATA.  
YOUR ALTERNATE HYPOTHESIS IS A REASONABLE EXPLANATION FOR THE  
EXPERIMENTAL RESULTS.**