The Effects of Increased Plasma Homocysteine Levels on Murine Birth Weight

Presented to the faculty of Lycoming College in partial fulfillment Of the requirements for Departmental Honors in Biology

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Abstract

Homocysteine (Hcy) is a non-essential, thiol containing, amino acid. It is produced in the body as an intermediate during the break down of methionine. Hcy has been associated with several diseases including Alzheimer’s disease, stroke, cardiac disease, neural tube defects, and low birth weight. In this study C57 mice were broken up into three groups. One group received 1mg/mL Hcy in phosphate buffered saline (PBS), another received 2mg/mL Hcy in PBS, and the last group received PBS. The average weights of the three groups were 1.2±0.1 g, 1.2±0.1 g, and 1.2±0.1 g respectively. The data was analyzed using a student t test, an ANOVA test, and others. It was determined that there was no statistical difference among the birth weights in the three groups. While reasons for this are not exactly known, possible explanations could include a dosage that was not consistent, improper injections, excess intake of B vitamins, or Hcy concentrations that were below the critical threshold for adverse effects.

Introduction

Homocysteine (Hcy) is a non-essential, thiol containing amino acid. It is produced in the body as an intermediate step during the break down of an essential amino acid, methionine, in order to produce cysteine. High plasma Hcy concentrations are associated with several diseases including cardiac disease, renal disease, stroke, and pregnancy complications (1-4). There are several factors that have been shown to increase plasma Hcy levels. These factors include low intake of folic acid, riboflavin, cobalamin, genetic variation in the enzymes responsible for regulating homocysteine concentrations, and
poor life style choices (1, 2, 5-9). This study focuses on the effects of high Hcy concentrations in pregnant females on birth weight and litter size in C57 black mice.

Homocysteine, as shown in figure 1, can be found in the plasma in different forms. The most common form is protein bound. Hcy can bind to cysteine that is located on the outside of a protein via a disulfide bond. Hcy can also be found in the oxidized state where it is linked to a cysteine, or another Hcy that is not associated with a protein. Very little of the Hcy found in the plasma is in the reduced sulphydryl form. (Figure 2) (2, 4). Normal plasma Hcy concentrations in humans range from 5 to 15 umoles/L, however, Robinson et. al. found that levels over 6.3 uM significantly increases an individual’s risk for heart disease (10). A vitamin B deficiency can indicate that a person’s Hcy concentrations could be too high because folic acid, B12, and B6 act as coenzymes during the break down of Hcy. Hcy can be broken down in two different ways. It can be remethylated back into methionine, or it can be converted into cysteine via transsulfuration.

\[
\begin{align*}
  &\text{Figure 1: The amino acid Homocysteine} \\
  &
  \begin{array}{c}
  \text{H} \\
  \text{H}_2\text{N}^+ \text{N} \text{C} \text{O} \\
  \text{\_CH}_2 \\
  \text{\_CH}_2 \\
  \text{\_SH}
  \end{array}
\end{align*}
\]
Plasma concentrations of folic acid and Hcy are inversely related such that high concentrations of folic acid decrease Hcy concentration while low concentrations of folic acid increase Hcy concentrations. This is due to the fact that folic acid is needed in the metabolism of Hcy. Folic acid is a water soluble B vitamin that is used to form a coenzyme. These coenzymes produced by folic acid aid in the synthesis of purines and pyrimidines, erythropoiese, and methionine (11). Because of its important role, the Food and Drug Administration (FDA) recommend that pregnant women consume 0.4 mg of folic acid per day. In 1996 the FDA issued a regulation effective January 1998 that all grain products such as flour, rice, pasta, and cornmeal must contain 140 ug of folic acid per 100 g (12). This requirement was established in order to help reduce the number of neural tube defects caused by a lack of folic acid intake.
Other B vitamins like B6 and B12 can also play a role in plasma Hcy concentrations (2, 9, 11). The vitamin B6 refers to a group of vitamins that are nitrogen containing compounds with three forms pyridoxine, pyridoxal, and pyridoxamine. Vitamin B6 is water soluble, and can be found in a variety of foods including poultry, fish, meat, legumes, nuts, potatoes, and whole grains. Vitamin B6 assists in more than 100 enzymatic reactions, and it is recommended that a person consume 2 mg daily (11). Vitamin B6 deficiencies are very rare because they are found in such a wide variety of foods (11).

Vitamin B12 is also water soluble, and can be found in animal products such as meat, eggs, and milk. It is recommended that a person ingests 6 ug of vitamin B12 daily. Vitamin B12 plays an important role in enzymatic reaction for fat and carbohydrate metabolism, protein synthesis, and hematopoiesis. Usually only strict vegetarians are at risk for a B12 deficiency, and there are no adverse effects of an increased B12 intake (11).

Hcy is produced in the body as an intermediate during the biochemical reaction that converts methionine into cysteine (Figure 3). Methionine first gets converted into S-Adenosylmethionine (SAM) by the addition of an adenosine from ATP. The enzyme that catalyzes this reaction is methionine adenosyltransferase. Methyl transferase then removes a methyl group from SAM, and transfers it to an acceptor such as a phospholipid, DNA, or another protein. The removal of the methyl group converts SAM into S-Adenosylhomocysteine. The adenosine is removed thus producing Hcy (9).

Hcy can be metabolized via two different pathways. The remethylation of Hcy produces methionine, and transsulfuration produces cysteine (2, 6). During the
transsulfuration of Hcy cystathionine β-synthase (CBS) converts Hcy into cystathionine, this is then changed into cysteine via Cystathionase (Figure 3) (6, 9). Both CBS and cystathionase require the vitamin B6 as a coenzyme. The remethylation of Hcy involves both B12 and folic acid as coenzymes (6, 9). When folate is consumed it is transferred into 5, 10-methylene-tetrahydrofolate through a series of reactions. Methylene-tetrahydrofolate reductase (MTHFR) then transfers this into 5-methyl-tetrahydrofolate. This 5-methyl-tetrahydrofolate is the essential methyl donor during the remethylation of Hcy (Figure 4). (2, 6). A DNA polymorphism in either the MTHFR or CBS can cause Hcy levels to be elevated in an individual.

Figure 3: The Metabolic pathway of Hcy (9).
Figure 4: 5-Methyl-tetrahydrofolate is used as the methyl donor during the remethylation of Hcy to methionine. Methyl synthase (MS) and Cobalamin (Methyl-Cbl) are needed for the reaction (2).

The most common inherited form of hyperhomocysteinemia is caused by a polymorphism in the MTHFR gene. This enzyme is responsible for producing the folic acid that is used as the methyl donor during remethylation of Hcy (Figure 3). The polymorphism is a C to T transversion in the gene at position 677. This 677C>T transversion causes an alanine to valine substitution in the catalytic portion of the enzyme. Heterozygotes of this 677T allele have about a 35% increase in Hcy levels while homozygotes of the allele have an increase of 75% (6, 9). Another less common polymorphism on the MTHFR gene is a 1298A>C transversion which substitutes the amino acid glutamate with an alanine. This affects the catalytic activity of the enzyme, but does not affect MTHFR as severely as the 677C>T transversion (9).

Along with genetics, there are also lifestyle choices that can affect Hcy concentrations. The Hordaland Homocysteine Study (HHS), the largest Hcy study ever completed, found that several different factors can affect plasma Hcy concentrations. The most significant factors that affect Hcy concentrations include sex, age, folate intake,
smoking status, and coffee intake (refer to figure 5) (8). A combination of the three modifiable factors, low folate intake, smoking, and large coffee intake, showed the highest Hcy concentrations while individuals who have high folate intake, do not smoke or drink, and drink little coffee showed the lowest Hcy concentrations. This suggests that a simple change in life style, especially for those already at risk for hyperhomocysteinemia, can make a dramatic difference in Hcy levels (8).

Figure 5: The affects of several different factors on total plasma Hcy concentrations (8). The shaded area represents the 95% confidence interval.
Obesity is another lifestyle choice that has been shown to increase Hcy levels. Hcy concentrations are positively associated with sugar intake and increased body mass index (2). According to Bjorke *et. al.*, there are two possible mechanisms for this increased Hcy concentrations in obese children (2). The first is diet. One can assume that obese children consume high fat and low folate diets. Secondly there may be an insulin resistance because insulin has been shown to lower Hcy concentrations (2). According to Horadaland Homocysteine Study, the lack of physical activity may also play a role in the higher Hcy concentration in obese children. Although a gastroplasty may solve the problem of obesity, Borson-Chazot *et. al.* showed that a gastroplasty can also increase Hcy concentrations (13). This is most likely due to the decrease in the amount of food consumed thus decreasing the amount of folate consumed.

High Hcy levels are also very common in renal transplant recipients, and patients with chronic renal failure (2). While the overall cause of the increased Hcy concentrations is not known, it is known that high Hcy concentrations can be an indicator of possible renal distress, or cardiac distress. It is also know that giving folic acid supplements to patients who have high Hcy levels and renal failure can decrease Hcy levels, but it can not normalize them (2). It has also been shown that by giving folic acid supplements to pediatric patients who have renal failure can improve the flow mediated dilation (2).

The current study examines how Hcy affects birth weight and litter size in C57 black mice (1). Thirty C57 mice were injected with either Hcy solution or PBS. It was hypothesized that increased concentrations of Hcy in pregnant females would cause decreased birth weights, and decreased litter size. It was predicted that the mice who
received the highest dosage of Hcy would give birth to the smallest young, and have smaller litters than the other groups.

C57 black mice were used because of they are one of the most common inbred strains used in studies. There are several characteristics that are specific to the C57 mouse strain. C57 mice have a very low susceptibility to tumors. They do however have a high susceptibility to obesity, hyperglycemia, and hyperinsulinemia. They have a high incidence of eye defects, and a resistance to audiogenic seizures. Finally, C57 mice have low bone density, and late onset of hearing loss (14). Their average body weight changes throughout their lifetime, refer to figure 6 for a graph of body weight vs. age in black C57 mice.

![Figure 6: Mean body weight of C57 mice throughout their life time (14).](image)
Methods

Stock solutions of Hcy were prepared before the start of the experiment. 40 mL of 1mg/mL Hcy in PBS, 2 mg/mL Hcy in PBS, and PBS solutions were prepared. These solutions were sterilized in a cell culture hood by filtering the solutions through a 0.2 um filter. The solutions were placed in vacutainers, and stored at 4°C.

Forty-five C57 black mice, 15 males, and 30 females, were ordered from Jackson Laboratory. The mice were kept in the Lycoming College mouse house in the Heim Biology and Chemistry Building. Here they lived under a 12 hour day and 12 hour night cycle. The mice were fed and watered daily, and also had several health checks during the course of the experiment. After the mice adjusted to their new living environment, breeding cages were set up that contained one male and two females. The females were not introduced into the breeding cages until the males had been acclimated in the cages for 2-3 days. This allowed the males to become comfortable with their new environment and make their nests before the females were introduced.

After the breeding cages were set up, vaginal plugs, an indication that the females had been inseminated, were checked every morning before 10:00am. After a plug had been detected, it was assumed that the female was pregnant, and these females were randomly assigned to one of the three groups, the 1 mg/mL group, the 2 mg/mL group, or the control that just received PBS. The mice were injected daily with an intraperitoneal injection of 0.1 mL of a solution with a 26 gauge tuberculin syringe. Around the expected day of birth, 18-20 days after a plug was detected, the mice were checked several times a day so that the newborns could be counted, and weighed as close to birth as possible. Once data had been collected on the newborns, they were sacrificed by decapitation.
Results

There were a total of 205 newborn pups that were analyzed in the current study. There were 65 pups in the control group, 71 pups in the 1 mg/mL groups, and 69 pups in the 2 mg/mL groups. The average weights of the newborn mice in the different groups were 1.2±0.1 g for the control group, 1.2±0.1 g for the 1mg/mL group, and 1.2±0.1 g for the 2mg/mL group. The 95% confidence interval for the control group, 1mg/mL group, and 2mg/mL group were 1.19-1.24 g, 1.18-1.22 g, and 1.21-1.25 g respectively. The weights ranged from 1.0-1.5 g. Refer to Table 1 for a list of these results.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1mg/mL</th>
<th>2mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Weight (g)</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td>1.19-1.24</td>
<td>1.18-1.22</td>
<td>1.21-1.25</td>
</tr>
<tr>
<td>Range</td>
<td>1.1-1.5</td>
<td>1.0-1.4</td>
<td>1.0-1.4</td>
</tr>
</tbody>
</table>

Table 1: List of mean, standard deviation, confidence interval, and range

A Student T test and an ANOVA test were done on the data. The T value for the control and the 1 mg/mL group was 1.05. The t value for the control and the 2 mg/mL group was 0.806. Finally the t value for the 1 mg/mL group and the 2 mg/mL group was 0.235. The P values for the three groups were 0.298, 0.815, and 0.422 respectively. The degrees of freedom for the three values are 132, 136, and 130 respectively. Refer to Table 2 for these results. The F value from the ANOVA test was 0.5824.
Finally frequency distributions were plotted in order to compare the three groups. Refer to figures 7 and 8 for the graphical representation of the weight distribution. The most frequent weight for the control and the 1 mg/mL group was 1.2 g, and the most frequent weight for the 2 mg/mL group was 1.3 g. The 2 mg/mL group had a decreased number of newborns that weighed 1.2 g thus causing a dip in the curve.

<table>
<thead>
<tr>
<th></th>
<th>Control and 1mg/mL</th>
<th>Control and 2mg/mL</th>
<th>1mg/mL and 2mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Value</td>
<td>1.05</td>
<td>0.806</td>
<td>0.235</td>
</tr>
<tr>
<td>Degrees of Freedom</td>
<td>134</td>
<td>132</td>
<td>138</td>
</tr>
<tr>
<td>Critical T value</td>
<td>1.660</td>
<td>1.660</td>
<td>1.660</td>
</tr>
<tr>
<td>P Value</td>
<td>0.298</td>
<td>0.815</td>
<td>0.422</td>
</tr>
</tbody>
</table>

Table 2: Student T Test values for Birth Weight

Figure 7: Bar Graph of the weight distribution.
Data was also collected on the number of newborns in each litter. There were a total of 32 litters born in this experiment. There were 10 litters in the control groups, and 11 litters in both experimental groups. The average number of pups born and the standard deviation in the control, 1 mg/mL, and 2 mg/mL were 6.5±1.3 pups, 6.45±0.93 pups, and 6.27±1.01 pups respectively (Table 3). The number of pups in each group ranged from 5-8 pups, and a frequency distribution was plotted. Finally a student t test and an ANOVA were performed on the data. The t values for the control and 1 mg/mL was 0.930, the t value for the control and the 2 mg/mL was 0.671, and the t value for the 1 mg/mL and 2 mg/mL group was 0.665 (Table 4). The F value from the ANOVA test was 0.1268.
<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1mg/mL</th>
<th>2mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>6.5</td>
<td>6.45</td>
<td>6.27</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.35</td>
<td>0.93</td>
<td>1.01</td>
</tr>
<tr>
<td>Range</td>
<td>5.0-8.0</td>
<td>5.0-8.0</td>
<td>5.0-8.0</td>
</tr>
</tbody>
</table>

Table 3: List of mean, standard deviation, and range on liter size data

<table>
<thead>
<tr>
<th></th>
<th>Control and 1mg/mL</th>
<th>Control and 2mg/mL</th>
<th>1mg/mL and 2mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Value</td>
<td>0.93</td>
<td>0.671</td>
<td>0.665</td>
</tr>
<tr>
<td>Degrees of Freedom</td>
<td>19</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Critical T value</td>
<td>2.093</td>
<td>2.093</td>
<td>2.086</td>
</tr>
</tbody>
</table>

Table 4: Student T Test Values for Liter Size

Discussion

Homocysteine (Hcy) is a non essential, thiol containing, amino acid that has been associated with negative reproductive consequences. This study was especially interested in the potential negative affects that Hcy has on birth weight and liter size. Pregnant C57 black mice were injected with different concentrations of Hcy, and the control group was injected with PBS. Newborn mice were weighed, the data was collected, and several statistical tests were done in order to determine if there was a significant difference between treatment groups and the control. After analyzing the data it was determined that there was no statistical difference between any of the groups.

The first statistical test done on the data was an analysis of variance test, which is better known as an ANOVA test. An ANOVA test is used to determine if there is a statistical difference among experimental groups. The tests in the ANOVA are based on the F-ratio. The F-ratio is the variation due to an experimental treatment divided by the variation due to experimental error. In this study the F value for the weight of the
newborn mice was 0.5824. Because the F experimental value is less than the F theoretical value, 3.94, the null hypothesis, that there is no difference among the groups, is not rejected, and therefore there was no statistical difference between the three experimental groups. The F value for the litter size was 0.1268. The F theoretical value for the litter size was 3.32. Since the F experimental value is less then the F theoretical value it can be assumed that there is no difference in litter size among the treatment groups.

Another test that was done was the student t test, refer to Table 2. The experimental t value for the control group and the 1 mg/mL group was 1.05. The experimental t value for the control group and 2 mg/mL group was 0.806. Finally the t value for the 1 mg/mL group and the 2 mg/mL group was 0.235. All three of these values were less than the critical cut off t value of 1.660 with a 95% significance level. Because the t statistic did not exceed the critical value, the null hypothesis could not be rejected. Therefore no statistical difference in birth weight between the groups was apparent. The probability value, or p value, was calculated with the student t test. All of the p values were greater then 0.05, and because of this the null hypothesis was once again not rejected for a 95% significance level. The student t value for litter size for the control and 1 mg/mL, the control and 2 mg/mL, and the 1 mg/mL and the 2 mg/mL were 0.93, 0.67, and 0.67 respectively. Because all the experimental t values for litter size were less than the theoretical t value, 2.086 with a 95% significance level, there was no statistical difference in litter size between any of the experimental groups.

Finally to help visualize that the null hypothesis was not rejected, two frequency plots were plotted, figures 7 and 8. The frequency plots show that the 2 mg/mL group appears to have the largest newborns while the control group appears to have the smallest
newborns. This finally helps to visualize the statistical analysis that shows there is no significant difference between the groups, and that the Hcy did not decrease the newborns birth weight as hypothesized.

Hcy is broken down in the body with the assistance of folic acid. Folic acid is used by the body to produce nucleic acids. It was hypothesized that increased Hcy levels would cause a decrease in birth weight (1). However in this study there was no significant difference among the birth weights of the mice that received Hcy injections and the control group. While the exact reasons for this are not known, there are a few possible reasons why there was no overall statistical difference among the three experimental groups.

One possible reason why there was no significant difference between the groups was because the dosage of Hcy the mice received did not remain constant through out the experiment. Before the mice were pregnant, they weighed about 16 g. At the end of their pregnancy, about 18-20 days, they weighed about 24 g. Even though the mice increased in weight, the amount of Hcy injected into the experimental mice did not change. This was done in order to be able to use data that was already collected during a previous trial. Ideally each mouse would have been weighed each day before injection. Depending on the dosage of Hcy the mouse was supposed to receive, the volume of Hcy solution injected would have been changed accordingly. This would have assured a constant amount of Hcy being injected into the mouse throughout the gestation. In this study the dosage of Hcy each mouse received decreased each day since the same volume, 0.1 mL, was injected each day. However the blood concentration of Hcy should still have been increased even though the dosage did not remain constant.
The average mouse has about 0.06 mL of blood per gram (15). For the 1 mg/mL group this means that in an absolute ideal situation with a 0.1 mL injection of 1 mg/mL Hcy the plasma Hcy concentrations in a 16 g mouse should have been about 700 uM. This should have been more than enough to cause a decrease in birth weight, and also possibly cause other problems. Since the concentration should have been sufficient, the Hcy that was injected into the mice was most likely not getting into the blood stream properly. This could have been caused by the way the Hcy was injected into the mice.

In this experiment the mice were injected daily with intraperitoneal injections (IP). The peritoneal cavity is the area that surrounds the abdominal cavity. An IP injection is done when an intravenous injection cannot be done. Because of the size of mice, the IP injection is preferred. However, Miner et. al. has shown that on average 24% of IP injections are not placed correctly in the peritoneal cavity (16). The most common incorrect placement of the injection occurred in the stomach and in the lumen of the intestines (16). The study examined the placement of an IP injection using the same size needle used in the current study. It found that whether injecting as quickly as possible or being as careful as possible there is still a 24% error in placement. This could mean that 24% of the injections done in the current study were placing the Hcy into the digestive system. If the Hcy was placed in the large intestines it could have been flushed from the system before it was absorbed into the blood stream.

Another possible reason that there was no significant difference in weight could have been caused by the intake of B vitamins in the mouse’s diet. All of the mice in the Lycoming College Heim mouse house eat Purina Rodent Chow Lab Diet 5001. Lab Diet 5001 is a constant nutrition formulation that is specifically for mice, rats, and hamsters. It
is designed to minimize variability in nutritional intake thus making it good for long term studies.

Purina Rodent Chow Lab Diet 5001 contains many different proteins, minerals, and vitamins. Specifically the diet contains five different B vitamins. The B vitamins are riboflavin (B2), niacin (B3), folic acid (B9), pyridoxine (B6), and cobalamin (B12). A list of these B vitamins and the amount of each found in the diet can be found in Table 5. It was possible that the intake of these B vitamins was enough to reduce the Hcy levels in the mice. B vitamins, especially B6, B12, and folic acid, act as coenzymes during the metabolism of Hcy. Lower concentrations of these vitamins in the plasma can cause an increase in Hcy concentrations. Even though it is not known how much each mouse eats specifically, this amount of B vitamins might have been enough to counter-act the increased Hcy that was given to the mice.

Table 5: The B vitamins and the amounts found in Purina Rodent Chow Lab Diet 5001

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin (B2)</td>
<td>4.5ppm</td>
</tr>
<tr>
<td>Niacin (B3)</td>
<td>120ppm</td>
</tr>
<tr>
<td>Folic Acid (B9)</td>
<td>7.1ppm</td>
</tr>
<tr>
<td>Pyridoxine (B6)</td>
<td>6.0ppm</td>
</tr>
<tr>
<td>Cobalamin (B12)</td>
<td>50ug/kg</td>
</tr>
</tbody>
</table>

Normal Hcy levels in humans can range from 5uM-15 uM, however it has been shown that even a plasma Hcy concentration of 6.3 uM can have significant risk factors associated with it (10). Since the Lab Diet 5001 is not designed to maximize breeding in
mice by having increased B vitamins and other nutrients (17), it is more likely that the original hypothesis is wrong and that the null hypothesis is correct. Hcy may not cause low birth weight by decreasing the amount folic acid present. Instead Hcy could be an indicator that folate levels are low. It is these low folate levels, and not the high Hcy levels that can cause low birth weight. Genetics can play a role in decreasing folic acid levels thus increasing Hcy concentrations (18).

Much like people, mice also have the MTHFR enzyme that catalyzes the irreversible reaction that converts 5-10 methyl-tetrahydrofolate into 5-methyl-tetrahydrofolate. In order to study the importance of this gene Ghandour et. al. produced MTHFR knock out mice in order to study its relationship with Hcy (18). The homozygous MTHFR knockout mice had a ten fold increase in plasma Hcy levels relative to wild type mice (18). The homozygous knockout mice had an increase in tetrahydrofolate because it could not be converted into the 5-methyltetrahydrofolate, for which the knockout mice also had a deficiency. Since the mice in this study did not appear to be affected from the increased Hcy, it can be assumed that the mice in general did not suffer from the MTHFR 677C>T transversion.

Prior to the current study a previous study was done that was based on determining plasma Hcy concentrations using UV-HPLC. Several studies have been done that show that using HPLC is an efficient way to determine plasma Hcy concentrations (19-22). Several hours were spent using a Waters 510 HPLC with a Waters 991 photodetector trying to successfully separate Hcy and cysteine in albumin solutions. Since the HPLC system and computer were at least 15 years old, there were several problems with the separations. Had this experiment been successful it would have been
possible to determine the plasma Hcy concentrations in the mice before and after injections. Because this was not possible it was impossible to determine the significance of the doses of Hcy given to the mice. Even without this advantage, it was possible to calculate relative concentrations given to the mice on a daily bases.

High homocysteine levels in plasma have been shown to cause low birth weight and neural tube defects. However in the present study no such relationship was found. There was no statistical difference between the control group and the two experimental groups in birth weight or litter size. This could have been due to several factors including inconsistent dosage, extra intake of B vitamins, error in the injections, or not enough Hcy given to the mice. However it is more likely that Hcy does not cause low birth weight. Instead, Hcy is just an honest indicator of low folate concentrations, and it is this that causes the low birth weight. Future work could include determining the average plasma Hcy concentration in the mice before the start of the experiment because this was not done prior to this study. With this value known, proper Hcy dosages could be given to the mice in order to more precisely examine the affects of Hcy on birth weight. It would also make it easier to exceed the critical threshold limits. Also MTHFR knock out mice could also be injected with Hcy. The affect of Hcy injected into these mice would be amplified since they lack the ability to produce the methyl donor required to break down Hcy.
References


