Eight adolescent males (14 to 18 years old) were evaluated before and after 50 minutes of exercise on a bicycle ergometer at 60 percent of their maximal heart rate to investigate the relationship between blood magnesium status and the derangement of other serum electrolytes in the etiology of "sports anemia." Criteria of assessment included changes in serum concentration and total serum content of magnesium, sodium, potassium, calcium, and red blood cell magnesium concentration, urinary magnesium excretion, hematocrit, hemoglobin, mean corpuscular volume, red blood cell count, osmotic fragility, reticulocytosis, and spherocytosis. A significant reduction in serum sodium concentration was found at post-exercise, whereas, serum calcium and potassium concentrations rose 3.8 percent and 7.7 percent, respectively. Total serum content of magnesium and sodium was significantly reduced by 4.9 percent and 9.0 percent, respectively, at post-exercise. Red blood cell magnesium dropped 3.1 percent at
post-exercise. Following a one-hour recovery, serum magnesium concentration had fallen significantly (10.3 percent) and red blood cell magnesium concentration was 2.6 percent higher than the pre-exercise concentration. Although there was no evidence of red blood cell hemolysis, red cells did show spherocytosis and a tendency toward increased osmotic fragility. In addition, the changes observed in total serum magnesium content were significantly correlated to changes in total serum calcium at post-exercise and to total serum potassium content at recovery. The spherocytosis and decreased osmotic resistance appear to result from the impairment of magnesium-dependent adenosine triphosphatase, which is responsible for the active transport of sodium and potassium across the erythrocyte membrane.
Magnesium and Red Blood Cell Fragility
Following Heavy Exercise of Moderate Duration
in Untrained Teenage Boys

by

Christina Scribner Reiter

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Magnesium and Red Blood Cell Fragility
Following Heavy Exercise of Moderate Duration in Teenage Boys

INTRODUCTION

Statement of the Problem

Millions of Americans are involved in the craze to become physically fit. The current fitness boom has been growing for over a decade and may be recognized as a national obsession (1). The most obvious evidence of America's preoccupation with body improvement is the marketplace. Five billion dollars a year are spent on health foods and vitamins; $50 million for diet and exercise books; $6 billion for diet drinks; another $5 billion for health clubs and corporate fitness centers and $9 billion more for sporting gear and gadgetry (1). Polls also point to a dramatic increase in exercise. A 1977 Gallup Poll (2) found that 47% of adults exercised daily, which was twice that reported in 1961, and a 1978 Louis Harris survey indicated that 59% of adults exercised regularly (3). A Washington Post-ABC News poll taken in October 1982 reported that 53% of the respondents over age 17 years exercised "strongly" each day (3).

Perhaps the best indicator of the fitness boom is participation in athletics. In 1971, 233 runners entered the New York City Marathon, compared to the 25,000 runners who applied for the 16,000 places in 1981 (1). Fourteen million people over 18 years of age, or about nine percent of the United States adult population, were playing tennis as a regular activity in 1979 (4) and by 1983, 38% of the adult population was playing tennis (5).
Running and tennis do not stand alone in the exercise revolution. Participation in bicycling, swimming, weight lifting, and even walking are among the activities credited for transforming the life of Americans (1).

The goal of increased physical fitness is not necessarily winning a trophy. Today, the goal is better health (6). The claimed benefits of physical fitness include reduced risk of heart disease, high blood pressure and obesity, and development of increased lean body mass, muscular strength, and maximal oxygen consumption (1,5,6,7,8). Psychological changes resulting from physical fitness are generally thought to be positively correlated with well-being (1,6,8).

The prevailing dogma that exercise is good under any condition, however, can be challenged since physical exertion can also do a lot of damage (9). The most common negative effects result from sudden and excessive demands made on an unconditioned body and include sprains, twisted knees and elbows, torn ligaments or cartilage, broken bones (10), and an unusual type of transient anemia termed "sports anemia" (11,12,13,14). This type of anemia has been attributed to increased fragility of red blood cells and frequently includes significant decreases in hemoglobin concentration, hematocrit, mean corpuscular volume, and osmotic resistance (12-18).

Research Hypothesis and Objectives

The mechanism for the increased destruction of red blood cells following physical activity has not been identified. Decreased concentrations of magnesium in serum and red blood cells have been
reported following exercise and may play a central role in hemolysis through impairment of magnesium-dependent adenosine triphosphatase (ATPase) in the red blood cell. Impairment of ATPase may cause an imbalance in the sodium and potassium concentrations of the serum and red blood cell (19, 20, 21) resulting in spherocytosis and hemolysis (19,22).

The research described in this report is directed towards identifying a possible effect of moderate exercise in untrained adolescent boys on magnesium status, red blood cell morphology, and red cell fragility. It is hypothesized that changes in magnesium status following exercise may play a key role in explaining the derangement of other serum electrolytes and the development of sports anemia. The specific objectives of this study are:

1. To identify the effect of frequent exercise on magnesium status.
2. To determine if a relationship exists between serum or red blood cell magnesium concentration and the fragility of erythrocytes.
3. To indicate a mechanism for the occurrence of the anemia following physical exertion termed "sports anemia."
"Sports Anemia" - What is it?

In 1959, H. Yoshimura (15) coined the term "sports anemia" to describe a mild, transient anemia (13, 14) that was observed in athletes following prolonged or heavy physical exercise. Although it was hypothesized that this type of anemia, previously known as "March Hemoglobinuria," (23) resulted from the destruction of erythrocytes, it is not yet clear as to which type or level of work is required to induce sports anemia (13), nor is the etiology of sports anemia completely clear. Examples of hypotheses are: 1) preferred utilization of hemoglobin by the working muscles including the heart, spleen, and bone marrow; 2) deviations in red cell structure; and 3) derangements of iron status (13, 15).

Studies relating changes in hematologic status to the development of sports anemia have provided important insight into understanding at least part of the mechanism. Among the first of these studies was a hematological screening of the Netherland Olympic candidates for the 1968 Olympic Games in Mexico City. Four and one-half percent of the males and 7.5% of females were iron depleted as evidenced by less than 50 ug iron/100 ml serum (16), compared to a normal value of 60-170 ug iron/100 ml (24). A Swedish investigation of eight male long distance runners in training produced a controlled survey of iron status which included peripheral blood values, bone marrow iron, hemoglobin iron, and plasma clearance of iron. The
resulting values for hemoglobin, hematocrit, and serum iron were all considered to be normal. Three of the eight subjects had plasma clearance of iron greater than 60 minutes, which is the lower limit of normal. Bone marrow aspirates completely lacked iron in five of the subjects, and three subjects had only traces of iron (11). Overall, this study indicated suboptimal iron status but not overt anemia.

Radomski et al. (13) showed five to six hours of exercise at 35% maximal oxygen consumption for six days to result in at least a 12% drop in hemoglobin compared to a non-exercise control group, but the difference remained within the physiological range of normal. Significant decreases in hematocrit and red blood cell counts were also attributed to the exercise, but again the results were within the physiologic range of normal. Unfortunately, these investigators failed to measure pre-exercise values but noted increases of 5% in hematocrit and 8% in the red blood cell count from post-exercise through four days of recovery. In addition, examination of the size distribution of red blood cells revealed two populations where 80% of red blood cell counts were small cells (mean size 68 u) and 20% of the red cell counts were large cells (mean size 123 u^3). Although a significant decrease of 5% in mean corpuscular volume was identified as a preferential destruction of large blood cells, the original proportion of cells by size returned to normal by the second day of recovery. In another study, Costill et al. (25) showed mean corpuscular volume to decrease 5.7% following exercise which resulted in a 5.8% dehydration. Average hemoglobin concentration dropped nearly 5% among athletes one hour after a 25 kilometer run (12). However,
this decrease remained within the physiologically normal range (26).

Evidence collected by Halicka et al. (18) suggests that athletes may exhibit prolonged deviations in the morphology of red cells. Studies on 20 men and 20 women, all of which were performing athletics at the Academic School of Physical Education in Warsaw, Poland, indicated significantly lower than normal erythrocyte osmotic resistance in 0.45%, 0.50%, 0.55%, and 0.60% sodium chloride solutions. Red blood cell count was depressed and the red blood cell diameter, mean corpuscular volume, and mean corpuscular hemoglobin were raised in both sexes. Analysis of the data, however, revealed that the control levels used for comparison were simply the median of the normal range and that all results remained within normal physiological ranges. Because Halicka et al. utilized a post-test only design, the lowering of osmotic resistance was the only empirically valid result obtained in the study.

Yamada, as cited in an abstract by Yoshimura (15), also indicated that athletes in training for one week exhibited decreased osmotic resistance and sports anemia. Lieberman and Acel found osmotic resistance to decrease in both animals and humans over the first few minutes of strenuous exercise and then return to normal during rest (12). Hiramatsu reported the mean life of red blood cells to decrease 38% from 52.5 days during rest to 32.3 days in rats running on a treadmill at 1.3 km per hour for two hours in a four week long experiment (15).

Since the hematological changes observed in the studies cited may be due to a derangement in red cell metabolism, other investigators have looked for changes in blood electrolytes following exercise as a
factor supporting this possibility.

**Blood Content of Sodium, Potassium, and Calcium Following Exercise**

Exercise of intensity greater than 60% maximal oxygen consumption is often reflected in a decrease in plasma volume. Plasma concentrations of sodium have been shown to rise from 1 to 4%, and plasma chloride concentrations have been shown to increase 8% in response to exercise on the bicycle ergometer, on the treadmill, or following skiing in both trained and untrained subjects (27-30). The potassium concentration of plasma has been reported to rise from 8.5% to 13% in response to similar exercises and may be larger than can be explained by volume changes alone (27-31), suggesting the release of potassium from the red blood cell. Serum calcium concentrations do not generally increase by significant margins in response to exercise (27,30).

While fluid loss and decreased plasma volume are commonly recognized as results of muscular activity, little data are available on the changes in total plasma concentration of electrolytes. Difficulties in making accurate and frequent measurements of plasma volume changes have contributed to this lack of information. However, it has been demonstrated that hemoconcentration can be calculated accurately from changes in hematocrit (28). Using hematocrit readings, vanBeaumont et al. (28) calculated total plasma content of electrolytes and found that maximal exercise on a bicycle ergometer induces a decrease in total plasma sodium and chloride, with a fall in
potassium occurring following an initial increase immediately after cessation of the work. Plasma calcium content was not measured.

**Changes in Magnesium Following Exercise**

Plasma magnesium concentrations have been reported to decrease as a result of vigorous exercise. Rose and his associates report that 10 to 40 minutes of exercise produced an increase in serum magnesium concentration whereas exertion of longer duration was accompanied by a 21% drop in serum magnesium concentrations (31). The decrease was assumed to be due to magnesium uptake by red blood cells, muscle cells, or to losses in sweat (31).

Sixteen well-trained male long-distance cross-country skiers participated in 70 and 90 kilometer races. Serum magnesium concentration decreased 8 and 11%, respectively; red blood cell magnesium content increased 4 and 6%, respectively; there was no change in whole blood magnesium content (30). No explanation for the increase in red blood cell magnesium content was offered. The serum magnesium of eight participants in the Boston marathon, a 26.2 mile race, was reported to drop an average of 21%, which was attributed to sweat losses (27).

Beller et al. (32) produced serial changes in serum magnesium concentration in males during treadmill exercise. Nine subjects exercised for 45 and 90 minutes at a mean increase in heart rate of 74 beats per minute. There was a 1% decrease in serum magnesium concentration following 45 minutes of exercise and a decrease of 4% following 90 minutes of exercise, both of which were statistically
Muscle electrolytes, measured by needle biopsy, have also been under study in response to physical exertion. Eight men performing exercise on a bicycle ergometer at 70% maximal oxygen consumption followed by exercise in an environmental chamber to obtain a desired weight loss produced significant decreases in magnesium from muscle tissue (lateral aspect of the thigh) at various levels of dehydration (2.2%, 4.1%, and 5.8%). Plasma magnesium concentrations also decreased 14.6, 18.7, and 18.2 mg/dl following dehydrations of 2.2% 4.1%, and 5.8%, respectively (25).

Potential Significance of Changes in Magnesium Status Following Exercise to the Etiology of Sports Anemia

Several observations suggest that changes in magnesium status following moderate to severe exercise may be an important factor to explain the development of sports anemia.

Magnesium is the second major cation within cells, exceeded only by potassium (19). It is an important activator for many enzyme systems that are involved in the transfer and release of phosphate groups (alkaline phosphatases, pyrophosphates, acid phosphatase, and ATPase) (33). ATP is required for impulse generation, muscle contraction, membrane transport, oxidative phosphorylation, amino acid activation, acetate and succinate activation, and synthesis of nucleic acids, coenzymes, fat, and proteins (33). Magnesium is involved in all reactions in which ATP participates and may serve as substrate and activator of magnesium-dependent sodium, -potassium,
-ATPase (33). Magnesium is required by creatine kinase, pyruvate kinase, cyclic AMP-dependent protein kinases, as well as by adenylate cyclase which mediates a number of peptide hormones. Phosphoglucomutase requires magnesium, as do many more enzymes (33).

Concern over magnesium status arises due to dietary surveys and analysis of typical sample meals of children and adults which show that most Americans' magnesium intakes do not meet the National Research Council's recommended dietary allowance (NRC-RDA) (34). In a study of 37,785 individuals by home economists in the U.S. Department of Agriculture, magnesium was listed as a problem nutrient (intake < 70% of the 1980 NRC-RDA) for 41 to 62% of females over 11 years of age and 43 to 44% of males between the ages of 12 and 22 and over 74 years (35). Furthermore, there is evidence in human subjects undergoing growth and repair that high magnesium intakes result in the retention of large amounts of magnesium. This evidence suggests that the optimal magnesium intake of pregnant or lactating women, adolescents, athletes, and convalescents needs further study (34).

Magnesium deficiency resembles sports anemia with respect to hematological changes. Anemia resulting from magnesium deficiency has been characterized by decreases in the red cell size and life span, hemolysis, spherocytosis, decreased osmotic resistance and reticulocytosis in both man and animals (19,22,36). The mechanism of the anemia is unknown (19,37), but decreased erythrocyte survival time may be affected synergistically by decreases in both red cell magnesium concentration and plasma magnesium concentration. Of these two, the diminished concentration of magnesium in the plasma seems
to be more important in producing a decrease in the red cell life span (19).

Young male rats fed magnesium-deficient diets containing 40, 60, or 80 ppm magnesium had reticulocytosis, microspherocytosis, shortened cell survival, and anemia after seven weeks when compared to controls fed a diet containing 690 ppm magnesium. A decrease in plasma magnesium, red blood cell magnesium, and red blood cell ATP was also observed in rats fed the magnesium-deficient diets (37).

In addition to red blood cells, various other cell types may respond to magnesium deprivation through cell shape changes. Cultured ovarian granulosa cells deprived of calcium and magnesium exhibit rounding which is reversible after the addition of the ions (40).

A proposed sequence of events resulting in red blood cell hemolysis due to magnesium deficiency may involve either spherocytosis or a loss of membrane integrity (19). In magnesium deficient rats, the loss of membrane integrity has been associated with a developmental defect, and it is characterized by the presence of intramembraneous plaques. Spherocytosis results when the red blood cell becomes less elastic (19). Magnesium-activated ATPases are responsible for the active transport of calcium, sodium, and potassium across membranes (19,30). Of the three ATPases in human red blood cells identified by White and Ralston, one has an absolute requirement for magnesium (19,31). It is, therefore, possible that in a magnesium-deficient environment, the energy production in the red blood cell is impaired and the active transport of the ions across
the membrane is disrupted. This would result in an increased concentration of sodium and a decreased concentration of potassium in the red blood cell leading to spherocytosis and eventually to hemolysis (19). Although reports concerning the effects of magnesium deficiency on plasma concentrations of sodium and calcium are conflicting (42), hypocalcemia and hypokalemia are frequently found in magnesium deficiency (37).

Hemostasis of magnesium is poorly understood, but it seems to rely on urinary excretion since it is linearly related to serum magnesium concentration (33, 50, 51). Maintenance of serum magnesium appears to be dependent upon regular ingestion of the ion, regulated excretion by the kidney, and coarse control of intestinal absorption related to the level of dietary intake (33).

**Summary of Research Findings and Research Question**

In summary, the current literature neither defines nor explains the condition termed "sports anemia." Data which demonstrate a genuine anemia, defined as a sub-normal hemoglobin concentration (40), do not appear in the present literature. The current literature does, however, support examples of reductions in red blood cell count, hemoglobin concentration, and hematocrit, and an increase in the osmotic fragility of erythrocytes, with some hemolysis, following physical exertion. One goal of the present study is, therefore, directed towards clarification of the mechanism of decreased osmotic resistance.

Various changes in serum electrolytes occur following exercise.
These changes are compounded by hemoconcentration, but serum sodium, chloride, and potassium concentrations generally rise, whereas serum magnesium concentrations fall. Evidence comparing the changes in total plasma or serum content of electrolytes is scarce, but there appears to be a decrease in sodium, potassium, and chloride. On the other hand, red cell magnesium concentration has been shown by some investigators to increase, but by others to decrease (31).

As shown in Figure 1 (modified from Elin, reference 19), the theory underlying the present study is that changes in blood electrolytes may be a clue towards the cause of the increased osmotic fragility observed in red cells of persons engaging in physical activity. Exercise leads to a loss of plasma volume and a concomitant increase in the serum concentrations of sodium, potassium, and chloride. In contrast, the concentration of magnesium in serum may fall which might lead to reduced magnesium-dependent ATPase and result in a reduced sodium, potassium pump activity. Spherocytosis, and eventually increased hemolysis, of red blood cells should result. Figure 1 does not indicate the theoretical changes in total serum electrolyte concentrations. Based on the present theory, total serum magnesium content will decrease and be followed by a decrease in total serum sodium content and an increase in total serum potassium content.
Figure 1
Hypothesized Flow of Events Resulting in Red Blood Cell Hemolysis Following Physical Exertion

Physical exertion

↓
Loss of plasma volume

Increased serum sodium and calcium concentration

Increased serum potassium concentration

Increased urinary magnesium excretion

Decreased serum and red cell magnesium concentration

Impaired magnesium-dependent ATPase activity

Decreased sodium-potassium pump activity

Increased serum potassium concentration and

Decreased serum sodium concentration

Spherocytosis

Increased red blood cell hemolysis
METHODS

This chapter covers the methods of data collection and analysis conducted in this project. Methods have been subdivided into five subsections: 1) subject selection and recruitment, 2) test setting, 3) sample collection, 4) laboratory analyses, and 5) data analysis.

The initial step in this study was to request permission to use human subjects. This application was submitted to, and granted by, the Oregon State University Committee for Protection of Human Subjects. The request included: 1) a description of methods and procedures to be used, 2) a list of the risks and/or benefits to the subjects, 3) a copy of the informed consent document, 4) a description of the method by which anonymity of the subjects would be maintained, and 5) a copy of the diet record form to be used.

An application for use of the Oregon State University Stress Physiology Laboratory and equipment was also submitted and granted. This document required that the exercise be performed in the presence of a person certified in cardio-pulmonary resuscitation.

The applications to the Oregon State University Committee for Protection of Human Subjects and to the OSU School of Health and Physical Education are included in Appendix A and B, respectively.

Subject Selection and Recruitment

It was statistically determined that between six and ten subjects would be required to produce data with statistical
significance \((p \leq 0.05)\). This calculation was based on the assumption that the between-subject variation which occurred in this project would be much lower than that observed by Rose et al. (27) in marathon runners and would be similar to that observed by Beller et al. (32) following 45 minutes of treadmill exercise. In an effort to reduce variance, the subjects were recruited on the basis of homogeneity.

The general subject criteria were:

1) Each subject had to be a male within the ages of 15 and 18 years in order that the subjects represent the group for which the NRC-RDA for magnesium is highest (41).

2) Each subject must have supplied information which assured their good physical health prior to the onset of data collection. This was met by the physical examination required for participation on the high school tennis team.

3) Each subject must have returned the consent form (Appendix C) signed by himself and his parent prior to the onset of data collection.

All of the selection criteria were met except for age. One subject was 14 years old but was accepted into the study based on his sincere interest. Table 1 describes the subject characteristics.

All eight subjects in this project were recruited from the Crescent Valley High School Tennis Team in Corvallis, Oregon. With the cooperation of the team's coach, the entire team met to learn about the prospective experiment. Eight of the team members volunteered to participate. They were strongly encouraged to fulfill
Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean</th>
<th>Standard Deviation</th>
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<td>1.3</td>
<td>14-18</td>
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<tr>
<td>Height (cm)</td>
<td>181.7</td>
<td>11.9</td>
<td>169.6-205.7</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
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<td>6.7</td>
<td>53.0-73.5</td>
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<tr>
<td>post-exercise</td>
<td>65.1</td>
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<td>1.1</td>
<td>0.5</td>
<td>0.7-1.7</td>
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<td>148</td>
<td>2</td>
<td>145-152</td>
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</table>
their commitment to this study by their coach. Each subject performed all of the tasks required by this study.

Test Setting

All data was collected in Langton Hall, Exercise Physiology Laboratory, Oregon State University. The sample collections were made on two consecutive days due to personnel and equipment limitations. Five subjects appeared on one day and three on the next. Each subject was teamed with a laboratory technician who was experienced with the set-up and operation of electrocardiography (ECG) equipment.

The data collection required approximately 2.5 hours time for each subject and ECG technician per day. All samples were collected between 7:00 a.m. and 10:30 a.m. on the two days to avoid diurnal variation.

Sample Collection

Heart Rate Determinations

Heart rate generally increases linearly with oxygen consumption and workload (8). This linear relationship allows one to use heart rate to estimate how strenuous an activity or exercise is. The lifting of a specific weight represents an absolute workload. Absolute work is dependent on strength and endurance (8). Since it was desirable to expose each subject in this study to equivalent workloads even though they may not have identical strength or
endurance levels, the principle of relative workloads was utilized. Sixty percent of the maximal heart rate was selected as the workload to be maintained during exercise in this study. This heart rate is slightly higher than the lower limit suggested for aerobic training among the age group selected (42).

During the week prior to data collection, the subjects kept a record of their resting heart rates. The subjects were instructed to gently feel the pulse in their neck for six seconds at three times during the day on two separate days when relaxed and write it down. The mean of these six values was used to determine the resting heart rate for each subject. This resting heart rate was used in the Karvonen formula (43) for determination of the heart rate equivalent to exercise at 60% maximal heart rate, as shown below:

\[
\text{\{ (220 - age) - resting heart rate\} X 0.60 + resting heart rate}
\]

Three-Day Diet Records

The diet record forms and instructions for recording the diet were distributed to the subjects two weeks prior to the date of data collection. Each subject was instructed to keep a record of his diet on three separate days. These three days were specified to include a non-school day, such as Saturday or Sunday, and the two school days preceding the day of laboratory data collection. These diet records were intended to be a tool in explaining individual deviations in urinary or blood magnesium values and for comparison of the subjects with the normal population.
Each subject was contacted at least one time over the diet record period to answer any questions and remind the subjects of the importance of recording their diet and resting heart rates as accurately as possible. The diet records were returned to the researcher at the laboratory prior to exercise. The diet records were examined by the researcher while the subjects performed the exercise. After each subject completed his exercise and was in the recovery phase, the diets were reviewed by the subject together with the researcher.

Overnight Fast and Exercise Attire

In order to work with a more homogeneous sample and limit sources of variability in subject response, all samples for biochemical analysis were collected from the subjects following a fast of at least ten hours. The subjects were instructed to urinate after rising in the morning, to avoid all strenuous activity during the ten hours prior to data collection, and drink 1.5 cups of water (as measured with a household measuring cup) approximately 45 minutes prior to arriving at the test setting. Each subject was instructed to dress in light shorts, socks, t-shirts, and tennis shoes. Each subject was contacted by telephone the evening of the fast to confirm the procedure to be followed for testing.

Weight and Urine Collection

After arriving at the Exercise Physiology Laboratory, the subjects produced a urine sample which was collected in four ounce sterile specimen cups (VWR Scientific, San Francisco, CA) with
emphasis on completely emptying their bladder. The subjects were then weighed on an Accu-Weigh scale (Model BD-400 PK, Metro Equip Corp., Sunnyvale, CA) and allowed to stretch as they liked.

Blood Collection

A certified medical technician drew blood from the right or left antecubital vein with a 20 gauge multiple sample needle (Becton-Dickinson, Rutherford, NJ) directly into seven ml Vacutainer tubes (Sherwood Medical Industries, St. Louis, MO). One blood sample was obtained in a silicone coated tube for collection of serum. A second blood sample was collected in a tube containing 10.5 mg tripotassium ethylenediamine tetraacetic acid (EDTA). EDTA was selected over heparin as the anticoagulant because it is recommended for general use and has the least overall effect on formed elements and retains hemoglobin, hematocrit, and red blood cells for 24 hours (44). Although heparin is recommended for the osmotic fragility test due to its low hemolytic effect on red blood cells, it is poor for Wright Stained blood films (44).

Bicycle Ergometers

A dividing wall was placed between each Monark bicycle ergometer (Model GCI, Monark-Crescent AB, Vargerg, Sweden) so that subjects could not see each other. The resistance setting on the ergometer was hidden from view of the subjects by placing tape over the scale. This precaution was taken to avoid the possibility of altering the subject's heart rate via perceived workload or competition. The
testing environment was maintained within the range 18 to 22°C (64 to 72°F) with relative humidity below 60% and in still air in accordance with a World Health Organization recommendation (45).

The subjects were instructed to begin pedaling on the bicycle at a rate of approximately 50 cycles per minute. The subjects cycled in this manner while the assisting technician gradually adjusted the resistance so that the subject reached his designated heart rate within two minutes.

Monitoring Heart Rates

Three disposable ECG pre-gelled silver/silver chloride electrodes (Model 177-005 Quinton Instrument Co., Seattle, WA) were attached to each subject with a Blackburn CM-5 Lead System in the Lead II position (46). The CM-5 Lead is regarded as a very reliable and popular system because it gives the greatest amplitude in QRS segments of an electrocardiogram (46). Heart rates were monitored with ECGs recorded with either a Sanborn, Model 51 (Sanborn Co., Waltham, MA), a Birtcher, Model 244 (Birtcher Corp., El Monte, CA), or a Parke-Davis, Model 2100 (Parke-Davis Pharmaceutical, Warner Lambert Co., Morris Plains, NJ), Electrocardiograph. The heart rate was checked and recorded at two to three minute intervals for 50 minutes following the two minute adjustment period.

There was some difficulty in maintaining contact with the electrodes due to sweat. This was satisfactorily remedied by having the subjects exercise without shirts and placing a thin elastic
bandage with spandex fiber around the subject's chest and blotting the sweat with paper towels intermittently.

Post-exercise and Recovery Procedures

A second blood sample was drawn immediately after the subjects had completed their 50-minute ride on the bicycle ergometer. The subject remained on the cycle and proceeded to undergo a five-minute cool-down by pedaling more slowly to recover a baseline heart rate.

Following the exercise period, the subjects relaxed in the laboratory for 55 minutes. At the end of this time, height and weight were recorded for each subject prior to the collection of a third blood sample and a second urine sample.

Sample Preparation and Storage Procedures

Each Vacutainer tube was marked with an identification number and code prior to sample collection. After the blood was drawn, the Vacutainers and urine samples were refrigerated at 4°C until the researcher returned to the nutrition laboratory with the samples.

In the laboratory, the blood to be used for serum samples was set aside to clot for about four hours, and the urine samples frozen at approximately -15°C. Since the osmotic fragility of erythrocytes must be determined within six hours of blood collection (36), whole blood was immediately tested for osmotic fragility. When the blood in the siliconized Vacutainers had completely clotted, the samples were centrifuged (International Equipment Co., Boston, MA, model
PR-2) for 15 minutes at 4°C and 2000 X g. Serum was removed with a disposable pasteur pipet and frozen at -15°C for future analysis of serum sodium, potassium, and magnesium concentrations.

**Laboratory Analyses**

The experimental procedures followed in this study were initially not familiar to the researcher and were practiced and revised prior to the onset of this project. The blood samples used during trial runs were donated by the Oregon State University Student Health Center.

All glassware used for analysis was soaked in 10% nitric acid for at least four hours, rinsed with redistilled-deionized water, dried and stored in polyethylene bags until used. All dilutions were made with redistilled-deionized water unless otherwise noted. Analyses were performed in duplicate. If the duplicate analysis did not agree, a third sample was analyzed. The mean of the duplicates was accepted as the true value.

**Determination of Red Blood Cell Osmotic Fragility**

The osmotic fragility test is based on the principle that spherocytes have a "tight" surface area with a decrease in the surface:volume ratio. The membrane is more permeable to sodium ions. Thus, in order to maintain osmotic stability, the cell must spend more energy pumping sodium back out. As sodium enters the cell, so does water. If the cell can not effectively pump sodium out, the cell swells. The opposing forces of a "tight" membrane
and water logging cause rupture of the cell (48).

The osmotic fragility test employs a series of sodium chloride solutions which are hypotonic (< 0.9% sodium chloride) and will cause cells to swell. The spherocytes, with their already reduced surface:volume ratio, lyse in concentrations of hypotonic saline solutions which fail to lyse normal cells (47).

The osmotic fragility of red blood cells was determined on pre- and post-exercise whole blood samples according to the method of Dacie and Lewis (36) and using hypotonic dilutions as modified by Miale (48). Blood was added to twelve hypotonic solutions ranging from 0.10% sodium chloride to 0.85% sodium chloride. The amount of red blood cell lysis was determined from the supernatant of each sample and compared to the 100% lysis sample (0.10% sodium chloride) via a Beckman Spectrophotometer (Model DU, National Technical Laboratories, South Pasadena, CA). The use of a Gilford Rapid Sampler (Model 2443-A, Gilford Instrument Laboratories, Inc., Oberlin, OH) greatly facilitated the 192 total dilutions required for the determination.

Determination of Red Blood Cell Count, Hemoglobin, Hematocrit, and Mean Corpuscular Volume

The red cell indices were assessed with a Coulter Counter (Model AB1, Coulter Diagnostics, Inc., Hialeah, FL) and Coulter MVC/HCT/RBC computer, Model MHR. The Coulter Counter draws 0.5 ml blood diluted with ISOTON II (Coulter Diagnostics, Inc.) through its aperture and produces a pulse for each red blood cell. The
amplitude of each pulse is proportional to the volumetric size of the cell it represents. When the flow is completed, the computer component of the instrument determines the hematocrit by multiplying the corrected red cell count and mean corpuscular volume.

Hemoglobin was determined with a Coulter Hemoglobinometer (Coulter Diagnostics, Inc.). Normal and abnormal controls (Dade, CH-60, Miami, FL) were used for instrument calibration.

**Determination of Spherocytes**

Smears from whole blood collected at pre-exercise, post-exercise, and recovery were examined for the presence of spherocytes under the light transmission microscope (Zeiss, NY, NY) and under the scanning electron microscope.

Samples for scanning electron microscopy were prepared as air-dried blood smears on 1 by 3 inch glass microscope slides. The slides were mounted on aluminum scanning electron microscope plancets using DuPont Model Cement. After the glue dried, the samples were placed in a VARIAN VE-10 Vacuum Evaporator (Varian Vacuum Division, Palo Alto, CA), brought to a vacuum of approximately 1 x 10^-5 Torr and coated with approximately 100 Å of 60:40 gold/palladium by the vacuum evaporation of this alloy while the sample was rotated and tilted with respect to the evaporating metal source.

All specimens were examined in an AMRAY 100-A Scanning Electron Microscope (Amray, Inc., Bedford, MS) at 30° tilt, 20 kv accelerating
voltage, secondary electron mode. Images were recorded on Polaroid Type 55 positive negative film at a magnification of 700X. The scanning electron microscopy was performed by Al Soeldner, Director of the Electron Microscope Facility at Oregon State University.

**Determination of Serum Calcium and the Magnesium Content of Serum, Whole Blood, and Urine**

Serum calcium and magnesium concentrations were determined using atomic absorption spectrophotometry (Perkin-Elmer 403, Norwalk, CT). The samples and standard were prepared with 0.1% Lanthanum diluent to control absorbance interference by phosphorus. The samples were diluted 1:50 and analyzed according to standard conditions described by Perkin-Elmer (49).

Whole blood magnesium concentration was determined with atomic absorption spectrophotometry as described by Refsum et al. (30). The whole blood was hemolyzed by freezing in an Ultra Low (Revco Inc., West Columbia, SC) freezer at $-60^\circ C$ followed by thawing three times. The hemolyzed whole blood was diluted 1:100 and analyzed according to standard conditions (49).

Urinary magnesium concentration was determined as a fraction of urinary creatinine (mg magnesium per mg creatinine). Urinary creatinine was determined according to the method of Folin and Wu (52). Urine was diluted either 1:250 or 1:50 and the concentration was determined with the Beckman Spectrophotometer previously described.
Urinary magnesium was determined using an atomic absorption spectrophotometer (Perkin Elmer 403, Norwalk, CT). The samples were diluted either 1:250 or 1:500, depending on the concentration of magnesium present in the urine. The samples were analyzed according to standard conditions (49).

Determination of Serum Sodium and Potassium

Serum sodium and potassium concentrations were determined using a flame photometer (Perkin-Elmer Coleman, Model 51-Ca, Maywood, IL). The samples were diluted 1:100 and analyzed under standard conditions as described by Coleman (53).

Data Analysis

Magnesium intake was estimated from the three-day diet records using Nutrient Analysis, which is part of the Ohio State University data base.

Calculations

The actual loss or gain of serum electrolytes following exercise was calculated from equations derived by vanBeaumont et al. (28). This calculation enabled the researcher to determine whether the changes in serum concentration were simply a result of hemoconcentration.
1) The proportional changes in plasma volume (ΔPV) were calculated from the hematocrit (Hct) as follows:

\[
\% \Delta PV = \frac{100}{100 - \text{Hct}} \times \frac{100(\text{Hct pre} - \text{Hct post})}{\text{Hct post}}
\]

where Hct pre = hematocrit from pre-exercise and Hct post = hematocrit from post-exercise or recovery.

2) The expected concentration (Ec) of the plasma electrolyte, correcting for the change in plasma volume, was calculated as follows:

\[
\text{Ec} = \frac{\text{Hct post} (100 - \text{Hct pre}) \times C}{\text{Hct pre} (100 - \text{Hct post})}
\]

where C = pre-exercise serum concentration of the electrolyte.

3) The change in total serum electrolyte content is the difference between Ec and the measured concentration (Mc):

\[
\Delta C = \text{Ec} - \text{Mc}
\]

The percent change in total serum electrolyte content can be calculated from:

\[
\% \Delta C = \frac{\text{Mc} - \text{Ec}}{\text{Ec}} \times 100
\]

The magnesium content of red blood cells (RBC Mg) was calculated from whole blood magnesium (WB Mg) and serum concentrations as described by Archer et al. (54). The calculation was as follows:

\[
\text{RBC Mg} = \frac{\text{WB Mg} - \text{Serum Mg} (1 - \text{Hct})}{\text{Hct}}
\]

Statistical Analysis

The changes from pre-exercise (control) to post-exercise or recovery were considered significant at p ≤ 0.05 and were determined
using a one-tailed, paired student t-test. Multiple correlation was applied to examine joint relationship, and a scatter diagram and simple regression evaluated in those relationships for which the absolute value of the Pearson product moment correlation coefficient (r) was larger than 0.600. The statistical tests were made using Minitab, a statistical program at Arizona State University, copyrighted by Penn State University, University of Toledo version PDP-11.

Three multiple correlations were performed: 1) the change in osmotic resistance, measured as percent hemolysis, in 0.45% sodium chloride solution between pre-exercise and post-exercise samples; 2) the percent change in total serum magnesium content at pre-exercise and post-exercise to urinary magnesium excretion and dietary magnesium; and 3) absolute and percent changes in the concentrations of the electrolytes measured at both pre-exercise and recovery.
RESULTS

A list of normal physiologic values are provided in Appendix C for comparison to the biochemical results obtained from this study.

Experimental Observations

At the onset of exercise, most subjects felt as though they would be unable to complete the 50-minute exercise period. However, after approximately 20 minutes had passed, the subjects seemed to be exercising without excessive stress and believed they could complete the test.

Dietary Magnesium

The three-day diet records indicate that seven of the eight subjects consumed diets which contained approximately 250 to 390 mg of magnesium per day, which is less than the 1980 NRC-RDA of 400 mg/day (20). Three subjects had intakes of magnesium which were more than 30% below the NRC-RDA. One subject consumed considerably more magnesium (641 mg/day) than the other subjects. The mean intake for all subjects was 351 mg magnesium per day (SD = 129).

Heart Rate

Heart rates maintained during the 50-minute exercise period were divided into thirds (beginning, middle, and end) for comparison to the "target heart rate" (60% maximal heart rate) which was calculated for each subject and as a group. None of the subjects varied significantly from their target heart rate during any part of the
50-minute exercise period.

**Hematological Measurements**

Table 2 summarizes hematological changes. The red blood cell count, hematocrit, and hemoglobin concentration increased by significant margins ($p < 0.05$) from pre-exercise to post-exercise and returned to the pre-exercise level at recovery. Plasma volume, as derived from hematocrit values, decreased 2.6% at post-exercise ($p < 0.001$, SD = 1.6) and by a non-significant 0.92% at recovery (SD = 6.9). All eight subjects experienced the drop in plasma volume at post-exercise, ranging from 0.67 to 5.4%. At recovery, six subjects had plasma volumes lower than at pre-exercise (range = 0.22 to 11%), where the plasma volume in three subjects had begun to return toward the pre-exercise level and the plasma volume of two other subjects overshot the pre-exercise level by 4.5 and 12%. Mean corpuscular volume decreased, but not by a significant margin, during both post-exercise and recovery as compared to pre-exercise.

No significant differences were found in the spherocyte counts from blood samples collected pre-exercise versus those collected post-exercise. Red blood cells analyzed by scanning electron microscopy, however, showed a pronounced deviation from normal immediately after exercise in all subjects. As shown in Figure 2, red cells collected at pre-exercise (A) and recovery (C) had central depressions which are characteristic of normal biconcave red cells. Red cells collected at post-exercise (B) exhibited
Table 2

Influence of Exercise on Red Blood Cell (RBC), Mean Corpuscular Volume (MCV), Hemoglobin (Hb), and Hematocrit (Hct)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC x 10^6/ul</td>
<td>5.08^1</td>
<td>5.21^2</td>
<td>5.08</td>
</tr>
<tr>
<td></td>
<td>± 0.21</td>
<td>± 0.28</td>
<td>± 0.23</td>
</tr>
<tr>
<td>MCV (m^3)</td>
<td>85.3</td>
<td>84.7</td>
<td>84.9</td>
</tr>
<tr>
<td></td>
<td>± 5.2</td>
<td>± 4.5</td>
<td>± 4.3</td>
</tr>
<tr>
<td>Hb (g/100 ml)</td>
<td>16.1</td>
<td>16.4^2</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>± 1.1</td>
<td>± 1.1</td>
<td>± 1.0</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>45.8</td>
<td>47.0^2</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>± 3.3</td>
<td>± 3.4</td>
<td>± 2.4</td>
</tr>
</tbody>
</table>

^1 Mean ± Standard Deviation (n = 8)

^2 Significantly different from pre-exercise
Figure 2

The Influence of Exercise on Red Blood Cell Morphology at Pre-exercise (A), Post-exercise (B), and 55 Minutes Post-exercise (C). Magnification = 700 X, Micron bar = 10 u.
smooth surfaces which are typical of spherocytes. These deviations in red blood cell structure were not visible through the light transmission microscope. The example shown is representative of all eight subjects.

The percent hemolysis in 0.45, 0.50, 0.55, and 0.60% sodium chloride solutions at pre-exercise was compared to the percent hemolysis at post-exercise and was not found to be significantly different for the group as a whole. Nevertheless, the percent hemolysis tended to be higher at post-exercise in 0.45 and 0.50% sodium chloride solutions. Hemolysis increased from 80% at pre-exercise to 85% at post-exercise in 0.45% sodium chloride and increased from 20 to 36% in 0.50% sodium chloride.

**Electrolytes**

The results of analyses for magnesium, sodium, potassium, and calcium follow and are summarized in Table 3.

**Serum Magnesium**

Five of the subjects experienced decreases in serum magnesium concentration from pre-exercise to post-exercise of 0.02 to 0.12 mg/100 ml, while three subjects experienced increases of 0.03 to 0.21 mg/100 ml. Six subjects showed a decrease from pre-exercise to recovery ranging from 0.02 to 0.53 mg/100 ml, while two subjects, who had previously shown an increase, showed no difference. Exercise led to an insignificant decrease in serum magnesium concentration from pre-exercise to post-exercise for the group which became
Table 3
Influence of Exercise on Blood Electrolytes and Urinary Magnesium

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Measured in Laboratory</th>
<th>Value derived by calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exercise</td>
<td>Post-exercise</td>
</tr>
<tr>
<td>Serum magnesium mg/100 ml</td>
<td>1.93</td>
<td>1.92</td>
</tr>
<tr>
<td>Whole blood magnesium mg/100 ml</td>
<td>3.55</td>
<td>3.50</td>
</tr>
<tr>
<td>Red blood cell magnesium mg/100 ml</td>
<td>5.45</td>
<td>5.28</td>
</tr>
<tr>
<td>Urine magnesium mg/mg creatinine</td>
<td>1.40</td>
<td>ND</td>
</tr>
<tr>
<td>Serum sodium mEq/l</td>
<td>153</td>
<td>149</td>
</tr>
<tr>
<td>Serum potassium mEq/l</td>
<td>±5.89</td>
<td>±14.4</td>
</tr>
<tr>
<td>Serum calcium mg/100 ml</td>
<td>9.27</td>
<td>9.62</td>
</tr>
</tbody>
</table>

1 Mean ± Standard Deviation (n = 8).
2 Significantly different from measured pre-exercise value.
3 Not determined.
4 Significantly different from calculated post-exercise value.
5 Calculated from equations derived by vanBeaumont et al.(28).
significant at recovery.

Because of hemoconcentration, the calculated serum magnesium concentration was 4.93% higher than that actually measured at post-exercise. If no loss of magnesium had occurred, there should have been an increase in serum magnesium concentration from 1.93 mg/100 ml at pre-exercise to 2.03 mg/100 ml at post-exercise due to hemoconcentration alone. The difference found in this study between the measured and expected changes in serum magnesium concentration signifies an actual 4.93% loss of magnesium from the serum. This loss of total serum magnesium occurred in seven subjects and ranged from 0.03 to 0.38 mg/100 ml.

The difference between measured and expected concentrations of serum magnesium at recovery is more pronounced than that at post-exercise, but is obscured by individual subject variation. Seven of the subjects experienced actual losses of magnesium from the serum at recovery which ranged from 0.01 to 1.34 mg/100 ml.

Whole Blood Magnesium

Six of the eight subjects experienced a drop in whole blood magnesium concentration of 0.02 to 0.08 mg/100 ml from pre-exercise to post-exercise, and two subjects showed increases of 0.08 and 0.15 mg/100 ml. The whole blood magnesium concentration increased from pre-exercise to recovery in three subjects by 0.01 to 0.22 mg/100 ml, dropped by 0.02 to 0.52 mg/100 ml in four subjects, and was not different in one subject. There were no significant changes in the group whole blood magnesium concentrations during either the
post-exercise or recovery phases of this study.

Red Blood Cell Magnesium

Six of the subjects experienced decreases in red blood cell magnesium concentrations at post-exercise from 0.04 to 0.76 mg/100 ml, and two subjects showed increases of 0.06 to 0.27 mg/100 ml. At recovery, the red blood cell magnesium concentration showed an overall rebound in five subjects, ranging from 0.01 to 0.64 mg/100 ml above pre-exercise concentrations. In three other subjects, recovery levels of red blood cell magnesium remained at 0.08 to 0.64 mg/100 ml below pre-exercise concentrations. For the group, there was a non-significant decrease in red blood cell magnesium concentration of 0.13 mg/100 ml at post-exercise and a non-significant increase of 0.14 mg/100 ml at recovery relative to pre-exercise values.

Urinary Magnesium

All of the eight subjects experienced a decrease in urinary magnesium excretion from pre-exercise to recovery. Individual decreases in magnesium concentration ranged from 0.03 to 1.00 mg magnesium per mg creatinine. As shown in Table 3, the group showed a significant ($p < 0.0001$) decrease in urinary magnesium from 1.40 (SD = 0.22) mg magnesium/mg creatinine at pre-exercise to 0.80 (SD = 0.22) mg magnesium/mg creatinine.

Serum Sodium

Exercise resulted in a group decrease of three mEq/L in serum
sodium concentration at post-exercise. This apparent drop in serum sodium was characterized by a large degree of individual variation. Four subjects experienced decreases of 1 to 33 mEq/L in serum sodium concentration at post-exercise, while four other subjects experienced increases of 1 to 10 mEq/L.

At recovery, the group showed an increase of 5.05 mEq/L from the pre-exercise concentration. Five subjects experienced increases in serum sodium concentration at recovery which ranged from 4 to 16 mEq/L. Three subjects showed decreases in serum sodium ranging from 2 to 6 mEq/L.

Comparison of the expected concentration of serum sodium to that measured at post-exercise reveals a significant (p < 0.01) decrease of 13 mEq/L. All eight subjects showed this loss of total serum sodium ranging from 3 to 38 mEq/L. There was not a significant change in total serum sodium at recovery for the group. However, four of the five subjects who showed an increase in serum sodium concentration also showed an increase in total serum sodium, ranging from 2.18 to 38.8%.

**Serum Potassium**

Serum potassium concentration was significantly higher than the pre-exercise level at both post-exercise (p < 0.01) and recovery. Post-exercise serum potassium concentrations were elevated over the pre-exercise concentrations by 0.29 to 0.59 mEq/L among all eight subjects. At recovery, seven subjects had serum potassium concentrations elevated over control levels by 0.02 to 0.48 mEq/L and one
subject experienced a decrease of 0.12 mEq/L. Seven of the eight subjects also had elevations in total serum potassium content of 0.02 to 0.59 mEq/L at post-exercise. At recovery, five of the subjects still had serum potassium concentrations higher than expected by 0.01 to 1.18 mEq/L. Increases in total serum potassium content, however, were not statistically significant for the group.

Serum Calcium

The group experienced a significant elevation in serum calcium concentration at post-exercise of 0.35 mg/100 ml. Six subjects showed increases of 0.07 to 1.31 mg/100 ml in serum calcium concentration; one subject experienced a drop of 0.29 mg/100 ml; and there was no measurable change for one subject. Six subjects, however, had a loss of total serum calcium content at post-exercise of 0.76 to 1.40 mg/100 ml; but the change was not significant.

At recovery, three of the subjects had serum calcium concentrations elevated over the pre-exercise level by 0.14 to 0.52 mg/100 ml. Serum calcium concentration dropped by 0.24 and 1.67 mg/100 ml in two subjects and returned to the control level in one subject. There was a loss of total serum calcium in four subjects of 0.65 to 3.44 mg/100 ml, an increase in two subjects by 0.10 to 1.46 mg/100 ml, and no change in two subjects.

Summary

Figure 3 summarizes the combined effects of exercise on electrolyte concentrations and total serum content of electrolytes
Figure 3

Percent Changes in Electrolyte Concentration, Total Electrolyte Content, and Plasma Volume at Pre-exercise (A), Post-exercise (B), and Recovery (C)
observed in this study. The decrease in the magnesium concentration
of serum, red blood cell and urine, decrease in sodium content, and
increase in serum potassium content support the hypothesis of this
study that magnesium is a key factor in the etiology of "sports
anemia".

Multiple Correlation and Regression

Decreases in serum magnesium concentration from pre-exercise
to recovery are positively related \( p \leq 0.05, r^2 = 58.5\%, \ SD = 0.18 \)
to decreases in urinary magnesium excretion. However, the clumping
together of data renders the relationship imprecise.

At post-exercise, the percent change in total serum magnesium
was related to the percent change in total serum calcium \( p \leq 0.01, r^2 = 86.3\%, \ SE = 2.82, y = 0.674x - 0.884 \). As shown in Figure 4,
magnesium and calcium were similarly related at recovery \( p \leq 0.01, r^2 = 93.6\%, \ SD = 4.123, y = 0.729x + 1.16 \). The percent change in
total serum magnesium content was also related to the percent change
in total serum potassium \( p \leq 0.01, r^2 = 85.8\%, \ SD = 7.73, y = 0.882 + 11.1 \) at recovery, as shown in Figure 5. These plots
illustrate positive relationships between actual changes in serum
content of magnesium versus calcium and potassium among individuals
which are not obscured by hemoconcentration.
Figure 4

Relationship Between Percent Change in Total Serum Magnesium Content and Percent Change in Total Serum Calcium Content From Pre-Exercise to Recovery
Figure 5

Relationship Between Percent Change in Total Serum Magnesium Content and Percent Change in Total Serum Potassium Content From Pre-exercise to Recovery
DISCUSSION

The results of this study support the hypothesis that the development of the condition called "sports anemia" can be explained by a transient impairment of magnesium status, as summarized in Figure 1. Reductions in magnesium status at post-exercise, as evidenced by serum and red cell magnesium concentrations, were associated with reduced serum sodium concentrations, elevated serum potassium concentrations, spherocytosis, and increased osmotic fragility, which are consistent with a probable impairment in red cell magnesium-dependent ATPase activity. Following a one-hour recovery, red blood cell magnesium, serum potassium, and serum sodium concentrations were returning to pre-exercise levels, as did red blood cell shape. A reduction in magnesium status during moderate exercise, therefore, appears to be a key factor in explaining the etiology of sports anemia, at least in athletically untrained, teenage boys.

The destiny of the magnesium lost from the serum and red cell is unknown. Based on studies involving intravenous injections of sodium, calcium, or lactate, it was postulated that increases in serum sodium and calcium concentrations would result in an increase in urinary magnesium excretion (19, 37). Serum calcium concentrations did rise slightly, but urinary magnesium decreased. The decrease in urinary magnesium excretion is explained by the positive correlation between urinary excretion of magnesium to serum magnesium concentration, which supports the current knowledge of the
homeostatic mechanism of magnesium. Previous research (25) suggests that the magnesium is not taken up by the exercising muscle, but the parameters measured in this study do not provide a basis for one to speculate on whether there was a significant loss of total body magnesium through sweat or simply a migration of magnesium to highly active tissue.

Results of the three-day diet records suggest that there is not an abundant amount of dietary magnesium to replace magnesium lost during moderate exercise in teenage boys, since seven of the eight subjects in this study did not have an optimal dietary intake of magnesium. The results of the three-day diet records support the evidence presented by Pao and Mickle (35) which indicate that meeting the NRC-RDA for magnesium is a problem, at least for males between 14 and 18 years of age, and that the dietary magnesium intake of the subjects in this study is representative of typical male adolescents in the United States.

Unlike previous research (12, 13, 18, 25), this study did not produce sustained decreases in red blood cell count, hematocrit, or hemoglobin concentration. However, a direct comparison of the results from other researchers is not necessarily valid due to differences in experimental design, including intensity and duration of exercise, and, most importantly, the frequent use of a post-exercise only design. Although red blood cell count, hematocrit, and hemoglobin concentration became elevated with exercise, those parameters rapidly returned to pre-exercise levels at 55 minutes post-exercise.
which suggests that these changes were due to hemoconcentration. Thus, the use of changes in hematocrit to calculate the percent changes in plasma volume was justified.

The tendency towards a decrease in mean corpuscular volume is not explained by the results of this study. A drop in mean corpuscular volume has previously been attributed to increased plasma osmolality when a change in the hematocrit-to-hemoglobin ratio occurs (26). However, no change in the hematocrit-to-hemoglobin ratio was present in this study. Radomski et al. (13) noted a preferential destruction of large red blood cells following repeated prolonged exercise which lowered the mean corpuscular volume, but the conditions present in this study do not support such an explanation. The slight drop in mean corpuscular volume observed in this study appears to be inconsequential.

The entire group of subjects in this study exhibited spherocytosis similar to that found by Oken et al. (22) in rats fed magnesium-deficient diets. However, the normal biconcave shape of the red cells returned to normal after one hour of recovery from the exercise, as did red cell magnesium concentrations. The change in red cell shape cannot be attributed to increased plasma osmolality because spherocytes retain their volume and have reduced diameters due to their increased thickness (26). Furthermore, all eight subjects exhibited the spherocytosis, and all cells returned to normal at recovery, even though plasma volume continued to drop in three of the subjects.
Spherocytes are known to have a lowered osmotic resistance in hypotonic solutions (26). A decrease in osmotic resistance at post-exercise could not be verified by the present study due to variability of subject response. However, the substantial increase in percent hemolysis in 0.45 and 0.50% sodium chloride solutions cannot be overlooked when coupled with the evidence of spherocytosis from scanning electron microscopy.

A significant decrease in serum sodium concentration at post-exercise was observed in this study. This finding contradicts previous reports (27, 29, 30) of increased serum sodium concentration following moderate to heavy exercise and supports the work of Costill et al. (25). Costill et al. (25) observed plasma sodium concentration to drop 1.9% thirty minutes after exercise on a bicycle in an environmental chamber to a weight loss of 2.2%. The mean weight loss in this study was 1.1% and the mean drop in serum sodium concentration was 2.5%. However, direct comparison of the results from this study to the work of Costill et al. is not logical due to differences in experimental design; Costill et al. standardized their study on response to physical exertion (dehydration) rather than on relative workload (percent maximal heart rate).

A reduction in total serum sodium content was seen in this research, which is in agreement with the results of van Beaumont et al. (28) following maximal exercise on a bicycle ergometer. Again, direct comparison of the magnitude of change observed in the present
study to that of van Beaumont et al. is hindered by the differences in exercise intensity and duration.

The dramatic increase in serum potassium concentration at post-exercise agrees with previous studies (25, 27, 30, 37) and supports the proposal of potassium loss from the red blood cell. The maintenance of stability in total plasma or serum potassium content was observed in this study and by Costill et al. (25). The postulated explanation is that the large concentration of potassium in interstitial fluids provides a readily available source of potassium exchange (25).

In contrast to previous reports (27, 30), the increase in serum calcium concentration following exercise was significant. At recovery, a loss in total serum calcium content occurred; but due to individual variations, the mean value failed to reach significance. The losses in total serum calcium were significantly correlated with the losses in total serum magnesium between subjects. The meaning of this relationship is unknown. However, it is known that severe magnesium deficiency may result in hypocalcemia in humans (37). Although the cause of hypocalcemia in magnesium deficiency remains a question, magnesium deficiency does impair parathyroid hormone secretion and action. Since the stimulation of parathyroid hormone secretion is contingent upon action by magnesium-dependent adenylate cyclase, parathyroid hormone action is probably disrupted by an impaired adenylate cyclase-cyclic AMP system in the human parathyroid gland (37). Reduced adenylate cyclase activity may also cause
skeletal resistance to parathyroid hormone (37). It seems logical to suggest that the low serum magnesium levels observed in this study may have affected parathyroid hormone activity within the hour following exercise, resulting in the low serum calcium concentration observed at recovery.

As with the results of Refsum et al. (30), whole blood magnesium concentration remains essentially unchanged following exercise. The apparent stability of whole blood magnesium may be explained due to the relative concentrations of magnesium in the serum and red blood cell and the loss of plasma volume which typically accompanies physical exercise.

However, this research clearly illustrates a loss of magnesium from the blood (red blood cell and serum) and conservation of magnesium by the kidneys. The loss of magnesium from serum was most dramatic at recovery, whereas red blood cell magnesium concentrations showed a slight rebound over pre-exercise levels. Previous research has indicated that short-term exercise produces increases in serum magnesium concentration; whereas, moderate to long-term (over 40 minutes) exercise causes reductions in serum magnesium concentration (31). However, to the author's knowledge, gain or loss of total serum magnesium content based on the change in plasma volume has not been previously assessed. Thus, this research provides valuable information regarding the actual magnesium status of serum which is not obscured by hemodilution. In addition, the positive correla-
tions observed between the loss of total serum magnesium and total serum calcium and potassium losses supports previous work indicating the maintenance of a steady ratio between the concentrations of magnesium and calcium, and between magnesium and potassium in the plasma (33). The pre-existing theory is that hypocalcemia and hypokalemia result from severe magnesium deficiency (37). However, the evidence presented here suggests that the relationship between the severity of magnesium depletion and potassium or calcium status should be re-evaluated. It appears as if magnesium loss from the plasma during exercise also leads to loss of potassium and calcium.

Red blood cell magnesium concentrations are not generally considered to be a useful tool in assessing magnesium status following a brief period of magnesium deprivation (37, 47). This is, at least in part, probably due to the current controversy over the permeability of the red blood cell to magnesium (19). Since magnesium is primarily an intracellular ion which is involved in the maintenance of red blood cell structure and function (19, 37) and the concentration of magnesium in red blood cells has previously been altered following physical exercise (30), it was measured in this study.

The decrease in red blood cell magnesium concentration at post-exercise observed in this research, coupled with the decreases in serum magnesium concentration and total serum magnesium content, refute the assumption that serum magnesium lost during exercise is taken up by red blood cells (31) and indicates the presence of a
rapid exchange of magnesium between erythrocytes and plasma. Previously, exchange of magnesium between plasma and red blood cells was thought to occur only after prolonged magnesium depletion (37). The return of red cell magnesium, but not serum magnesium, to control levels at recovery cannot be explained except to suggest that magnesium returns to the metabolically active red cell faster than to the serum.

The results from this study suggest that fifty minutes of heavy physical exertion result in sub-optimal levels of magnesium in serum and red blood cells, resulting in temporary spherocytosis and increased osmotic fragility. Although the frequency and duration of physical exertion required to induce electrolytic imbalance in the serum and red blood cell were not evaluated, it seems logical to assume that the availability of magnesium to the red blood cell is directly related to these aspects of exercise. Thus, the importance of this study to individuals intensifies with respect to the person's devotion to physical fitness. If training is carried out very intensively, spherocytosis and decreased osmotic resistance of red blood cells may become pronounced and result in hemolysis.

This research was not directed toward the relationship of various levels of dietary magnesium intake on changes in serum electrolytes and red cell morphology. Thus, it does not provide evidence that increased magnesium intake is effective in evading suboptimal magnesium status due to physical exertion. However, the
diet records from this study and the work of Pao and Mickle (35) do provide grounds which enable the researcher to suggest that dietary intakes of magnesium for American males between the ages of 14 and 19 years may need improvement.
SUMMARY

By definition, the evidence supporting the condition termed "sports anemia" does not represent a genuine anemia. Rather, "sports anemia" appears to be a state of altered serum electrolytes capable of provoking red blood cell spherocytosis and hemolysis through impairment of magnesium-dependent sodium-potassium ATPase.

This research clearly shows that fifty minutes of heavy physical exercise results in a transient spherocytosis and increased erythrocyte fragility. Reduced serum and red blood cell magnesium concentrations, decreased serum sodium concentrations and total serum sodium content, and increased serum potassium concentration support the theory underlying this study (refer to Figure 1) that "sports anemia" results from impaired magnesium status during physical exertion, and a subsequent derangement in serum sodium and potassium reduces red blood cell osmotic resistance via spherocytosis.

Spherocytosis following physical exertion has not previously been associated with impaired magnesium status. In addition, alterations in calcium and potassium, which accompany exercise, have not been previously related to concomitant changes in magnesium status.

The following recommendations are made for future research. The wide range of normal values and between-subject variability were obstacles in evaluating the results of this research. The variance in plasma volume changes may, in part, be the source of
this problem. For purposes of future research, standardization of subject response to absolute stress may be effective in reducing variance, rather than standardizing the exercise itself. For example, utilization of a range of reductions in plasma volume, as estimated from weight loss (dehydration), may be preferred to exercise at a designated maximal heart rate or maximal oxygen consumption.

An estimation of total body loss of magnesium following exercise would allow one to determine whether the requirement for magnesium is increased during physical exertion. This could be accomplished by washing the subjects and their clothing before and after exercise to collect sweat and desquamated skin, followed by analysis of the wash for magnesium content. In addition, urine collection should be extended for several days before and after exercise. Dietary magnesium could also be controlled at various levels of intake to explain fluctuations in urinary magnesium loss and provide insight towards the effectiveness of dietary alteration on avoiding or replacing magnesium loss. The possibility of magnesium migration could also be investigated by employing muscle biopsy. Of course the above mentioned techniques would require substantial financial support and technical assistance.

A stronger conclusion would be facilitated if changes in serum and red cell magnesium status were correlated to red blood cell sodium and potassium concentrations, and red blood cell ATPase activity. The preparation of microscope slides for scanning electron
microscopy should be modified from the technique used here to include washing-out excess plasma and, thus, avoid clumping of red blood cells. In addition, a more complete picture of the changes occurring within each subject could be provided by increasing the number of blood samples. The collection of three or four samples during a one and one-half to two-hour period following exercise would provide insight as to variance in subject response to and recovery from exercise.
REFERENCES


APPENDICES
APPENDIX A

Application to the Oregon State University
Committee for Protection of Human Subjects

OREGON STATE UNIVERSITY
APPLICATION FOR APPROVAL OF THE HUMAN SUBJECTS BOARD

Principal Investigator* Florian L. Cerklewski
Department Foods and Nutrition Phone 754-3561
Project Title Effects of Exercise on Magnesium and its Relationship to Red Blood Cell Fragility in Teenage Boys Present or Proposed Source of Funding Dept. of Foods and Nutrition (30-050-1113)

Type of Project ______Faculty Research Project
XX Graduate Student Thesis Project* (Student's name Christina S. Reiter)

The following information should be attached to this form. All material, including this cover sheet, should be submitted IN DUPLICATE to the Office of the Dean of Research, AdS A312. Feel free to call extension 3437 if you have questions.

1. A brief description of the methods and procedures to be used during this research project.

2. A list of the risks and/or benefits (if any) to the subjects involved in this research.

3. A copy of the informed consent document and a description of the methods by which informed consent will be obtained. (Information concerning the "Basic Elements of Informed Consent" is reproduced for your information on the back of this form.)

4. A description of the method by which anonymity of the subjects will be maintained.

5. A copy of any questionnaire, survey, testing instrument, etc. (if any) to be used in this project.

6. If this is part of a proposal to an outside funding agency, attach a copy of the proposal.

Signed Florian L. Cerklewski Date 3/6/82
Principal Investigator

*Note: Graduate Student Thesis projects should be submitted by the major professor as Principal Investigator.

R-5-79 mep
The informed consent of subjects will be obtained by methods that are adequate and appropriate. Informed consent is the agreement obtained from a subject, or from his authorized representative, to the subject's participation in an activity.

The basic elements of informed consent are:

1. A fair explanation of the procedures to be followed, including an identification of those which are experimental;
2. A description of the attendant discomforts and risks;
3. A description of the benefits to be expected;
4. A disclosure of appropriate alternative procedures that would be advantageous for the subject;
5. An offer to answer any inquiries concerning the procedures;
6. An instruction that the subject is free to withdraw his consent and to discontinue participation in the project or activity at any time.
7. With respect to biomedical or behavioral research which may result in physical injury, an explanation as to whether compensation and medical treatment is available if physical injury occurs and, if so, what it consists of or where further information may be obtained.

In addition, the agreement, written or oral, entered into by the subject, should include no exculpatory language through which the subject is made to waive, or to appear to waive, any of his legal rights, or to release the institution or its agents from liability for negligence.
I. Brief description of the methods and procedures:

This project is intended to test the effects of exercise of moderate duration on magnesium status in serum, red blood cells and urine. Specifically, the objective is to relate magnesium status to red blood cell fragility and the occurrence of anemia among athletes. Between 6 and 10 male volunteers between the ages of 15 and 18 years will be needed, as determined through the work of Rose et al. and Beller et al. A sample size of 6 should yield results which occur 70% of the time and are significant at p<0.05 via a 1-tailed T test with a mean difference of 0.24 mEq/L magnesium in serum. Nine subjects should provide results which are significant 80% of the time. Human subjects are best for this study because of the volume of blood required and the elimination of drawing inferences from animals to humans.

The subjects will be contacted through a local high school coach, probably the Crescent Valley tennis team. They will all be considered to be in good physical health, as indicated by the physical required by the school for participation in sports. In addition, the subjects and their parent or legal guardian will return the enclosed consent form.

The test setting will be in Langton Hall, room 134, Oregon State University. Three or four persons will be tested at one time in the presence of a person certified in CPR. Each subject will have completed a three day diet record to provide a general idea as to the dietary intake. The subjects will arrive at the test site in at least a 10 hour fasted state, having drank exactly 1 1/2 cups (12 oz.) water approximately 1 hour earlier.

Each subject will serve as their own control. Immediately prior to exercise each subject will provide a urine sample, measure of body temperature from under the arm, body weight, and a 15 ml blood sample. The blood sample will be drawn by a medical technologist from the Foods and Nutrition department using sterile disposable needles and vacutainer tubes. Each blood sample will be taken from the right or left anticubital vein. Ten ml of the blood will be used for whole blood analysis and 5 ml for serum analysis.
Test samples will be collected immediately following 50 minutes of exercise on a Monarch bicycle ergometer at 60% maximal heart rate, as calculated by the Karvonen formula\(^1\) which includes resting heart rate and age considerations. For example, a 17 year old with a resting heart rate of 75 beats per minute (bpm) would exercise at a heart rate of 151 bpm. This value is similar to that found in Beller's\(^1\) work and is slightly higher than the lower limit (140-147 bpm) suggested for aerobic training among this age group.\(^4\) The heart rate will be maintained at this level via EKG monitoring and appropriately adjusting the resistance of the ergometer. A second blood sample identical to the first will be drawn and body temperature recorded.

A third group of samples will be collected which are identical to the first, one hour following the exercise and will represent recovery levels.

The following analyses will be made: Hemoglobin; Hematocrit; Red blood cell count; Reticulocyte count; Spherocyte count; Serum calcium, potassium, magnesium, sodium; Osmotic resistance of red blood cells; Red blood cell magnesium; urinary magnesium; urinary creatinine.

II. List of risks and/or benefits to the subjects:

The subjects will benefit from this study through: A) the experience of EKG monitoring, B) the educational benefit of participation in a controlled study, C) the analysis of their dietary record, and D) the access to the test results.

III. See attached letter to parent and consent form.

IV. Anonymity will be maintained by assigning each subject a number which will be confidential and known only by the researcher.

V. See attached Diet Record form.
Appendix A (continued)

References


Appendix A (continued)

Dear Parent:

A nutrition-exercise project is being initiated by Christina Reiter, Masters Degree student in the Department of Foods and Nutrition of Oregon State University. The purpose of this study is to evaluate magnesium status as a result of exercise in teenage subjects. Teenage males between the ages of 15 and 18 years have the highest requirement for magnesium. Magnesium is important for energy production by the body and may be an important factor in the development of anemia among those frequently involved in strenuous physical activity. A study such as this will be valuable in providing further information on the dietary requirements for nutrients, particularly for persons in a state of rapid growth and engaging in athletics.

We are requesting your permission for your son to participate. The following procedure will be used:

1. Prior to the study each subject will supply information which provides assurance as to their good physical health. This will be met by the physical examination required for participation in athletics.

2. The subjects will arrive at the lab in a fasting state (10 hours), provide a urine sample, be weighed, temperature measured, and provide a 15 ml sample of venous blood.

3. Exercise on a bicycle ergometer for a total of 50 minutes, immediately followed by the drawing of a second blood sample and recording of body temperature.

4. One hour following exercise a third blood sample will be drawn, a urine sample collected, and the subject weighed.

The blood will be drawn by a registered and experienced medical technologist and the exercise performed in the presence of a person certified in CPR cardio-pulmonary resuscitation. The frequency and amount of blood drawn should not be a physical stress for a normally active person. If injuries are suffered as a result of this project through fault of the University, its officers, or its employees, they are covered by the State Liability Fund.

All information obtained will be kept confidential and the data from the study made available to each participant.

This study has been approved by the Oregon State University Committee on Human Subjects. If you have further questions please contact me. A consent form is enclosed for you and your son if you wish to participate. Thank you for your consideration.

Sincerely,

Christina S. Reiter
Department of Foods and Nutrition
Phone (days): 754-3561
(evenings): 754-6267
CONSENT FORM

I give my consent as the parent or legal guardian of ________________
______________________ for his participation in a nutritional-exercise
study. I further give my consent to allow 3 collections of blood
samples (15 ml each), 50 minutes of exercise, and 2 consecutive urine
samples. The blood will be drawn by a medical technologist using
sterile disposable needles and vacutainers. All of my questions
regarding this study have been satisfactorily answered. I understand
that I may withdraw consent from the project at any time.

Parent/Guardian_______________________________________________

Date_________________________________________________________

Witness_______________________________________________________

I give my consent to participate in this study. I agree to provide
the required blood, urine, and information involving my diet and
use of food supplements. The details of this study have been explained
and all of my questions regarding this study have been satisfactorily
answered. All information will be confidential. I understand that I
may withdraw from the study at any time.

Subject_______________________________________________________

Date_________________________________________________________
Appendix A (continued)

Instructions for Diet Record

1. Record each food and beverage you consume on a separate line. Be sure to include water, snacks, sauces, milk on cereal, sugar, etc.

2. Record the foods in reasonably exact amounts, for example:
   Liquids in-- cups, ounces, milliliters
   Vegetable or Fruits in-- cups or using the ruler on the record sheet.
   Bread in-- slices
   Meats in-- ounces or using the ruler.
   * when it is not practical to measure foods at certain meals, make a mental note of the portion size and measure a comparable food later.

3. Describe the food- fresh, canned, frozen, barbequed, baked, boiled, fried, or brand of food, non-fat, low-fat, wholewheat, etc.

4. Food Mixtures should be described by listing each ingredient (soups, salads, sandwiches, casseroles).

5. If possible specify if food is fortified.

6. Include any other information you may feel is helpful.
### DIET RECORD SHEET

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<thead>
<tr>
<th>FOOD</th>
<th>SOURCE</th>
<th>BRAND</th>
<th>PREPARATION</th>
<th>AMOUNT</th>
<th>FOR OFFICE USE</th>
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*LEAVE A BLANK SPACE BETWEEN EACH MEAL.*

*USE A SEPARATE SHEET FOR EACH DAY.*

*FOOD* specify each food or beverage on a separate line

*SOURCE* canned, dried, fresh etc.

*BRAND* be specific

*PREPARATION* fried, baked, raw etc.

*AMOUNT* measure in cups, inches etc.

*FOR OFFICE USE* AMT., WT., code code
OREGON STATE UNIVERSITY
Committee for Protection of Human Subjects

Chairman's Summary of Review

Title: Effects of Exercise on Magnesium and Its Relationship to Red Blood Cell Fragility in Teenage Boys

Program Director: Florian L. Cerklewsik, Foods and Nutrition

Recommendation: * Approval

Remarks: The informed consent forms obtained from each subject need to be retained for the long term. Archives Division of the OSU Department of Budgets and Personnel Service is willing to receive and archive these on microfilm. At present at least, this can be done without charge to the research project. Please have the forms retained in Archives as well as in your files.

Date: April 9, 1982

Signature

If the recommendation of the committee is for provisional approval or disapproval, the program director should resubmit the application with the necessary corrections within one month.
APPENDIX B

SCHOOL OF HEALTH & PHYSICAL EDUCATION
EXERCISE
REQUEST FOR USE OF PHYSIOLOGY LABORATORY
INSTRUMENTS &/OR PERSONNEL

Investigator: Florian L. Cerklewski
Department: Foods and Nutrition

Office: Telephone Ext.: 754-2131

Project Title: Effects of Exercise on Magnesium and Its Relationship to Red Blood Cell Fragility in Teenage Boys

Type of Project: ___ Faculty research
___ Instruction activity (Course number: )
___ Graduate thesis project (Student's name: Christina S. Reiter)
___ Other; specify exactly:

Equipment to be used or facility being requested:

1. 3-4 EKGs (Lead 3 and Recorder)
2. 3-4 bicycle ergometers
3. scale for body weight
4. 

Date(s) facility and equipment is desired:

<table>
<thead>
<tr>
<th>Hour</th>
<th>Date</th>
<th>Month</th>
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<tr>
<td>6:00am</td>
<td>19th</td>
<td>May</td>
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<tr>
<td>9:30am</td>
<td>19th</td>
<td>May</td>
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<tr>
<td>6:00am</td>
<td>21st</td>
<td>May</td>
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<tr>
<td>9:30am</td>
<td>21st</td>
<td>May</td>
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</table>

IF faculty research or Graduate thesis project is indicated, include copy of Proposal for Project Involving Human Subjects and/or copy of Human Subjects Committee approval. REQUEST WILL BE DENIED WITHOUT COMMITTEE APPROVAL.

IF instructional activity is indicated complete the following:

1. What is the specific nature of activity that is to be conducted? (Present BRIEF narrative that will be meaningful to naive reader)

2. How does this activity fulfill general and specific course objectives?

3. What are the RISKS to the subjects that will be participating in the laboratory activity?

4. What are the BENEFITS to the subjects that will be participating in the laboratory activity?

5. Will a CPR trained person be in attendance______ Who__________
APPENDIX C

List of Normal Physiologic Values (26, 30)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>Serum magnesium concentration</td>
<td>1.8 to 3.1 mg/100 ml</td>
</tr>
<tr>
<td>Red blood cell magnesium concentration</td>
<td>5.2 to 7.5 mg/100 ml</td>
</tr>
<tr>
<td>Serum sodium concentration</td>
<td>135 to 155 mEq/L</td>
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<tr>
<td>Serum calcium concentration</td>
<td>9.0 to 11.0 mg/100 ml</td>
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<tr>
<td>Serum potassium concentration</td>
<td>3.6 to 5.5 mEq/L</td>
</tr>
<tr>
<td>Hemoglobin concentration (males)</td>
<td>14.0 to 17.0 gm/100 ml</td>
</tr>
<tr>
<td>Hematocrit (males)</td>
<td>42 to 53%</td>
</tr>
<tr>
<td>Red blood cell count</td>
<td>4.6 to 6.2 million/ul</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>82 to 96 u³</td>
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</table>