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The Effect of Dietary Supplemented Vitamin E and Selenium on Experimental Chagas' Disease

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THE EFFECT OF DIETARY SUPPLEMENTED VITAMIN E AND SELENIUM ON EXPERIMENTAL CHAGAS' DISEASE

A Thesis
Presented to the Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Brenda J. Bennett
August, 1995
THE EFFECT OF DIETARY SUPPLEMENTED VITAMIN E AND SELENIUM
ON EXPERIMENTAL CHAGAS' DISEASE

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Dietary supplementation with increased levels of vitamin E can enhance the immune system of C3HeB/FeJ mice to enable many of them to survive an otherwise lethal dose of *Trypanosoma cruzi*, the causative agent of Chagas’ disease. To study the role of diet on the course of experimental Chagas’ disease, C3HeB/FeJ mice were fed pelleted, synthetic food containing no vitamin E and selenium, 800 IU/kg vitamin E, 2 ppm selenium, or food containing 800 IU/kg vitamin E plus 2 ppm selenium. Mice on synthetic food were compared to control mice fed a commonly used Purina rodent chow # 5001. After diet supplementation for a minimum of 14 days, mice were infected with $10^3$ blood-form trypomastigotes of *T. cruzi*. The mean peak parasitemia for all mice fed synthetic food was $2.56 \times 10^6$ parasites per ml of blood compared to $1.12 \times 10^7$ parasites per ml of blood for the infected control group fed Purina rodent chow # 5001. This difference in parasitemia levels represents a fourfold lower mean peak parasitemia for mice fed synthetic food as compared to the infected control mice. Parasitemias were not only lower for all the mice fed synthetic food but also the typical sharp rise in parasitemia during the acute phase was not observed. After the peak in parasitemia, on day 44, the surviving mice had a gradual decrease in parasite numbers until there were no blood-form
trypomastigotes seen in the peripheral circulation. Statistical analysis showed that there was a close correlation between the replicate cages of mice fed diets deficient in vitamin E and selenium and the mice fed diets containing 2 ppm selenium. There also was a close correlation between the mice fed diets containing 800 IU/kg vitamin E plus 2 ppm selenium and the mice fed diets containing 800 IU/kg vitamin E only. By day 70, the percent survival for mice receiving diets deficient in vitamin E and selenium was 37.5% (n=8) while the percent survival for mice receiving diets containing 2 ppm selenium was 44.5% (n=9). In contrast, mice receiving diets containing 800 IU/kg vitamin E plus 2 ppm selenium had a percent survival of 77.8% (n=9) while the mice receiving diets containing 800 IU/kg vitamin E had a percent survival of 80.0% (n=10). The infected control mice that were fed Purina rodent chow # 5001 exhibited 0% survival by day 46. Parasite specific antibody levels were measured by ELISA to determine any existing pattern that could be correlated to parasitemia and mortality. In all treatment cages of mice, antibody levels increased by day 35 post-infection (acute phase) and remained elevated through day 53 post-infection (chronic phase). Mice receiving synthetic diets containing 800 IU/kg vitamin E not only maintained the lowest mean peak parasitemias but also maintained the highest percent survival. These results suggested that increased dietary intake of vitamin E can have a pronounced effect on the course of experimental Chagas’ disease.
INTRODUCTION

Chagas’ Disease

Chagas’ disease or American Trypanosomiasis was first described in 1909 by Carlos Chagas and today the disease infects approximately 10-24 million people in South and Central America (Wendel and Gonzaga, 1993; Molyneux, 1993). The etiological agent of Chagas’ disease, Trypanosoma cruzi, is a eukaryotic, protozoan parasite that also can infect between 100-150 species of wild and domesticated mammals. Besides humans, natural reservoirs for T. cruzi are skunks, opossums, marmots, armadillos, sloths, bats, foxes, jaguars, ferrets, raccoons, rabbits, mice, hamsters, squirrels, monkeys and marmosets. It is not certain how many of these mammals act as reservoirs of human infection but the armadillo, which has a long lifespan, is important because it can harbor the T. cruzi infection for years (Molyneux, 1993).

Life Cycle of T. cruzi

The vectors are hematophagous bugs of the family Reduviidae, and these Reduvid bugs commonly infest huts and human dwellings in Central and South America. The three genera of the family Reduviidae that are important in the spread of the human disease are Panstrongylus, Triatoma and Rhodnius. The Reduvid bugs are commonly referred to as “kissing bugs” because they normally bite humans on the uncovered face while they are asleep. After the “kissing bug” punctures the skin and takes a blood meal, the infected bug defecates on the skin. The feces contain infective trypomastigotes which enter the
host by localized scratching or through mucous membranes near the feeding site. Once
the trypomastigotes invade the host, they enter various cells for which they have a
particular affinity for cells such as macrophages, muscles cells and nerve cells. The
trypomastigotes transform into the amastigote stage within the host cell; it is this form that
enables the parasite to multiply. The ultimate result of the multiplication of amastigotes is
lysis of the host cell and release of the blood-form trypomastigotes (BFT) into the
peripheral blood stream. The circulating trypomastigotes are now infective for any of the
31 species of the Reduvid bugs that can transmit Chagas' disease when a blood meal is
obtained from the vertebrate host. The blood-form trypomastigotes can also invade other
host cells and repeat the vertebrate host cycle (Bogitsh and Cheng, 1990; Wendel and
Gonzaga, 1993). Figure 1 shows the life cycle of *T. cruzi* in humans and in the insect
*Panstrongylus megistus* ("kissing bug").
Figure 1. Life cycle of *Trypanosoma cruzi* in humans and in the insect *Panstrongylus megistus* (Bogitsh and Cheng, 1990).
**Pathology of Chagas’ Disease**

The amastigote evades destruction within macrophages by escaping from the phagosome before lysosomal fusion occurs. Once the amastigote is safely in the cytoplasm of the infected macrophage, the parasite will multiply until the cell ruptures and releases the trypomastigote stages of the parasite. The nervous system, reproductive systems, intestines and bone marrow are sometimes invaded by the parasite (Bogitsh and Cheng, 1990; Molyneux, 1993).

In early stages of Chagas’ disease, the parasites are numerous in the infected tissues and the peripheral blood. As the infection commonly moves toward the chronic phase, the number of parasites in the circulation decreases drastically, until the parasites are no longer found in the peripheral circulation. In acute cases, about one to three weeks after infection, fever, headache and enlargement of the liver and spleen are examples of some of the physiological changes caused by the parasite (Bogitsh and Cheng, 1990). *T. cruzi* causes long term autoimmune reactions which can lead to the death of infected and uninfected host cells. These autoimmune reactions can lead to cardiac failure if the heart becomes infected with the parasite. Chagas’ disease is further complicated by the fact that the parasite expresses antigens on it’s surface that can cross-react with those of the host muscle and nerve cells, also leading to autoimmune reactions (Molyneux, 1993).

Besides evading the lysosomal action of the infected cells, some trypomastigotes can cleave immunoglobulin molecules and inhibit complement lysis. *T. cruzi* can also suppress Interleukin 2 (IL-2) synthesis and down regulate IL-2 receptor expression which contributes to the immunodepressed state that classically occurs with the infection.
Amastigotes within macrophages can be killed if the host cells are activated by Interferon-
gamma, but this mechanism does not occur in muscle or nerve cells, much to the
advantage of the parasite (Molyneux, 1993). Cytotoxic T-lymphocytes and natural killer
cells are primarily responsible for lysis of the parasite; however, all of the phagocytic
immune cells can play a role in the engulfment and killing of the various stages of the *T.
cruzi* parasite (Kuhn, 1989).

There are no known cures for Chagas’ disease, although a few therapeutic
approaches are successful at relieving symptoms if used early in the onset of the acute
phase of infection. Once an infection has moved from the acute phase to the chronic
phase, there are no guaranteed treatments to successfully relieve the enlargement of
infected organs, or megasymphdromes, that can occur (Wendel and Gonzaga, 1993).

**Transmission of Chagas’ Disease**

Wendel and Gonzaga (1993) report that there may be 50,000 immigrants infected
with *T. cruzi* living in the United States alone. They also noted that in Europe, there
might be greater than 300,000 Brazilian immigrants and in Japan there are over 150,000
Brazilian immigrants. *T. cruzi* can be transmitted through blood transfusion or blood
components with the exclusion of blood derivatives subjected to sterilization procedures.
Other methods of transmission of Chagas’ disease include congenital transmission or
breast feeding (Skolnick, 1991). Blood transfusion is the second most important mode of
transmission in endemic areas. Transfusion transmitted Chagas’ disease may be changing
its geographical limits as emigration, especially to North America, increases. At the
present time, American Red Cross (ARC) blood banks are not required to screen blood or
blood components for the presence or past-presence of Chagas’ disease. There are no serological procedures to screen for Chagas’ disease that have approval of the Federal Drug Administration (FDA), and this fact undermines any efforts at eliminating transmission of Chagas’ disease by blood transfusion or transfusion of blood components. Organ donations from *T. cruzi* infected individuals can also be involved in transmission of Chagas’ disease. The difference in the types of transmission noted above versus insect transmission is that the chagoma of inoculation, a characteristic swelling of the skin, face, or eyelids, is not seen. Fever is the most common and often the only symptom of Chagas’ disease transmitted by organ donations, blood or blood component transfusion (Wendel and Gonzaga, 1993).

*T. cruzi* is viable in refrigerated whole blood for 18 days or more and has been shown to survive freezing temperatures for at least 24 hours. Hemophiliacs have been infected when treated with cryoprecipitate (Factor 8) that has been in the blood bank freezer for 24 hours or less (Wendel and Gonzaga, 1993). A small number of parasites must survive the initial humoral and cell-mediated response in order to establish an active infection in their mammalian hosts.

**Vitamin E and Selenium**

Vitamin E and selenium are essential for the integrity and optimal functioning of all mammalian cells since both are involved in the stability of biological membranes (Sheffy and Schultz, 1979). Selenium, long known for its toxicity, was shown in 1969 to be an essential trace element in the nutrition of chicks and rats (Thompson and Scott, 1969; McCoy and Weswig, 1969). Selenium is now an approved additive to animal feeds
(Jenkins and Hidiroglou, 1972). Even before an absolute requirement for selenium was established, small amounts of selenium were found to protect rats against liver necrosis brought on by vitamin E deficiency (Spallholz et al., 1973). Several diseases of domestic and research animals, such as exudative diathesis in chicks, necrotic liver disease in swine, white muscle disease in ruminants and myopathy of the heart and gizzard in poultry, responded favorably to selenium as well as vitamin E supplementation (Nockels, 1979; Vergroesen and Crawford, 1989).

The metabolic role of selenium is linked with that of vitamin E in that the two elements act in a synergistic manner to protect body cells from oxidation which can lead to cell death. Some diseases associated with low concentrations of selenium (less than 0.1 ppm) are similar to disease conditions caused by vitamin E deficiency and are usually manifested under conditions of low intake of vitamin E. In some instances, these syndromes have been observed to respond fully to the administration of vitamin E -- while in others, selenium was substantially more effective or induced an enhanced response. For example, white muscle disease in lambs is readily prevented by administering selenium to the ewe, whereas vitamin E is essentially ineffective. Limited placental transfer of vitamin E evidently is one factor in this difference in efficacy, since vitamin E is active when given to lambs directly in therapeutic doses. Selenium is not only more efficiently transferred across the placenta and secreted in the milk but is also more actively retained in the tissues of the young. There are inconsistent reports that administration of selenium induces increased growth in lambs receiving adequate vitamin E. On the other hand, selenium is not effective against other aspects of this syndrome, notably the susceptibility of
erythrocytes to hemolysis. Maximal overall response appears to depend on the presence of adequate amounts of both nutrients (Beeson et al., 1971; Vergroesen and Crawford, 1989).

At least a partial explanation for the relationship between selenium and vitamin E has been suggested by experiments on exudative diathesis in chicks. Like liver necrosis in rats, this disease has been induced by feeding a diet low in selenium, vitamin E, and protein (sulfur amino acids). It has been a general observation that these diseases can be prevented by administering either selenium or vitamin E. However, experiments indicate that when chicks are fed a diet extremely low in selenium (less than 0.005 ppm), vitamin E no longer prevents kidney degeneration and mortality even when given in high concentrations. Exudates only occur after the body stores of vitamin E have become depleted as a result of poor absorption of the vitamin. Under these conditions, the intestinal absorption of dietary lipids, including vitamin E, is severely inhibited. These observations suggest that selenium functions in the absorption of dietary vitamin E (Harik-Khan et al., 1993; Beeson et al., 1971).

Vitamin E is found in dark green vegetables, eggs, liver and other meats, wheat germ, vegetable oils, oatmeal, peanuts, and tomatoes. Selenium is found in food sources such as butter, wheat germ, apple cider vinegar, garlic, asparagus, smelt and other fish. Low-fat foods from the meat group, breads and cereals, and legumes are good sources of this vital mineral. However, because soil levels of this nutrient vary according to geographical region, eating a broad spectrum of the above foods may be advisable. Selenium deficient soils in China have been directly linked to Keshan disease, a
cardiomyopathy that is endemic in large areas of China. Keshan disease is especially harmful to young children in that it alters the normal development of the heart tissue (Weiner, 1986).

**Biological Mechanisms of Vitamin E and Selenium**

The biological interrelationship between vitamin E and selenium was elucidated by Rotruck et al. (1973) who showed selenium to be an integral part of the enzyme glutathione peroxidase. Vitamin E is a collective name for a group of closely related lipids called tocopherols, which are very important because of their antioxidant action *in vitro* (Combs et al., 1975).

Selenium and vitamin E are both believed to act as antioxidants which function as one of the body’s primary defenses against free radicals and reactive oxygen molecules (Combs et al., 1975; Bostick et al., 1993). Free radicals are formed naturally in our bodies during metabolic activity by the addition of electrons to oxygen molecules. Free radical formation can be increased by smoking, poor dietary habits, disease states or the normal processing of the oxygen we breathe. Free radicals can react destructively with the cells and tissues of the body as a result of their oxygen toxicity. Antioxidants function by stabilizing volatile oxygen molecules (free radicals). Experts believe this “oxidative damage” contributes to many conditions including heart disease, cancer and even the basic process of aging (Barnett, 1995; Bostick et al, 1993). Free radicals and reactive oxygen molecules can initiate cell damage by reacting with unsaturated bonds in membrane lipids, denaturing proteins and altering nucleic acids (Bostick et al., 1993).

Vitamin E is the major lipid-soluble antioxidant found in cell membranes. It
prevents peroxidative damage to the cell membrane by trapping free radicals and reactive oxygen molecules thus inhibiting their destructive action. Vitamin E is also important in the protection of the subcellular organelles from free radical damage (Nemec et al., 1990; Bostick et al., 1993). In addition, it can also stimulate the immune system and may protect against the development of cancer by enhancing immune surveillance. Vitamin E reduces nitrite, thereby inhibiting the production of compounds that have been shown to induce tumors in experimental animals and possibly in humans (Combs et al., 1975; Bostick et al., 1993; Greeder and Milner, 1980; Poirer and Milner, 1983; Medina et al., 1983).

Selenium has a biological function related to vitamin E in that selenium is an essential component of glutathione peroxidase (GSHPx), which is an enzyme involved in the detoxification of hydrogen peroxide and lipid hydroperoxides (Rotruck et al., 1973). Selenium is non-lipid soluble, and its protective action is found in the aqueous cytosol within the cell. The antioxidative role becomes very important during the immune response when neutrophils produce large quantities of superoxide and hydrogen peroxide (examples of free radicals) from molecular oxygen to destroy ingested foreign microorganisms. Antioxidants help to rid the body of the excess free radicals once their job of killing the foreign microorganisms is finished. The lymphocytes seem to be especially susceptible to peroxidative damage because their membranes have a relatively high free fatty acid content. Cellular membranes are involved in the transport of soluble substances and are also actively involved in the binding of mitogens and antigens (Nemec et al., 1990; Combs et al., 1975; Sheffy and Schultz, 1979).
Vitamin E has been associated with a variety of functions affecting the reproductive, nervous, circulatory, muscular, skeletal, and hematopoietic cells (Sheffy and Schultz, 1979). Clinical signs of deficiency are degenerative in nature and often involve the stability of biological membranes. Vitamin E is more often thought of as an antioxidant and free radical scavenger than in the development and function of the immune system. Vitamin E protects against dangerous blood clots and affects blood cholesterol levels, blood flow to the heart, strength of capillary walls, and muscle and nerve maintenance (Weiner, 1986; Sheffy and Schultz, 1979).

The antioxidant protection of selenium may become defective during sickness, malnutrition, and old age. This decline in antioxidant activity may result in impaired cell function which could cause weakened immunity and overall a poorer state of health. With advancing age, the glutathione peroxidase-selenium enzyme does not work as efficiently and noxious oxidants accumulate in the body (Desowitz, 1987).

Selenium is extremely valuable for treating poisoning with heavy metals, such as mercury and cadmium, since it facilitates their excretion from the body. These toxic heavy metals have immunosuppressive effects on the body. However, too much selenium can also be dangerous. Weiner (1986) reported that in high doses selenium can produce symptoms such as loss of hair, nails, dizziness, fatigue, and dermatitis. However, he noted that in some cases high-dose supplementary selenium was tolerated over long periods without toxicity (Weiner, 1986).

**Selenium, Vitamin E and The Immune System**

In 1973, the accepted nutritionally adequate dietary level for selenium was 0.1
ppm. Selenium is less toxic in biological systems if given as a sodium salt of selenium (Na$_2$SeO$_3$). Spallholz et al. (1973) supplemented mice with dietary selenium in the range of 0.75 ppm to 10 ppm. Most of the mice were also supplemented with 70 ppm vitamin E or DL-$\alpha$-tocopherol acetate. Included in the study was a selenium-toxic diet for a group of mice that were fed 14 ppm selenium for two weeks and 42 ppm selenium for three weeks. The highest anti-sheep red blood cell antibody levels were found in mice given the 0.7 ppm and 2.8 ppm of selenium. The conclusion of that study was that dietary selenium at levels above that generally accepted as nutritionally adequate enhances the primary immune response in mice by increasing the numbers of plaque forming cells and antibody levels. Those authors reported that dietary selenite promoted immunoglobulin M (IgM) synthesis while vitamin E promoted immunoglobulin G (IgG) synthesis (Spallholz et al., 1973).

Vitamin E has been implicated in the stimulation of serum antibody synthesis, particularly IgG antibodies (Tengerdy et al., 1972, Tengerdy et al., 1973). Heinzerling et al. (1974a) collaborated with Tenderdy on a later study in which mice were supplemented with 180 milligrams of vitamin E (DL-$\alpha$-tocopheryl acetate) per kilogram of food. The mice were challenged with Diplococcus pneumoniae type I, and the phagocytic activity against this bacterial strain was measured. The phagocytic index of mice that had been immunized prior to being challenged with Diplococcus pneumoniae was four times higher in the 180 mg/vitamin E group than in a control group of infected mice. The results of that study indicated that increased macrophage activity probably aided by increased antibody production was the principal reason for the increased protection. Heinzerling et
al. (1974b) completed another study of vitamin E with chicks exposed to *Escherichia coli*. Supplemental dietary vitamin E of 150-300 mg/kg provided enhanced protection and immunity to the chicks. Yasunaga et al. (1982) reported that vitamin E can significantly enhance immune responses to the mitogens: phytohemagglutin, concanavalin A, and lipopolysaccharide.

An enhancing effect of vitamin E on antibody production by activation of the humoral immune system was observed in lambs challenged with parainfluenza virus. It was reported from this study that although the IgM antibody levels increased more substantially in selenium supplemented animals, the increase was greater with vitamin E supplementation after a secondary antigenic challenge (Reffert et al., 1988).

Interleukin-1 (IL-1), a cytokine primarily produced by activated monocytes and/or macrophages after antigenic challenge, is capable of activating T cells during antigen presentation, inducing IL-2 receptor expression by T helper cells, and enhancing B cell proliferation (Chandra, 1992; Corwin and Shloss, 1980). The research work of Romach et al. (1993) lends support to the theory that vitamin E can enhance the immune response in both cellular and humoral immunity. Vitamin E supplementation has been shown to enhance immunity by inducing macrophages to produce elevated levels of IL-1 and/or by down-regulating other immune factors that would inhibit the production of IL-1 (Romach et al., 1993).

In a double-blind, placebo-controlled study, Meydani et al. (1990) showed that supplementation of healthy elderly subjects, over 60, with vitamin E (800 IU/day of DL-α-tocopheryl acetate) for 30 days significantly improved delayed type hypersensitivity
(DTH) responses, lymphocyte proliferative responses to the T cell mitogen, concanavalin A, and concanavalin A stimulated IL-2 production. Furthermore, supplementation with 400 IU/day of vitamin E for six months produced a 91% increase in the DTH response of healthy elderly individuals. It was concluded from these studies that the improvements observed following short-term supplementation with vitamin E are maintained during longer periods of supplementation and that a dose of 400 IU/day of vitamin E is effective in inducing these changes. Vitamin E and selenium were both included in this study because they play a regulatory role in the immune response; however, the effect of selenium was not emphasized in this study (Meydani et al., 1990). Meydani (1993) proposed that vitamin and mineral supplementation exceeding the recommended dietary allowance significantly improves certain indices of the immune response in the elderly.

The results of Chandra (1992) agreed with the above findings and showed that immunological improvements following nutrient supplementation were associated with decreased frequency of infection-related illnesses in the elderly.

Moriguchi et al. (1993a) examined the effect of vitamin E supplementation on cellular immune functions that decreased with age. Their study used spontaneously hypertensive rats which were divided into two groups, one which was fed a 50 mg vitamin E/kg diet and the other which was fed a 585 mg vitamin E/kg diet. Spontaneously hypertensive rats represent accelerated disease and abnormality of cellular immune functions associated with aging (Moriguchi et al., 1993a). This research group reported that high vitamin E diets could enhance both splenic lymphocyte and alveolar macrophage functions in rats. The T cell functions appeared to be greatly affected by vitamin E
deficiency, and it was concluded that vitamin E plays an important role in T cell
differentiation in the thymus (Moriguchi et al., 1993a).

Another study by Moriguchi et al. (1993b) demonstrated the effects of vitamin E
on the differentiation of T cells in the thymus by monitoring the proportions of CD4⁺CD8⁻
and CD4⁻CD8⁺T cells and evaluated a possible association between the effect of vitamin E
on T cell differentiation in the thymus and the action of vitamin E as an antioxidant. The
F344 rats were divided into three groups: vitamin E-free diet, normal diet (50 mg vitamin
E) and high vitamin E diet (585 mg vitamin E). The results for the vitamin E-free group
and the high vitamin E group were compared to results obtained for the normal 50 mg
vitamin E group. The number of thymocytes was significantly lower in the vitamin E-free
group compared to the normal group. The ratio of CD4⁺CD8⁻/CD4⁻CD8⁺ T cells in the
spleen increased in the high vitamin E group and significantly decreased in the vitamin E-
free group. Production of IL-2 by thymocytes following stimulation with concanavalin A
for 48 hours increased approximately three-fold in the high vitamin E group.
Prostaglandin E2 production from thymocytes was significantly lower in the high vitamin
E group compared to the normal group of rats. These results of Moriguchi et al. (1993b)
suggested that vitamin E plays an important role in T cell differentiation in the thymus,
which may be related to the action of vitamin E as an antioxidant.

Deficiencies of vitamin E and/or selenium have been shown to cause suppression
of the immune system in small mammals. Both humoral and cellular immune responses are
enhanced by vitamin E and selenium supplementation (Wuryastute et al., 1993). The
purpose of the present study was to determine the effect of elevated levels of vitamin E
and/or selenium on the immune response of mice following antigenic challenge with a lethal dose of *T. cruzi*. Parasitemia, antibody production and mortality were measured in an effort to determine how each of these parameters would be affected by dietary supplementation with the antioxidants, vitamin E and selenium.
MATERIALS AND METHODS

Parasites

The Brazil strain of *T. cruzi* was used throughout this research project. The parasites were maintained by stock infections in C3HeB/FeJ mice in the blood-form trypomastigote stage (BFT). An infection inoculum of $1 \times 10^3$ BFT’S in 0.2 ml of Dulbecco’s phosphate buffered saline (DPBS, Sigma Chemical Co., St. Louis, Mo.) was administered intraperitoneally (IP) to each mouse.

Mice

Syngeneic female mice, strain C3HeB/FeJ (Jackson Laboratories, Bar Harbor, Maine), were maintained at room temperature (26°C) in accordance with NIH and local IACUC guidelines. The mice were four-five weeks old (born 4/2/94 and 4/3/94) upon arrival and were immediately switched to the synthetic food. Food and tap water were provided *ad libitum*. Age matched female mice that were fed a common Purina rodent chow, # 5001 (Purina, Richmond, IN.), served as the infected control mice. The nutritionally adequate amount in Purina rodent chow # 5001 fed to infected control mice was 0.2 ppm of selenium and 49 IU/kg of vitamin E (Purina, 1994). Strain C3HeB/FeJ mice are highly susceptible to infection with *T. cruzi* and death is normally expected by day 35 after the mice are injected IP with an infection inoculum of $1 \times 10^3$ BFT. Three identical trials of mice, Trial # 1, Trial # 2 and Trial # 3, were set up with four cages per trial for research in this order:
Cage A: Vitamin E and Selenium Deficient, n=8  
(Trial # 2 = 4 mice, Trial # 3 = 5 mice)

Cage B: 2 ppm Selenium, n = 9  
(Trial # 2 = 4 mice, Trial # 3 = 5 mice)

Cage C: 800 IU/kg Vitamin E plus 2 ppm Selenium, n = 9  
(Trial # 2 = 4 mice, Trail # 3 = 5 mice)

Cage D: 800 IU/kg Vitamin E, n = 10  
(Trial # 2 = 5 mice, Trial # 3 = 5 mice)

Cage E: Control Mice fed a common Purina Rodent Chow, # 5001, n = 3  
(T. cruzi infected control mice)

A third trial, Trial # 1, consisting of 20 mice was set up with 4 cages of 5 mice each. Cages A, B, C, and D were fed the synthetic diets in the same order as in the other 2 trials. The mice in Trial # 1 showed very few numbers of parasites in the peripheral circulation up through day 70 of the study due to an air conditioning malfunction. There was a substantial increase in temperature during several weeks at the start of the research. It has been documented by other research studies that a temperature increase can alter the course of experimental Chagas’ disease. When maintained at an elevated temperature of 36°C, infected mice show low parasitemia and low mortality as compared to mice maintained at room temperature (26°C), (Ming, 1994). The elevated temperature altered the normal course of Chagas’ disease in Trial # 1; therefore, these results are not included in the study.
Diets

Purified synthetic diets were prepared by Purina Test Diet Department in Richmond, Indiana. See Table 1 for diet formulations and Table 2 for diet specifications for the vitamin E and selenium purified diets.

Diet # 1: Vitamin E Deficient / Selenium Deficient
Diet # 2: 800 IU/kg Vitamin E / Selenium Deficient
Diet # 3: Vitamin E Deficient / 2 ppm Selenium
Diet # 4: 800 IU/kg Vitamin E / 2 ppm Selenium

ELISA (Enzyme linked immunosorbent assay)

A detergent extract of fibroblast-derived trypomastigotes (FDTS; 10 ug/ml concentration) was used as the source of antigen in 96 well microtiter plates. The mice were bled starting at day 20 and continued at 3-4 day intervals thereafter. Twenty ul (microliters) of blood were drawn from each mouse via a tail vein and diluted in 80 ul of DPBS. Diluted blood was then centrifuged for 10 min. at 8,000 revolutions per min. (rpm), and the diluted plasma was removed and stored at -20°C until batch testing could be performed at a later date. The mouse plasma, which contained the anti-\textit{T. cruzi} antibody, was used as the primary antibody in the 96 well ELISA plates. Plasma samples for each mouse within the same synthetic diet treatment cage were pooled. Two-fold serial dilutions of the mouse plasma were prepared in duplicate for each sample of pooled mouse plasma. The secondary antibody was goat anti-mouse immunoglobulin conjugated to horseradish peroxidase (HRP) (Fisher Diagnostics, Pittsburgh, Pa.). The substrate, O-phenylene-diamine (Sigma) was dissolved in Tris-citrate buffer and activated with 30 %
## DIET FORMULATIONS

<table>
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<tr>
<th>Ingredients</th>
<th>Diet #1</th>
<th>Diet #2</th>
<th>Diet #3</th>
<th>Diet #4</th>
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<td>Vitamin Free Casein (92% Protein)</td>
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<tr>
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### Table 1.
The four pelleted, synthetic diets formulated by Purina consist of the following: Diet # 1 contains 0 vitamin E / 0 selenium, Diet # 2 contains 800 IU/kg Vitamin E / 0 selenium, Diet # 3 contains 0 vitamin E / 2 ppm selenium and Diet # 4 contains 800 IU/kg Vitamin E / 2 ppm selenium.
**DIET SPECIFICATIONS**

<table>
<thead>
<tr>
<th>Diet Specifications *</th>
<th>Diet # 1</th>
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</table>

*Table 2.* Diet specifications for the four pelleted, synthetic diets. * Synthetic Diet

Food was stable for six months when stored in the refrigerator under cool and dry conditions.
Hydrogen Peroxide (H₂O₂). The optical densities in each well of the ELISA plates were measured with a Microplate reader (Courtesy of Vanderbilt University). The optical densities measured at 450 nm were plotted against the reciprocal of the dilutions. Parasite-specific antibody titers were determined from these plotted data. The parasite-specific antibody titer was calculated as the highest dilution that remains greater than +2 SD from the absorbance of the noninfected normal mouse serum.

**Monitoring Parasitemia and Mortality**

Parasitemias were determined at three to four day intervals starting at day 13 post-infection. Blood was obtained from the infected mice by clipping the tip of the tail and squeezing the tail until a uniform round drop of blood was expelled. Four µl of the infected mouse blood were collected and added to 96 µl DPBS which constituted a 1/25 dilution. A well mixed aliquot of the 1/25 dilution was placed on a hemacytometer counting chamber and the number of parasites in 4 large corner squares was counted. The calculation used was \[ \text{average # parasites/1 large square} \times \text{reciprocal of the dilution factor (25)} \times \text{reciprocal of the volume of 1 large square (}10^4\text{)} = \text{BFT /ml blood}. \] Calculations were made to determine BFTs per ml of peripheral blood. Data were collected using this procedure until the parasites were consistently not seen in the circulating blood from mice that survived the entire length of the research study. Mice were also monitored daily for clinical symptoms and mortality.

**Statistical Analysis**

All statistical analyses were performed using SYSTAT, Version 5.1 (Wilkinson, 1992). To check for normality of data distributions (data = parasitemia values for the
individual mice and the optical density (OD) of the antibody levels), Kolmogorov-Smirnov tests, Lilliefors' option, were performed within treatment trials. Both the parasitemia and optical density values were shown not to be normally distributed (p < 0.05). As values spanned several orders of magnitude, parasitemia and OD measurements were log transformed to restore linearity (Wilkinson, 1992).

To examine the relationship between antibody levels and parasitemias, the correlation coefficient was computed on the log-transformed data across all treatment cages. Due to lack of normality likely resulting from the pooling of treatment cages, the Spearman correlation coefficient was used (Wilkinson, 1992).

The possibility of pooling replicate treatment cages in the two trials was examined by regressing the parasitemia and antibody levels against the time period of infection using a least squares model. The differences between the slopes and intercepts of the best fit lines from replicate cages were then compared. There were no statistically significant differences detected for the replicate treatment cages in Trials # 2 and # 3, means ±2 SE overlapped for 8 of 8 pairs of intercepts and 7 of 8 pairs of slopes, indicating that the replicate treatment cages in the two trials could be combined for subsequent analysis (Wilkinson, 1992).

Significance of the relationship between the four different synthetic diet treatments was assessed using ANCOVA. Slopes were homogeneous across all treatment cages (p > 0.05). Since the assumption of homogeneity of slopes was satisfied in all cases, intercept differences between the cages of mice fed synthetic food were tested for significance using ANCOVA. To determine which cages differed significantly, pairwise comparisons were
made for the pooled treatment cages (A, B, C, D) using the conservative Tukey’s HSD
Multiple Comparison Test. This test corrects for Type I error level when multiple tests
are being applied (Wilkinson, 1992).
RESULTS

Determination of Parasitemia

Figure 2 shows the mean parasitemias for the replicate pooled cages of mice in Trials #2 and #3 in comparison to the infected control mice. The mean peak parasitemia of all the mice fed synthetic food was $2.56 \times 10^6$ parasites/ml of blood compared to $1.12 \times 10^7$ parasites/ml of blood for the infected control mice. The mean peak parasitemia for the mice fed synthetic food was fourfold lower than the mean peak parasitemia of the infected control mice. The mice that received the 800 IU/kg vitamin E had the lowest overall mean peak parasitemia of $1.26 \times 10^6$ parasites/ml of blood, which represents a ninefold lower mean peak parasitemia when compared to the infected control mice. The mean peak parasitemia of mice for the replicate treatment cages in Trials #2 and #3 were as follows: Cage A = $3.07 \times 10^6$ parasites/ml of blood (threefold lower than infected control mice), Cage B = $2.41 \times 10^6$ parasites/ml of blood (fourfold lower than infected control mice), Cage C = $3.48 \times 10^6$ parasites/ml of blood (threefold lower than infected control mice), Cage D = $1.26 \times 10^6$ parasites/ml of blood (ninefold lower than infected control mice) and Cage E (infected control mice) = $1.12 \times 10^7$ parasites/ml of blood.

Parasitemia levels showed significant differences among cages over time. The rates of increase in parasite numbers were heterogeneous among cages (cage x day interaction was significant; $F_{4,276} = 9.755, p < 0.001$). The T. cruzi infected control mice had a significantly higher slope than all synthetic treatment cages (slope means $\pm$ 2 SE did
Parasitemia

Figure 2. Parasitemia

Mean parasitemias of the pooled, replicate synthetic diet treatment cages in Trials # 2 and # 3 are shown versus days post-infection. Cage E represents *T. cruzi* infected control mice fed Purina rodent chow # 5001. Parasitemias are shown up to day 44 post-infection, which is the day that the number of blood-form trypomastigotes peaked in the peripheral blood. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n = 8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium (n = 9), Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium (n = 9), Cage D represents mice fed diets containing 800 IU/kg Vitamin E / 0 ppm selenium (n = 10) and Cage E represents *T. cruzi* infected control mice (n = 3).
Parasitemia

Blood Form Trypomastigotes

Days Post-Infection

Cage A  Cage B  Cage C  Cage D  Cage E
not overlap). Among the four different diet treatments, Cage B had a significantly greater slope than did Cage D. None of the other five comparisons were significant.

**Parasite-specific Antibodies**

Parasitemia and the anti-\textit{T. cruzi} antibody levels were significantly positively correlated across all days and treatment trials (Spearman correlation = 0.7, n = 228, p < 0.001). High parasitemia values were associated with high anti-\textit{T. cruzi} antibody levels.

Significant differences in antibody levels over time were detected among certain treatment cages. The assumption of homogeneity of slopes was satisfied for all treatments (cage x day interaction was nonsignificant: \(F_{3,791} = 0.399, p = 0.754\)). Tests for intercept differences using ANCOVA showed that there was a significant treatment effect on antibody levels (\(F_{3,294} = 9.894, p < 0.001\)). Pairwise comparisons of the four cages by Tukey's HSD multiple comparisons test indicated that mice in Cages A and B were not significantly different from each other; similarly, mice in Cages C and D were not significantly different. However, mice in Cages A and B were significantly different from mice in Cages C and D (Table 3).

All of the infected mice receiving synthetic food diets showed increases in antibody levels throughout the acute phase of the infection. Death by day 46 of the infected control mice marked the end of the acute phase for all the mice in the study. Figures 3, 4, 5 and 6 show the anti-\textit{T. cruzi} antibody levels measured by ELISA. Days shown post-infection are 20, 35, 44 and 53. Cage G is representative of a noninfected normal mouse serum. Plasma from mice in the non-infected control cage served as the baseline to detect
Tukey’s HSD Multiple Comparisons

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>------</td>
<td>0.037</td>
<td>-0.219</td>
<td>-0.273</td>
</tr>
<tr>
<td>B</td>
<td>0.953</td>
<td>------</td>
<td>-0.256</td>
<td>-0.310</td>
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<tr>
<td>C</td>
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<td>0.002</td>
<td>------</td>
<td>-0.054</td>
</tr>
<tr>
<td>D</td>
<td>0.001</td>
<td>0.000</td>
<td>0.866</td>
<td>------</td>
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</table>

Table 3. Tukey’s HSD comparisons tests for significant pairwise differences among synthetic diet treatments. Values obtained above are based on parasite-specific antibody levels. The values above the diagonal are pairwise mean differences and those below the diagonal are associated probabilities. Probabilities less than 0.05 are considered significant. Data are pooled across replicate diet treatment cages in Trials # 2 and # 3. For example, the pairwise mean difference between Cages A and D was -0.273 which corresponds to an associated probability of 0.001, indicating significant differences between Cages A and D. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium, Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium, Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium and Cage D represents mice fed diets containing 800 IU/kg vitamin E / 0 selenium.
increasing antibody titers.

**Mortality**

By day 70, percent survival for mice in the replicate treatment cages in Trials #2 and #3 was as follows: Cage A = 37.5%, Cage B = 44.5%, Cage C = 77.8%, and Cage D = 80.0%. The infected control mice, Cage E, fed Purina rodent chow #5001, exhibited 0% survival by day 46 (See Figure 7). Cage A represents 0 vitamin E / 0 selenium, Cage B represents 0 vitamin E / 2 ppm selenium, Cage C represents 800 IU/kg vitamin E / 2 ppm selenium and Cage D represents 800 IU/kg vitamin E / 0 selenium. The mice supplemented with diets containing 800 IU/kg vitamin E had approximately twice the number of survivors compared to mice supplemented with diets that were deficient in vitamin E. There was an overall synthetic food enhancement effect which is shown by the high percent survival (37.5%) of mice supplemented with diets deficient in vitamin E and selenium. Death is normally expected by day 35 post-infection with C3HeB/FeJ mice used in the present study.

**Figure 3.** Parasite-specific antibody levels on day 20 post-infection. Using a parasite-specific ELISA, levels of anti-\textit{T.cruzi} antibodies were measured in pooled plasma samples. Twofold serial dilutions were tested for the presence of parasite-specific antibodies in the ELISA microtiter plates. Pooled replicate cages are represented by Cages A, B, C and D. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n=8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium (n=9), Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium (n=9), Cage D represents mice fed diets containing 800 IU/kg vitamin E / 0 selenium (n=10) and Cage G represents noninfected normal mouse serum from mice fed Purina rodent chow # 5001 (n=5).
Parasite Antibodies: Day 20
Parasite-specific Antibody Levels / Day 35

Figure 4. Parasite-specific antibody levels on day 35 post-infection. Using a parasite-specific ELISA, levels of anti-*T. cruzi* antibodies were measured in pooled plasma samples. Twofold serial dilutions were tested for the presence of parasite-specific antibodies in the ELISA microtiter plates. Pooled replicate cages are represented by Cages A, B, C and D. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n=8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium (n=9), Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium (n=9), Cage D represents mice fed diets containing 800 IU/kg vitamin E / 0 selenium (n=10) and Cage G represents noninfected normal mouse serum from mice fed Purina rodent chow # 5001 (n=5).
Parasite Antibodies: Day 35

Absorbance at 450 nm

Reciprocal Dilution

- - - Cage A  - - Cage B  - - Cage C  - - Cage D  - - Cage G
Parasite-specific Antibody Levels / Day 44.

**Figure 5.** Parasite-specific antibody levels on day 44 post-infection. Using a parasite-specific ELISA, levels of anti-*T. cruzi* antibodies were measured in pooled plasma samples. Two fold serial dilutions were tested for the presence of parasite-specific antibodies in the ELISA microtiter plates. Pooled replicate cages are represented by Cages A, B, C and D. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n=8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium (n=9), Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium (n=9), Cage D represents mice fed diets containing 800 IU/kg vitamin E / 0 selenium (n=10) and Cage G represents noninfected normal mouse serum from mice fed Purina rodent chow # 5001 (n=5).
Parasite-specific Antibody Levels / Day 53.

Figure 6. Parasite-specific antibody levels on day 53 post-infection. Using a parasite-specific ELISA, levels of anti-\( T. cruzi \) antibodies were measured in pooled plasma samples. Twofold serial dilutions were tested for the presence of parasite-specific antibodies in the ELISA microtiter plates. Pooled replicate cages are represented by Cages A, B, C and D. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n=8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm selenium (n=9), Cage C represents mice fed diets containing 800 IU/kg vitamin E / 2 ppm selenium (n=9), Cage D represents mice fed diets containing 800 IU/kg vitamin E / 0 selenium (n=10) and Cage G represents noninfected normal mouse serum from mice fed Purina rodent chow # 5001 (n=5).
Parasite Antibodies: Day 53

Absorbance at 450 nm vs Reciprocal Dilution

- Cage A
- Cage B
- Cage C
- Cage D
- Cage G
Mortality.

Figure 7. Percent survival for the pooled, replicate synthetic diet treatment cages plus the *T. cruzi* infected control mice fed Purina rodent chow # 5001. Results shown are the combination of the pooled replicate treatment cages in Trials # 2 and # 3. Cage A represents mice fed diets containing 0 vitamin E / 0 selenium (n = 8), Cage B represents mice fed diets containing 0 vitamin E / 2 ppm Selenium (n = 9), Cage C represents mice fed diets containing 800 IU/kg Vitamin E / 2 ppm selenium (n = 9), Cage D represents mice fed diets containing 800 IU/kg Vitamin E / 0 selenium (n = 10) and Cage E represents *T. cruzi* infected control mice fed Purina rodent chow, # 5001 (n = 3).
DISCUSSION

If Chagas' disease could be effectively controlled by using natural vitamins or minerals, the implication in endemic areas of Latin America would be tremendous. Although Chagas' disease is endemic in Central and South America, there is a potential worldwide problem with the parasitic infection as individuals emigrate to new areas. Considering that the majority of infected individuals are asymptomatic and unaware they are infectious, the importance of an adequate therapeutic approach to Chagas' disease should be apparent.

As mammalian hosts have evolved new defense strategies to rid themselves of parasitic protozoans, the protozoans themselves have also co-evolved strategies to allow survival in their host. The protozoan parasite, *T. cruzi*, has developed several strategies to evade the immune response and survive in the mammalian host for an extended period of time in which the parasites can cause extensive damage to their host. Ultimately, the success of this research could make vitamin E and selenium effective tools to aid the immune response in the battle against Chagas' disease. To the author's knowledge, this is the first study designed to evaluate the effect of dietary antioxidants on Chagas' disease.

The results of the present study demonstrate that increased dietary intake of vitamin E can have a positive impact on the course of experimental Chagas' disease. Mice supplemented with synthetic diets containing 800 IU/kg vitamin E had the lowest overall parasitemia levels and also the highest percent survival. These mice had a ninefold lower
mean peak parasitemia level ($1.26 \times 10^6$ parasites / ml of blood) than the infected control mice ($1.12 \times 10^7$ / ml of blood). Eighty percent of the mice supplemented with synthetic diets containing 800 IU/kg vitamin E survived to day 70 post-infection compared to only 37.5% survival in the mice supplemented with synthetic diets deficient in vitamin E and selenium.

The combined mean peak parasitemia levels for all mice fed synthetic diets ($2.56 \times 10^6$ parasites / ml of blood) was fourfold lower than the infected control mice ($1.12 \times 10^7$ parasites / ml of blood). After the mice supplemented with synthetic diets had peaked in their parasitemia by day 44, there was a gradual decline in circulating blood-form trypomastigotes until none were evident in the peripheral circulation. The mice supplemented with synthetic diets also did not exhibit the typical sharp rise in parasitemias that was exhibited by the infected control mice. Furthermore, the peak in parasitemia for the infected control mice coincided closely with the days of their death. By day 46 post-infection, all of the *T. cruzi* infected control mice were dead.

Mice supplemented with synthetic diets containing 800 IU/kg vitamin E plus 2 ppm selenium developed higher parasitemia levels ($3.48 \times 10^6$ parasites / ml of blood) than the mice supplemented with dietary 800 IU/kg vitamin E only ($1.26 \times 10^6$ parasites / ml of blood). One mouse in the replicate cages fed the synthetic diet containing 800 IU/kg vitamin E plus 2 ppm selenium developed extremely high parasitemia levels that were sixfold higher than any of the other mice in the same representative cages.

Mice supplemented with synthetic diets containing 800 IU/kg vitamin E had an 80% survival by day 70 post-infection which was the highest percent survival of all the
mice. Mice supplemented with 800 IU/kg vitamin E plus 2 ppm selenium had the next highest percent survival of 77.8% by day 70 post-infection. These percent survival are relatively close and are representative of four cages of mice. The remaining mice supplemented with the other two synthetic diets showed a much lower combined percent survival of 41%. Therefore, there were approximately twice the number of survivors among the mice supplemented with diets containing an elevated level of vitamin E as compared to mice fed synthetic diets deficient in vitamin E. Mice supplemented with synthetic diets containing 2 ppm selenium exhibited 44.5% survival which is close to the 37.5% survival of the mice fed diets deficient in vitamin E and selenium. The percent survival of the infected control mice was 0% by day 46 post-infection. The death of all the infected control mice by day 46 marked the approximate end of the acute phase of the infection and the movement of the survivors into the chronic phase of the infection.

The C3HeB/FeJ mice used in this study are highly susceptible to *T. cruzi* infection and death is normally expected by day 35 post-infection. The death of the last infected control mouse by day 46 post-infection is suggestive of a decrease in virulence in the *T. cruzi* strain used in this study. The high percentage of survivors in this study suggests that dietary supplementation with increased levels of antioxidants can enhance the immune response of mice to Chagas’ disease. The mouse model shares many similarities with human Chagas’ disease; and therefore, the results of this study suggest that antioxidants may also improve the human response to Chagas’ disease.

In this research many of the mice that survived did not exhibit the classic Chagas’ disease anemia evidenced by a decrease in the circulating red blood cells. Profound
anemia was shown by the mice that died by evidence of the low percentage of red blood cells present in the hemacytometer counts. The majority of the surviving mice that were supplemented with synthetic diets remained active during the acute and chronic phase of infection and did not exhibit any classic pathological symptoms that are normally associated with this strain of mice. Further studies are needed to determine the numbers of amastigotes present in tissue samples of the mice supplemented with synthetic diets as compared to tissue amastigotes present in unsupplemented control mice. Histological studies would provide a more complete picture of the tissue pathology that allowed the mice supplemented with synthetic diets to survive and develop lower parasitemias than mice fed the commonly used Purina rodent chow # 5001 during the course of Chagas’ disease.

Vitamin E and selenium have been shown to enhance the immune system in a variety of ways. The synergistic effect vitamin E and selenium have on cells is essential for their ability to function optimally. Protection of cells against membrane damage resulting from uncontrolled lipid peroxidation depends upon the presence of vitamin E (Combs et al., 1975). The function of vitamin E and selenium in the maintenance of erythrocyte membrane integrity is vital since vitamin E is present within the membrane itself and selenium is associated primarily with the aqueous phase of the plasma membrane (Combs et al., 1975; Sheffy and Shultz, 1979). Erythrocytes and lymphoid cells originate from common pluripotential stem cells. This fact provided a basis for the concept that vitamin E and selenium may be associated with membrane fluidity of lymphoid cells, thus affecting immune response mechanisms as well (Sheffy and Shultz, 1979). Increased
intake of antioxidants like vitamin E and selenium, during stress or illness, may prove to be beneficial to overall health (Reffert et al., 1988).

After an extensive literature search, Desowitz and Barnwell’s research study (1980) was the only one found that addressed the antioxidant enhancement effect of selenium or vitamin E on mice challenged with a parasitic infection. The parasite used was the malarial parasite, *Plasmodium berghei*, which dramatically affects the red blood cell population of the host. These authors demonstrated that 2.5 ppm selenium can potentiate the protective effect of a killed *Plasmodium berghei* vaccine for Swiss-Webster mice (Desowitz and Barnwell, 1980).

Sheffy and Shultz (1979) concluded that the dietary levels of vitamin E and selenium most effective for immune response enhancement were those 10-30 times the dietary requirement, levels considered to be toxic for several mammalian species. Their research involved the measurement of antibody titers in dogs following vaccination with a canine distemper-hepatitis vaccine. The measurable antibody after immunization was depressed in the vitamin E and selenium deficient dogs.

High doses of vitamin E has been shown to enhance phagocytosis and to increase humoral antibody production against particulate and soluble antigens in various animal species (Vergroesen and Crawford, 1989; Marsh et al., 1981). Corwin and Schloss (1980) showed that cell-mediated immune functions were also stimulated by high dietary levels of vitamin E in research animals. Vitamin E deficiency has been shown to result in decreased lymphatic organ size, T and B cell proliferation, white blood cell function, inflammatory response, and host resistance to infection (Weiner, 1986; Unanue and Allen,
The research work of these authors is suggestive that elevated doses of vitamin E can play a part in immune system enhancement.

In the present study, it was expected that the mice receiving synthetic diets deficient in vitamin E and selenium would exhibit 0% survival. However, these mice had a higher than expected percent survival of 37.5%. In addition, parasitemia levels were threefold lower in these mice than in the infected control mice fed Purina rodent chow #5001. These parasitemia levels and percent survival results led to the conclusion that all the mice on the four synthetic diets had an overall enhancement effect that had to be taken into consideration. Technical personnel at Purina Test Diet Department in Richmond, Indiana, were consulted concerning the synthetic rodent chow. Purina reported that the synthetic rodent chow had a higher absorption rate than the commonly used Purina rodent chow #5001 (personal communication, 1994). In essence, the mice on the synthetic food were possibly more efficient in the uptake of vitamins and minerals from their food than were the mice on Purina rodent chow #5001. Statistical analysis on the SYSTAT Version 5.1 allowed for the elimination of the synthetic food effect so that the four different diet treatments could be compared to each other.

Slopes of the plotted lines using parasitemias versus cage x day post-infection showed a significant difference between all synthetic diet treatment cages (A-D) and the T. cruzi infected control mice. The parasitemias of the infected control mice increased steadily until death resulted by day 46 post-infection.

The correlation analysis showed a positive Spearman computation between the parasitemias and OD of the antibody levels, indicating that as the number of circulating
parasites increased the antibody levels also increased. Intercept differences of the OD of the antibody levels among all synthetic diet treatment cages were analyzed by ANCOVA and indicated a different response among one or more of the cages. The results of the parasite-specific antibody levels measured by ELISA showed an increase in antibody levels during the course of the parasitic infection. By day 20 post-infection, there were no measurable parasite-specific antibodies detected in plasma from mice in replicate cages in Trials #2 and #3. By day 35 post-infection, plasma from infected mice supplemented with synthetic diets showed parasite-specific antibody titers greater than 1/512. These antibody titers remained high in all infected mice supplemented with synthetic diets throughout the course of infection. There were no obvious differences detectable for the rise in antibody levels between replicate cages of infected mice supplemented with synthetic diets in Trials #2 and #3 (See Figures 3, 4, 5 and 6). This lack of obvious differences in antibody levels was addressed in the statistical analyses performed on SYSTAT Version 5.1. Tukey’s HSD comparisons tests is a highly conservative statistical analyses that allows pairwise differences that are not obvious between the synthetic diet treatments to be addressed. Tukey’s HSD comparisons tests measures the pairwise mean differences between the replicate cages and also calculates the associated probabilities of the closeness of the values. Any associated probability that is less than 0.05 is considered significant. Tukey’s HSD comparisons tests showed that mice supplemented with 800 IU/kg vitamin E, Cage D, had similar antibody levels as mice supplemented with 800 IU/kg vitamin E plus 2 ppm selenium, Cage C. Also, the mice supplemented with 2 ppm selenium, Cage B, had similar antibody levels as mice deficient in vitamin E and selenium,
Cage A. Cages A and B differed significantly from Cages C and D (Table 3). Thus, there were significant differences in the antibody levels between the synthetic diet treatment cages that was detectable by utilizing the Tukey’s HSD comparisons tests. The elevated vitamin E levels supplemented in the synthetic diets appeared to be the dividing factor in this study. Therefore, increased dietary vitamin E did have a significant effect on antibody level production during the course of Chagas’ disease in this study while the elevated selenium level did not have a statistically significant effect on the response of the mice.

The increase in antibody levels should directly reflect an increase in circulating peripheral parasites as the amastigote stage of the parasite changes into the infective trypomastigote and is released into the blood stream. Each new wave of trypomastigotes released after the onset of the initial infection would encounter an already primed immune response that would result in a stronger secondary response to the parasite. These results are suggestive that increased dietary intake of the antioxidant, vitamin E, can possibly enhance the humoral immune response of mice with Chagas’ disease.

Although the humoral immunity plays an important role in the protective response to Chagas’ disease, cell mediated immunity has also been shown to be crucial (Romach et al., 1993; Chandra, 1992; Corwin and Schloss, 1980, Tarleton, 1991). However, the effect of vitamin E and selenium supplementation on cell-mediated immunity was not addressed in the present study.

The percent survival of 44.5% for the mice supplemented with 2 ppm selenium was close to the percent survival of the mice fed the synthetic diet that was deficient in vitamin E and selenium. These percent survival results led to the hypothesis that the level
of selenium used in this study was perhaps too low for an enhancement effect to be noted. To circumvent the synthetic food effect, a second study using a selenium salt dissolved in the drinking water of the mice was undertaken by a separate research group. The highest percent survival observed in this study was for mice that received 8 ppm selenium in their drinking water. Previous research by Spallholz (1975) had documented hair loss and lethargy at elevated selenium levels of 16 ppm, but the only outward clinical symptom that was noted in the second study was hyperactivity. It is possible that optimal levels of selenium can be determined from the combination of the second study with the present thesis study.

The results from the present study demonstrate that increased dietary vitamin E can exert a significant positive impact on the course of experimental Chagas’ disease in C3HeB/FeJ mice resulting in a depression in parasitemia and an increase in survival. The mechanisms responsible for this alteration in the normal course of Chagas’ disease after infection with a lethal dose of *T. cruzi* were not addressed in this study.

The potential impact of the role of antioxidants on overall health and resistance to infectious diseases should be investigated further. The increasing numbers of people infected with diseases such as drug-resistant tuberculosis and Acquired Immunodeficiency Syndrome (AIDS) make the significance of this study more meaningful because of the emergence of “new” diseases and the resurgence of diseases once thought to be eradicated. Wang et al. (1994) reported that the immune response of female C57BL/6 mice with AIDS was enhanced by dietary vitamin E supplementation. This study measured specific cytokines that are known to be necessary in the response to Human
Immunodeficiency Virus (HIV) infection. The authors of that study suggested a combination of existing medical therapy with nutritional supplementation may provide a more successful treatment approach for HIV-infected individuals (Wang et al., 1994). Antibiotics are not as useful as they once were against many microorganisms; therefore, the immune enhancing ability of antioxidants, such as vitamin E and selenium, may become more important for humans in their war against pathogenic microorganisms.
LITERATURE CITED


Ming, Z. (1994). The role of CD8+ T cells in the immune response to Trypanosoma cruzi in mice held at elevated environmental temperature. Master’s Thesis. Western Kentucky University, Bowling Green, Ky. pp. 21-23.


glutathione peroxidase. Sci. 179, 588-590.


