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The Effect of Selenium Supplementation on the Immune Response of Mice with Experimental Chagas' Disease

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Spring 1995

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Table of Contents

Abstract ----------i

Introduction----------1

Materials and Methods----------13

Results----------18

Discussion----------22

Bibliography----------26

Appendix----------31

Figures

Figure 1---Mean Peak Parasitemia Levels (Comparison of Unsupplemented to Supplemented) p. 31

Figure 2---Mean Peak Parasitemia Levels (Comparison of Unsupplemented to Individual Treatment Groups) p. 32

Figure 3---Average Parasitemia Levels p. 33

Figure 4---Percent Survival (Comparison of Unsupplemented to Supplemented) p. 34

Figure 5---(a, b, c, d) Percent Survival (Comparison of Unsupplemented to Individual Treatment Groups) p. 35

Figure 6--Antibody Levels at Week 7 Post Infection p. 36

Figure 7---Antibody Levels at Week 8 Post Infection p. 37

Figure 8---Antibody Levels at Week 9 Post Infection p. 38

Abstract

Trypanosoma cruzi, the etiologic agent of Chagas' disease, can cause severe pathology in humans. Selenium (Se) has been found to function as an antioxidant which may enhance immunity to disease. To test the effect of Se on the immune response to Chagas' disease, 5 groups of C3HeB/FeJ mice were infected with 10^3 trypomastigotes while receiving Se supplementations of 2ppm, 4ppm, 8ppm, and 16ppm in tap water, with a control group receiving tap water only. After 64 days of infection, survival rates ranged from 0% (control) to 60% (4 and 8ppm). The Se-supplemented groups exhibited a mean peak parasitemia of 4.7 X 10^6 parasites/ml, while the unsupplemented group mean peak parasitemia was 7.0 X 10^6 parasites/ml. Serum antibody titers were measured by using an enzyme-linked immunosorbent assay. At week seven of infection, antibody titers were greatest in groups receiving 4ppm, 8ppm, and 16ppm of Se (titers of 1/128) compared to a titer of 1/64 for the group receiving 2ppm Se. These results demonstrate that Se supplementation can influence the survivorship and immune response of mice infected with a lethal dose of *Trypanosoma cruzi*.

Introduction

History of the Disease

American trypanosomiasis, commonly known as Chagas' disease, has historically been limited to Latin America but has started to show itself in North America. (Wendel and Gonzaga, 1993). There are as many as 16-18 million infected people in South America alone, and the number of immigrants to the United States increases each year, with unknown numbers being infected (Kingman, 1991). There is currently no cure for the disease.

In 1907, while working in Brazil, Carlos Chagas discovered, in certain human dwellings, a haematophagus bug carrying flagellates (later named *Trypanosoma cruzi*) in its hindgut. Through experimentation, he showed that these parasites could be found in the blood of animals that had been bitten by the bugs. In 1909, he found these same parasites in a young girl who was thought to be suffering from malaria. It was then that Carlos Chagas suggested that there might be an acute phase of the yet unknown disease. This led him to further experimentation, from

which he later described a chronic phase (characterized by the disappearance of flagellates from the blood), the agents, the vectors, and some of the signs and symptoms of the disease. (Wendel and Gonzaga, 1993)

The haematophagus bugs that Chagas described are commonly known as kissing bugs. They are from the family *Reduvidae* (subfamily *Triatominae).* When these bugs bite, they defecate, leaving the protozoans on the skin of the victim. Trypomastigote stages then usually enter the body through mucosal membranes. Once within host cells, these parasites will transform into the amastigote stage and differentiate back into trypomastigotes. With the release of these trypomastigotes into the bloodstream, the patient enters the acute phase of the disease. (Wendel and Gonzaga, 1993)

Stages, Signs, and Symptoms

The signs and symptoms of the acute phase of Chagas' disease are varied. Most patients develop a chagoma, a localized swelling that appears at the site where the trypomastigotes enter the body. Other symptoms may include fever, diarrhea, vomiting, enlarged liver and spleen, and electrocardiographic abnormalities. Symptoms usually vanish within four to eight weeks in most adults. However, the acute phase is very dangerous to children under the age of two; three percent of this age group experience mortality from acute myocarditis. Meningoencephalitis is another factor for young children, as mortality due to

meningoencephalitis has been shown to be as high as fifty percent. (WHO, 1991)

The chronic phase of Chagas' disease is preceded by an indeterminate phase, which can be relatively short or can persist indefinitely. There are no signs or symptoms in this stage, but the patient will still test positive for anti-*T. cruzi* antibodies in serological tests. (WHO, 1991)

The chronic phase can take several forms, the best known being the cardiac form. In addition to the hypertrophy of the heart, which weakens the walls of the heart, ventricular conduction defects and varying degrees of arrhythmia can occur (WHO, 1991). Death can be very sudden in this form, potentially due to the bursting of the already thinned walls of the heart. Another form affects the digestive tract and is marked by megaesophagus, causing regurgitation and dysphagia, and megacolon, causing severe constipation and diarrhea (WHO, 1960). The nervous system is the site of the third chronic form, where functional disorders of the cerebellum, alteration of dorsal root ganglions, and alteration of the sympathetic and parasympathetic nervous systems may occur (WHO,1991).

Transmission

Chagas' disease can be transmitted in ways other than by the reduviid bug, such as blood transfusions, congenital transmission, and breast feeding. Because of the increasing number of Latin American immigrants, infection due to blood

transfusions is North America's biggest *T. cruzi* threat. In Southern California alone, forty percent of all blood donors are Latin Americans. At present, blood banks are not mandated to test for *T. cruzi*, largely due to the fact that no commercial screening has been licensed by the FDA (Wendel and Gonzaga, 1993). Serological testing could be implemented, but is "laborious to perform and is not standardized" (Skolnick, 1991).

Los Angeles has started to implement a semi-screening of blood donors for Chagas' disease. Blood bank employees question potential donors about any associations they have had with areas in Latin America where *T. cruzi* is endemic. If the likelihood that these donors are infected with *T. cruzi* is great, then they are not allowed to donate. (Skolnick, 1991)

Treatment

Very little effective treatment is available for *T. cruzi*-infected patients. In the acute stage, nifurtimox and benznidazole may be administered. Both of these are effective against trypomastigotes, and nifurtimox has also been used in higher doses against meningoencephalitis. Allopurinol is another trypanosomicidal drug, but is still being tested for possible side effects. Other drugs, such as anticonvulsants, antiemetics, and antidiarrheals, can be used to offset the symptoms of the acute phase. "Restriction of sodium intake and administration of digitalis and diuretics" may be implemented to lessen the clinical manifestations due to myocarditis (WHO, 1991).

Sedatives and intravenous mannitol are used for acute meningoencephalitis. (WHO, 1991)

The chronic stage of the disease is harder to treat than the acute. Megaesophagus and megacolon are best treated by surgery. Cardiac problems may be treated by a variety of means. Antiarrhythmic drugs such as amiodarone can be used in conjunction with a pacemaker. Reduction of physical activity and peripheral vasodilators are also used. Patients are said to be cured when their serological tests are consistently negative for a year after treatment.

The body will mount its own attack against the disease during the acute phase. Antibody levels increase initially but fall during the course of infection. (WHO, 1960). Cytotoxic T-lymphocytes and natural killer cells are primarily responsible for lysis of the parasite, but "all phagocytic cells associated with the immune system have been found capable of engulfing and killing all developmental stages of *T. cruzi*" (Kuhn, 1989). Although these cells have the capability of destroying the parasite, *T. cruzi*-infected patients exhibit marked immunosuppression. The immunosuppression may, in part, be mediated by suppressor macrophages, which are responsible for the down regulation of helper T cells and the inability of T helper cells to produce interleukin 2 (Kuhn, 1989). Without interleukin 2 and T helper cells, the body is unable to defend itself successfully from the parasite. Selenium is a micronutrient that has been found to enhance the primary immune response and could

therefore be beneficial in the fight against *T. cruzi*.

The Significance of Selenium in Biological Systems

Selenium is an essential mineral that is required in the human diet for maintenance of proper health. It is believed that the role of selenium (Se) in the body is to function as an antioxidant, protecting the body against free radical energy and oxidant stress. The significance of Se can thus be found in its ability to prevent free radical damage and its possible role in preventing disease and other pathologies.

Free radicals arise from the single addition of electrons to an oxygen molecule. The resulting superoxoide anion serves as the parent structure for a group of reduced oxygen products that are associated with oxygen toxicity. The free radicals formed from this parent molecule, such as the hydroxyl radical, can then react destructively with many biological molecules. Another oxygen radical arises when a molecule of oxygen absorbs energy, thereby giving rise to a singlet oxygen. (Harris, 1992)

These molecules and other reactive free radicals may then target molecules in the body such as unsaturated lipids in cell membranes, amino acids in proteins, and nucleotides in DNA, causing disruption of normal cellular function (Harris, 1992).

Selenium Requirements in the Diet

Before the 1950's, the study of Se was largely limited to its toxic effects. After the discovery of several Se-responsive

conditions in animals in the 1960's, attention was brought to the nutritional properties of Se. One study by McCoy and Weswig in 1969 examined the offspring of Se-deficient mice, showing them to be hairless, sterile, and smaller in size than Se-sufficient mice. When in 1973 Se was found to be a constituent of glutathione peroxidase, additional attention was directed toward Se (Rotruck et al., 1973). Most data at that time suggested that a 0.1ug/g diet per day was sufficient for proper health. In 1989, after further research and dietary surveys, the recommended dietary allowance was set at 55ug/day for women and 70ug/day for men.

While the typical U.S. diet does supply the suggested levels of Se (Levander, 1991), there are a limited number of regions in the world in which Se levels have been found to be low. Se-deficient soils in China have been shown to be involved in Keshan disease, a cardiomyopathy that is endemic to broad areas of China (Yang et al., 1988). Finland has also been targeted as one of the low Se areas in the world. Because of these low levels, the Finnish government has added Se to soil fertilizers to increase levels in cereals and grains (Mussalo-Raurhamaa, 1993). Se bioavailability has also been shown to be low in the regional diet of Sao Paulo, Brazil (Cintra and Cozzolino, 1993).

The Functional Role of Selenium in the Cell

Se has a biological function in cells as an essential component of antioxidant enzymes. Included in the seleno-enzymes

is glutathione peroxidase, an enzyme that functions in detoxification of hydrogen peroxide and lipid hydroperoxides (Rotruck et al., 1973). Two additional seleno-enzymes that can also function in preventing oxidant stress are phospholipid hydroperoxide glutathione peroxidase and selenoprotein P. Phospholipid hydroperoxide glutathione peroxidase functions in metabolizing fatty acid hydroperoxides in phospholipids. Selenoprotein P, produced in the liver and secreted into plasma, may also protect against oxidant stress or serve as a Se-transport protein (Burk and Hill, 1992). These types of antioxidant enzymes provide a major cell defense by protecting membrane and cytosolic components from free radical damage. This type of protection becomes particularly important during the immune response when neutrophils and other phagocytic cells release increased quantities of superoxides and hydrogen peroxides during the digestion of foreign organisms (Combs et al., 1975; Sheffy and Shultz, 1979).

The Effect of Selenium on the Primary Immune Response

Se has been credited with the capability of inducing several immunologic responses. Among these is increased antibody production, including enhancement of both immunoglobulin M and immunoglobulin G (Spallholz, et al., 1973a). Along with antibody enhancement, Se may also alter the proliferative capability of T-lymphocytes (Roy et al., 1993).

Studies have indicated that dietary supplementation with

sodium selenate can cause an increase in the amount of sheep red blood cell agglutinating antibody in mice. Mice receiving dietary supplementation of 0.7 and 2.8ppm in chow diets exhibited an enhancement of antibody production as assayed by hemagglutination and plaque-forming cell tests (Spallholz et al., 1973b). An additional study by Spallholz et al., in 1973a, indicated that 3 or 5ug of Se injected intraperitoneally could enhance the primary immune response of mice to sheep red blood cell antigen. The most effective response was shown to occur when Se was administered prior to or simultaneously with sheep red blood cell antigen.

Another study, performed on human subjects, showed an increased ability of peripheral blood lymphocytes to respond to stimulation by phytohemagglutinin and alloantigen. The increased response of the lymphocytes resulted in an increase of high affinity interleukin 2 receptor (IL2-R) on their surface. An increased expression of IL2 receptor was previously found to affect the cell proliferation and clonal expansion of cytotoxic T-lymphocytes in mice. The same regulation of T-lymphocyte-mediated responses was found to occur in humans receiving Se supplementation in the form of a 200ug tablet taken daily (Roy et al., 1994).

Several additional studies have been done to examine the effect of Se on cancer and tumor growth. Schrauzer and co-workers (1976) found that Se supplementation administered in supply water prevented tumorigenesis in C3H/St mice. Examining

the effects of Se on Ehrlich ascites tumor cells, Greeder and Milner (1980) injected Se into mice with transplanted ascites tumor cells, and found that the mice receiving Se supplements had a decrease in tumor cell growth compared to mice without Se supplementation. The protective effect exhibited in the study was dependent on dose and chemical form (Greeder and Milner, 1980). The anti-tumorigenic effect of Se was again demonstrated by Poirier and Milner (1983), in a study utilizing Ehrlich ascites tumor-bearing mice. In this case, survival was found to be significantly increased in Se supplemented mice related to unsupplemented controls. Mammary tumors and mammary cell lines in monolayer cell culture have also been shown to exhibit a significant decrease in growth when supplemented with Se (Medina et al., 1993). This same study also examined the mechanism by which Se activates its chemopreventive effects, suggesting that tumor cells may be blocked in the S-G2 phase of the cell cycle (Medina et al., 1993).

No single mechanism for all cancer types can be applied to the chemopreventive effects of Se, though it has been noted that these effects can be attributed to glutathione peroxidase activity (Rotruck et al., 1973). Several additional mechanisms also have been shown to exert chemopreventive effects, including the inhibition of tumor virus replication, alteration of carcinogen metabolism, and protection against carcinogen-induced DNA damage (Schrauzer, 1992).

Other studies have demonstrated selenium's protective effect

during infectious disease. Desowitz and Barnwell (1980) demonstrated that Se, together with dimethyl dioctadecyl ammonium (DDA), has the ability to enhance the protective immunity of mice to *Plasmodium berghei* (malaria), when given in conjunction with the *P. berghei* vaccine. The Se used in the study was administered to the mice in their drinking water at 2ppm. Although Se alone showed no effect on the *P. berghei* infection without vaccine, mice which were given the Se administration along with the vaccine and DDA exhibited a 90% survival with low parasitemia values (Desowitz and Barnwell, 1980).

Another study examined the response of Se-deficient mice to *Candida albicans* infection. Neutrophils from Se-deficient mice were found to have impaired ability to kill the yeast, *C. albicans*. The authors speculated that this may have been due to decreased glutathione peroxidase levels. Examining microtubule concentrations in the neutrophils, the investigators found the concentrations of these cellular components to be less in Se-deficient mice that in Se-supplemented mice. Because the immune response to *Candida albicans* infection is greatly dependent on neutrophil activity, Se deficiency may result in an impaired immune response to the infection (Boyne and Arthur, 1986).

In the present study we have examined the effect of Se on infection of mice with the protozoan parasite *Trypanosoma cruzi*. Because of the findings of previous studies, we hypothesized that Se may also have an effect on the immune response to *T. cruzi*. By studying parasitemia levels, mortality, and antibody titers

during the course of *T. cruzi* infection in mice, the effect of various doses of Se supplementation on the course of experimental Chagas' disease could be determined.

Materials and Methods

Mice

Thirty female C3HeB/FeJ mice (Jackson Laboratories, Bar Harbor, Maine) were used in this study. The mice were divided randomly into six groups of five mice each and were given Purina Rodent Chow and tap water ad libidum throughout this study.

Selenium Supplementation

Mice in both the control group and group 1 were maintained on Purina Rodent Chow and tap water. Starting two weeks prior to infection, anhydrous sodium selenate was added to the drinking water of groups 2-5 throughout the course of the study as follows: Group 2-2ppm, Group 3-4ppm, Group 4-8ppm, and Group 5-16ppm. The Purina Rodent Chow contained an additional 0.2ppm.

Parasitemia Determination

Each mouse in groups 1-5 was infected on October 4, 1994, with 1000 blood-form trypomastigotes of *T. cruzi* (Brazil strain) by intraperitoneal injection. Weekly, beginning October 18th, 4ul of blood was collected from the tip of the tail of each mouse for a parasite count. The 4ul sample of blood was diluted with 96ul of Dulbecco's phosphate buffered saline (DPBS), (Sigma Chemical Co., St. Louis, MO), and the number of parasites counted using a hemacytometer.

Antigen Preparation

To prepare antigen for use in the enzyme-linked immunosorbent assay (ELISA), a PSC3H murine fibroblast cell line infected with *T. cruzi* trypomastigotes was cultured in RPMI-1640 medium supplemented with 20% newborn calf serum. The cell culture reagent RPMI-1640 was made by hydrating RPMI 1640 media (Sigma Chemical Co., St. Louis, Mo.) in distilled water. The medium was then supplemented with 20% newborn calf serum (Microbiological Associates), 25mM of HEPES (Sigma), and penicillin/streptomycin. The medium was then filter sterilized. Cells were cultured in sterile T-flasks and incubated in 6% CO_2 in air and high humidity.

The culture supernatants containing trypomastigotes were removed from each T-flask and filtered through a Whatman #1 filter paper. The filtered supernatant was then centrifuged for 20 minutes at 1800 RPM. The supernatant was removed and

discarded and the remaining pellet (containing parasites) was resuspended in 500ul of PBS. The parasites were then centrifuged again for 20 minutes at 1800 RPM. The supernatant was removed and the pellet resuspended in 500ul of PBS.

Following the final wash, the combined parasite pellet was resuspended in ice-cold PBS containing 0.5% Triton X (Sigma), placed on ice, and vortexed multiple times over a 30-minute period. The resulting solution was spun at 8,000 RPM for 20 minutes to remove non-soluble material; and the supernatant was collected. A protein assay was performed on the antigen solution to determine concentration by utilization of the Biorad Assay kit (Biorad), using bovine serum albumin as a standard, according to manufacturer's instructions.

Preparation of Mouse Plasma

Plasma from infected and non-infected mice was collected weekly during the course of the *T. cruzi* infection. Plasma was collected from each mouse in each group from weeks 3 to 9 post-infection. 20ul of blood was drawn from each mouse via a tail bleed and diluted in 80ul of DPBS. Diluted blood was then centrifuged at 8,000 RPM for 10 minutes. The diluted plasma was then removed and stored in a -20°C freezer until use.

For use in the ELISAs, plasma from all mice in each group was combined and used as a single sample for each week of infection. As a result, there were 5 samples for each week of infection, one from each of the groups. Groups containing fewer

than 200ul of serum on later weeks of infection, due to mortality, were not measured for antibody levels.

Indirect ELISA

Individual wells of 96-well microtiter plates were incubated with 100ul of a 10ug/ml concentration of *T. cruzi* antigen for approximately 48 hours at 4°C. After incubation with antigen, the plates were then rinsed 3 times with 1xPBS using a squeeze bottle, with equal pressure applied throughout when rinsing and flicking each plate dry in between washing. The plates were then patted dry. A 2.5% Carnation Instant Non-fat Milk solution in 1xPBS was used as a blocking solution. 200ul of blocking solution was put into each well and incubated for one hour at room temperature.

200ul of the 1:4 diluted plasma was diluted in 200ul of 2.5% milk blocking solution in order to produce a 1:8 dilution. A two-fold serial dilution was duplicated for every group each week that plasma was collected.

After all dilutions were made, plates were incubated at 37°C for 2 hours. After incubation, plates were washed 4X with 1XPBS as previously described. For use as a secondary antibody, a goat anti-mouse immunoglobulin conjugated with horseradish peroxidase (H+L)-HRP(Southern Biotechnology Associates, Inc.) was diluted 1:1000 in a 2.5% Carnation skim milk solution in PBS. 200ul of the conjugated secondary antibody solution was then added to each well and incubated at room temperature for one hour. After

incubation, plates were rinsed with an automated micro-plate rinser. o-Phenylenediamine dihydrochloride (Sigma) was used as the substrate. Each tablet contained 30 mg and was diluted in 10ml of Tris-citrate buffer. The substrate solution was then activated with hydrogen peroxide, and 50ul of the substrate was added to each well and incubated for 15 minutes at 37°C. After the incubation period, plates were read in duplicate by a Microplate reader (Property of Vanderbilt University) for absorbance values at 490nm. Antibody titers were determined according to the following equation:

Titer=Highest dilution of test serum in which the absorbance is > mean control absorbance $\pm$ 2 standard deviations and > .05nm.

Statistics

An ANOVA (analysis of variance) was done to test for treatment effects on parasitemia levels between groups. An A Posteriori test of each supplemented group versus control was performed to determine statistical differences in parasitemia and antibody levels between treatment groups. Because this analysis involved multiple tests, the Bonferrom correction was applied. Significance was assessed based on Bonferrom-adjusted criteria. ($P_{critical}$ = 0.5/# of tests = 0.05/4 = 0.0125) There was insufficient data available to perform a statistical correlation between antibody and parasitemia levels.

Results

Parasitemia Values

Mice infected with 10^3 blood-form trypomastigotes began exhibiting parasites on week 3 (day 35) of *T. cruzi* infection. Parasitemia levels were low (less than 3.0×10^5 parasites/ml) in the first four weeks of infection for all treatment groups and increased to peak levels on week 7 (day 50) post infection.

Mean peak parasitemia levels were determined for each treatment group by averaging the highest parasitemia value obtained during the first 8 weeks of infection for each mouse in that group. After averaging individual groups, parasitemia values for all supplemented groups were also averaged. The mean peak parasitemia for all supplemented groups was 3.9×10^6 parasites/ml compared to 7.0×10^6 parasites/ml for the group without Se supplementation. Supplementation of the mice infected with *T. cruzi* resulted in a 43.7% decrease in the number of parasites per ml in the combined treatment groups (Fig. 1).

The mean peak parasitemia levels for each group during the

entire course of infection were 7.0 X 10^6 parasites/ml for the unsupplemented infected group , 4.7 X 10^6 parasites/ml for the group receiving 2ppm Se, 2.75 X 10^6 parasites/ml for the 4ppm Se group, 5.26^6 X 10^6 parasites/ml for the group receiving 8ppm Se, and 3.0 X 10^6 in the group supplemented with 16ppm Se (Fig. 2).

Multiple A Posteriori comparison tests for significant differences between treatment groups indicated that the parasitemia levels of the Se-supplementation groups were significantly different ($p=.002$) from the groups without Se supplementation. The groups receiving 4ppm and 16ppm of Se were also found to be significantly different ($p=.001$ and $p\leq.001$, respectively,) from the unsupplemented group. Groups receiving 2ppm and 8ppm were not found to be significantly different from the unsupplemented group based on Bonferrom adjusted criteria ($p=.05$ for 2ppm; $p=.037$ for 8ppm).

Mean parasitemia levels for each week of infection were also determined for each group of mice. These values were obtained by averaging the parasitemia values of each mouse in each group for each week of infection. The progression of parasitemia levels during the course of infection is illustrated in Fig. 3.

Survival

Mice in group 1 (unsupplemented) began to show mortality by day 34 of infection and reached 0% survival by day 56. The percent survival was also determined for each Se treatment group throughout the study, and an average of percent survival for all

the supplemented groups was calculated. Percent survival for the unsupplemented group versus the average of the supplemented groups is shown in Fig. 4. The supplemented groups showed an average of 40% survival at day 69 of infection. Results for individual groups are shown in Fig. 5. Groups exhibiting the highest percent survival were those receiving 4ppm and 8ppm Se (60% survival at day 69). The mice in these two groups maintained 100% survival until day 37 and 43, respectively. Groups 2 (2ppm Se) and 5 (16ppm) exhibited the lowest survival (20%) of any of the supplemented groups. Group 2 reached 20% survival by day 56, and Group 5 reached this point by day 69.

ELISA

Antibody titers were determined for treatment groups during weeks 7, 8, and 9 of infection. Groups exhibiting high mortality before week 7 and having fewer that 200ul of plasma per sample were not measured for antibody titers.

Antibody titers were determined for groups receiving 2ppm, 4ppm, 8ppm, and 16ppm Se during week 7 of infection. The antibody titer for group 2 (2ppm) was 1/64, for group 3 (receiving 4ppm) was 1/128, for group 4 (8ppm) 1/128, and for group 5 (16ppm) 1/128 (figure 6).

Antibody titers for week 8 post infection were found to be 1/128 for group 3, 1/128 for group 4, and 1/64 for group 5 (figure 7).

Only two treatment groups remained at week 9 post infection.

Group 3 receiving 4ppm of Se again exhibited a titer of 1/128, while the group 4 receiving 8ppm exhibited a titer of 1/64 (figure 8).

Antibody levels in the control non-infected group were measured for each week of infection. All exhibited absorbance levels close to zero with small standard deviations.

Discussion

Previous studies have demonstrated that Se is beneficial in improving the immune response of mice. For example, Spallholz et al. (1973) demonstrated that the primary immune response to sheep red blood cells could be improved by supplementation with Se and found optimal Se levels to be at 0.7 and 2.8ppm to enhance antibody production, while Desowitz and Barnwell (1980) found 2ppm to have a positive effect on infection with *Plasmodium berghei*. In a more recent study by Roy et al. (1994), peripheral blood lymphocytes of humans showed an increased ability to respond to stimulation when given Se supplementation. Se has also been shown to have a positive effect on other infectious diseases and a chemopreventive effect on cancer in mice (Medina et al., 1983).

In the present study, the effects of Se on the course of

experimental Chagas' disease were examined. Mice in groups supplemented with Se exhibited mean peak parasitemias at levels 50% below the control group. C3HeB/FeJ mice are highly susceptible to *T. cruzi* and normally die during the acute phase of the disease. None of the supplemented groups exhibited 100% mortality. At the end of the study, parasitemia levels dropped to zero for three weeks in all surviving mice (supplemented groups only). As of April 26, 1995, these mice continued to remain alive and showed no visible sign of disease. Further experimentation would be necessary to examine the mice for pathology and to determine if any intracellular stages of the parasite remain. No mortality was observed after day 69 of infection, leaving groups 2 and 5 at 20% survival, and groups 3 and 4 at 60% survival.

Groups 3 and 4 proved to have the optimal levels of selenium supplementation, 4ppm and 8ppm respectively. Although the mean peak parasitemia for group 4 was not found to differ significantly from that of the control group, one mouse in group 4 had an uncharacteristically high peak parasitemia compared to the rest of the mice in the same group. Excluding the data for this mouse reduces the mean peak parasitemia for group 4 to 3.5 x 10^6 parasites per ml, which is statistically different from the control group. By contrast, although group 5 had a low mean peak parasitemia level, mortality in this group was 80%. It may be that Se supplementation initially improved the immune response in group 6, but the high levels of Se (16ppm) could have eventually

become toxic to the mice. Further testing will be required to evaluate this hypothesis.

The production of antibodies is an important mechanism in the body's immune response to Chagas' disease. Previous research has shown that Se supplementation does enhance antibody titers in mice (Spallholz et al., 1973). Results of the assay performed in this study do confirm that a parasite-specific antibody response was mounted in the mice infected with *T. cruzi*. However, the results obtained were inconclusive in determining the effectiveness of Se supplementation on antibody levels. Antibody titers were determined only for weeks 7, 8, and 9 of infection. Group 1, receiving no Se supplementation, exhibited a high mortality rate by week 7 and a 100% mortality rate by week 8 of infection. Antibody titers were therefore not determined for this group, and because of this, we were unable to make comparisons between the supplemented and unsupplemented groups.

Comparisons made between treatment groups receiving Se indicated that there were no statistically significant differences in absorbance measurements between any treatment groups. Despite this lack of significance, titers were found to be higher in groups 3, 4, and 5 than in group 2. Titers in group 3 remained consistent over the three weeks of infection, exhibiting a titer of 1/128. Titers in group 4 remained 1/128 until week 9 of infection. Antibody titers dropped in group 5 during week 8 of infection with a drop in survival rates also seen in this group.

These results may be inconsistent with the normal course of antibody levels in Chagas' disease. The World Health Organization, 1960, found that antibody levels drop during later stages of infection, typically during the chronic stage of infection in which there are no parasites found in the peripheral blood. The antibody titers obtained for the groups receiving 4ppm and 8ppm of Se remained high even when parasitemia levels reached zero in the peripheral blood. These results may suggest that Se does have some effect on antibody production, but further study is needed to examine more completely the effect of Se on antibody production.

Although Se may not be a cure for Chagas' disease, it appears to have had a protective effect in an experimental model of the disease, possibly by improving the immune response. Levels of 4ppm and 8ppm Se exhibited a positive effect on the parasitemia values, survivalship, and antibody levels examined in this study.

Further experimentation will be required to determine the mechanism(s) by which Se improves the immune response. Other immunologic responses should also be analyzed in order to more carefully monitor the effect of Se on this experimental disease model. Repeating this study, using higher and more closely controlled levels of Se could possibly reveal stronger correlations between Se and Chagas' disease.

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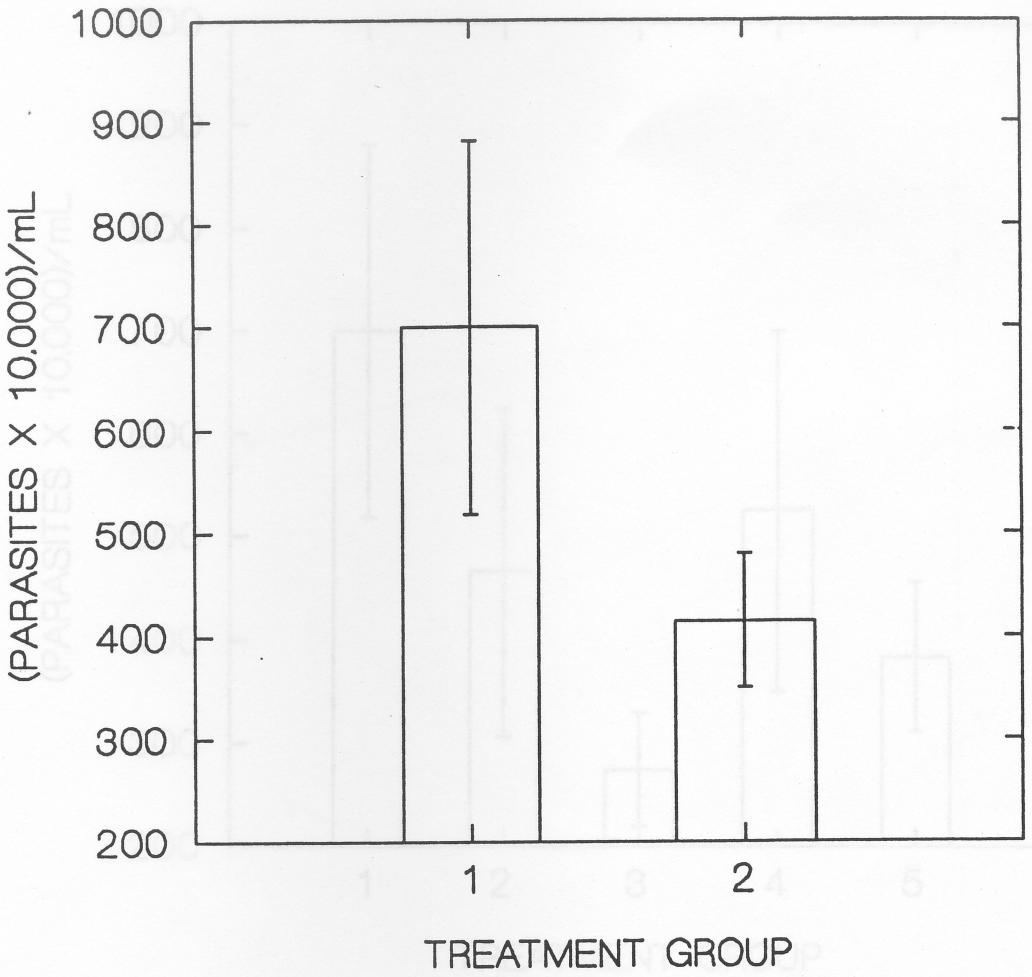

Figure 1. Mean Peak Parasitemia Levels. Mean peak parasitemia levels were averaged for each group through ten weeks post-infection. Group 1-unsupplemented, Group 2-supplemented. Bars represent SEM. SEM's reveal a statistically significant difference between groups.

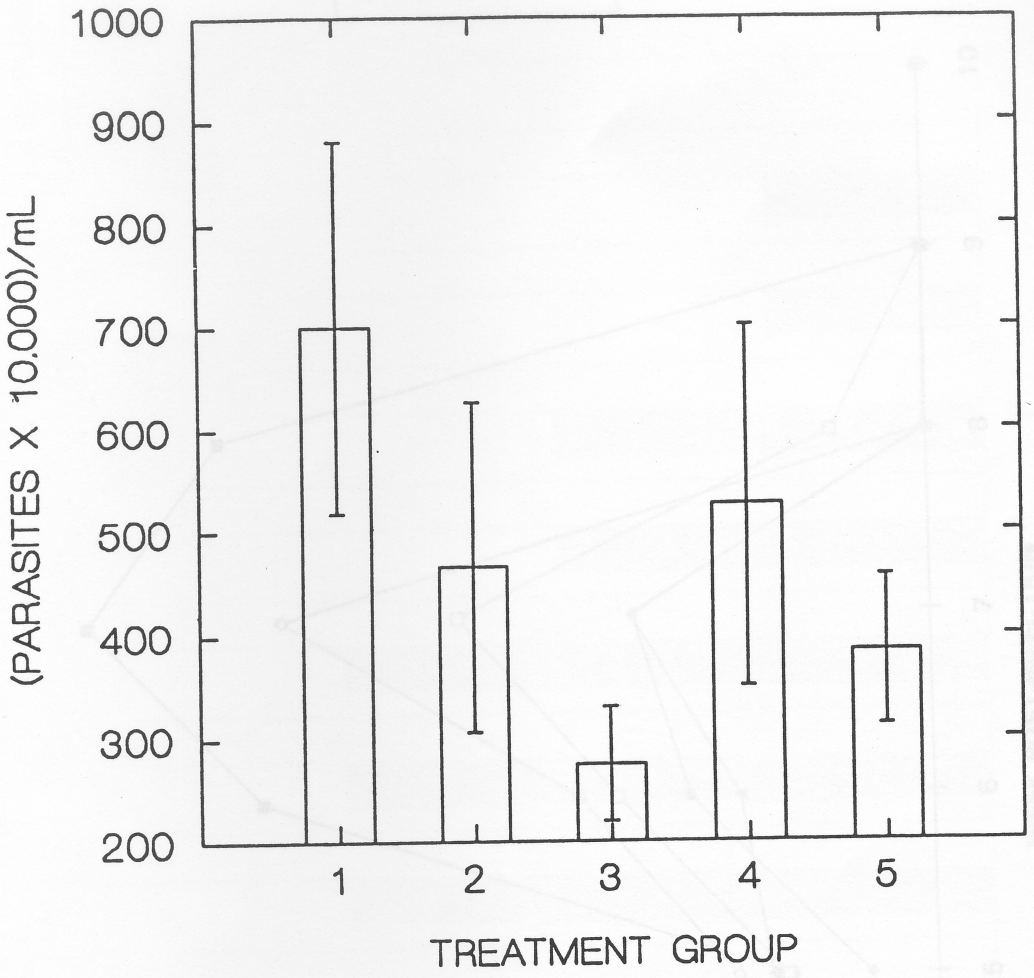

Figure 2. Mean Peak Parasitemia Levels. Graph shows a comparison of control to individual treatment groups. Group 1-unsupplemented, Group 2-2ppm, Group 3-4ppm, Group 4-8ppm, and Group 5-16ppm. Bars represent SEM. SEM's reveal statistically significant differences between groups 1 and 3 and groups 1 and 5.

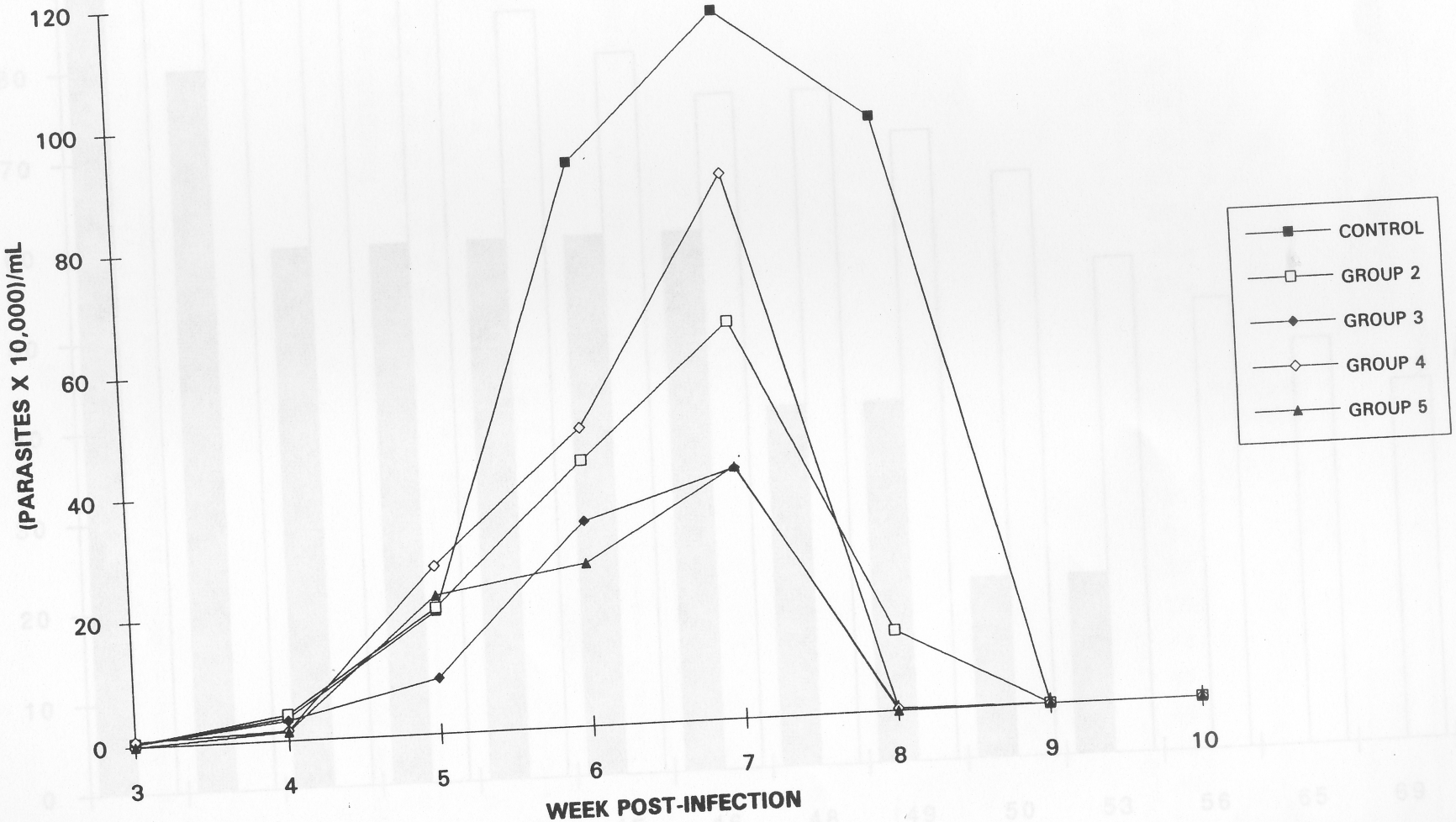

Figure 3. Average Parasitemia Levels. Average parasitemia levels for all groups through week ten post-infection. Control-unsupplemented, Group 2-2ppm, Group 3-4ppm, Group 4-8ppm, and Group 5-16ppm.

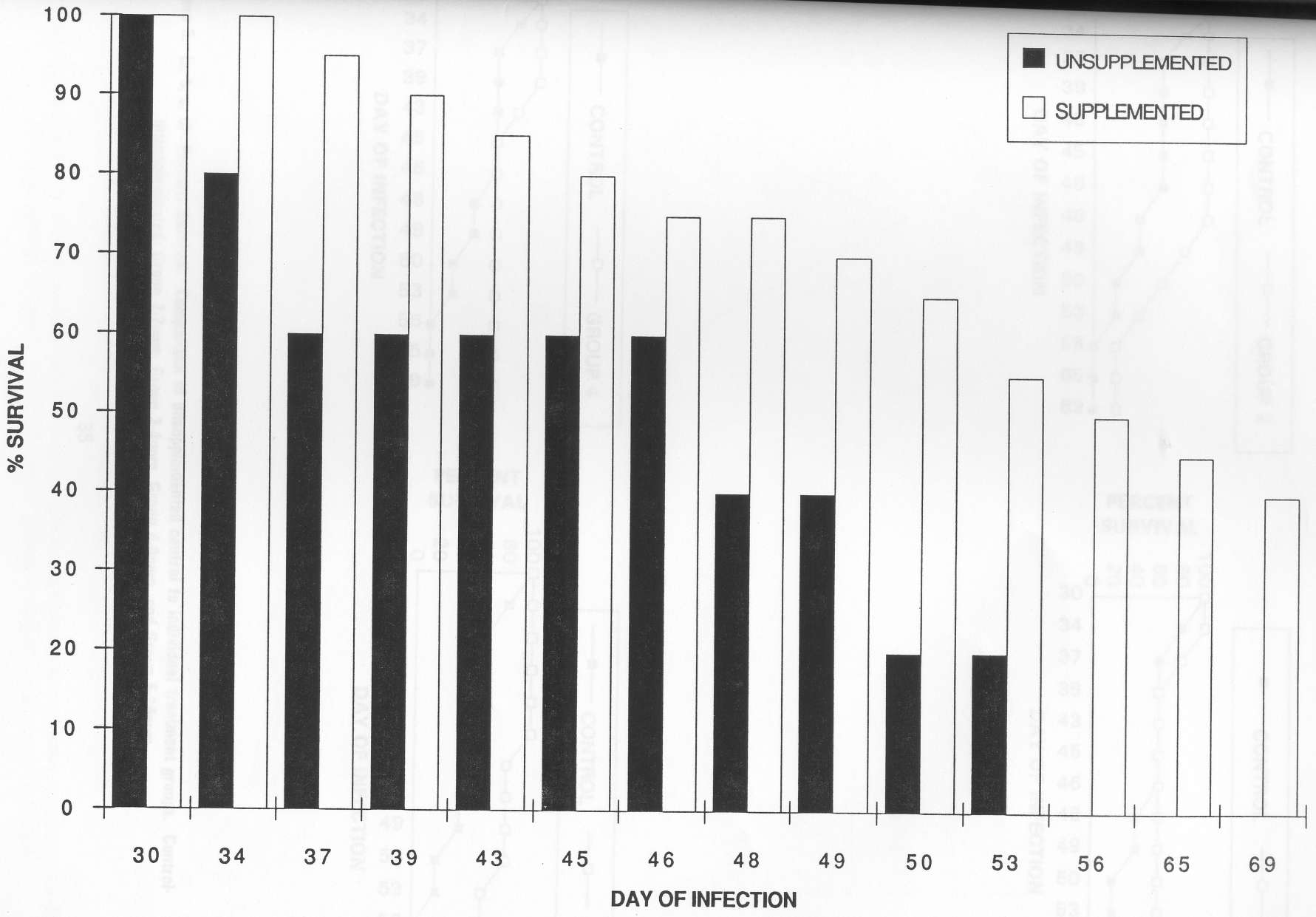

Figure 4. Percent Survival. Comparison of unsupplemented to the average of supplemented groups. Data for all supplemented groups pooled and the average percent survival was calculated for each day of infection.

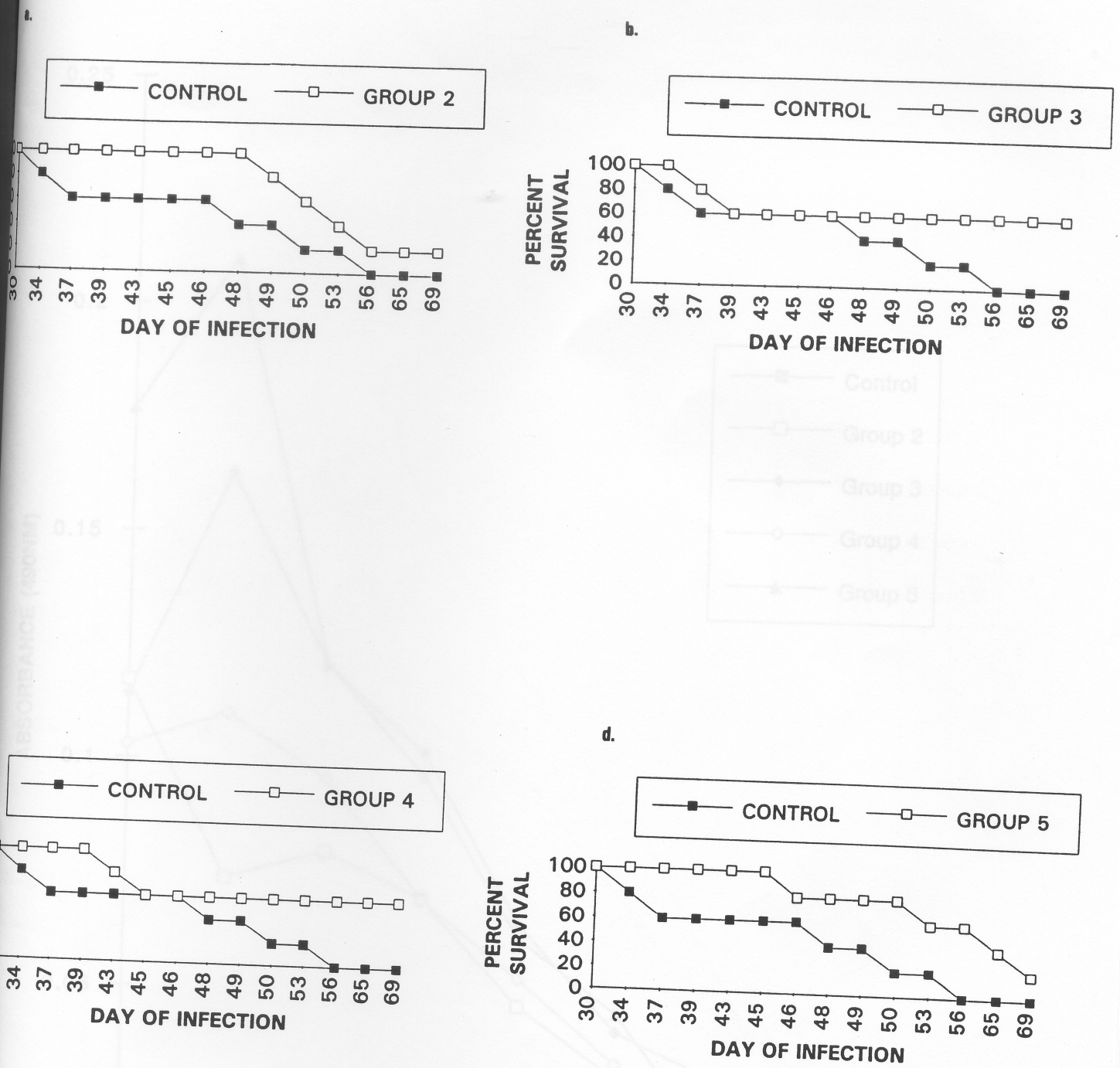

Figure 5. (a, b, c, d) Percent Survival. Comparison of unsupplemented control to individual treatment groups. Control-unsupplemented, Group 2-2ppm, Group 3-4ppm, Group 4-8ppm, and Group 5-16ppm.

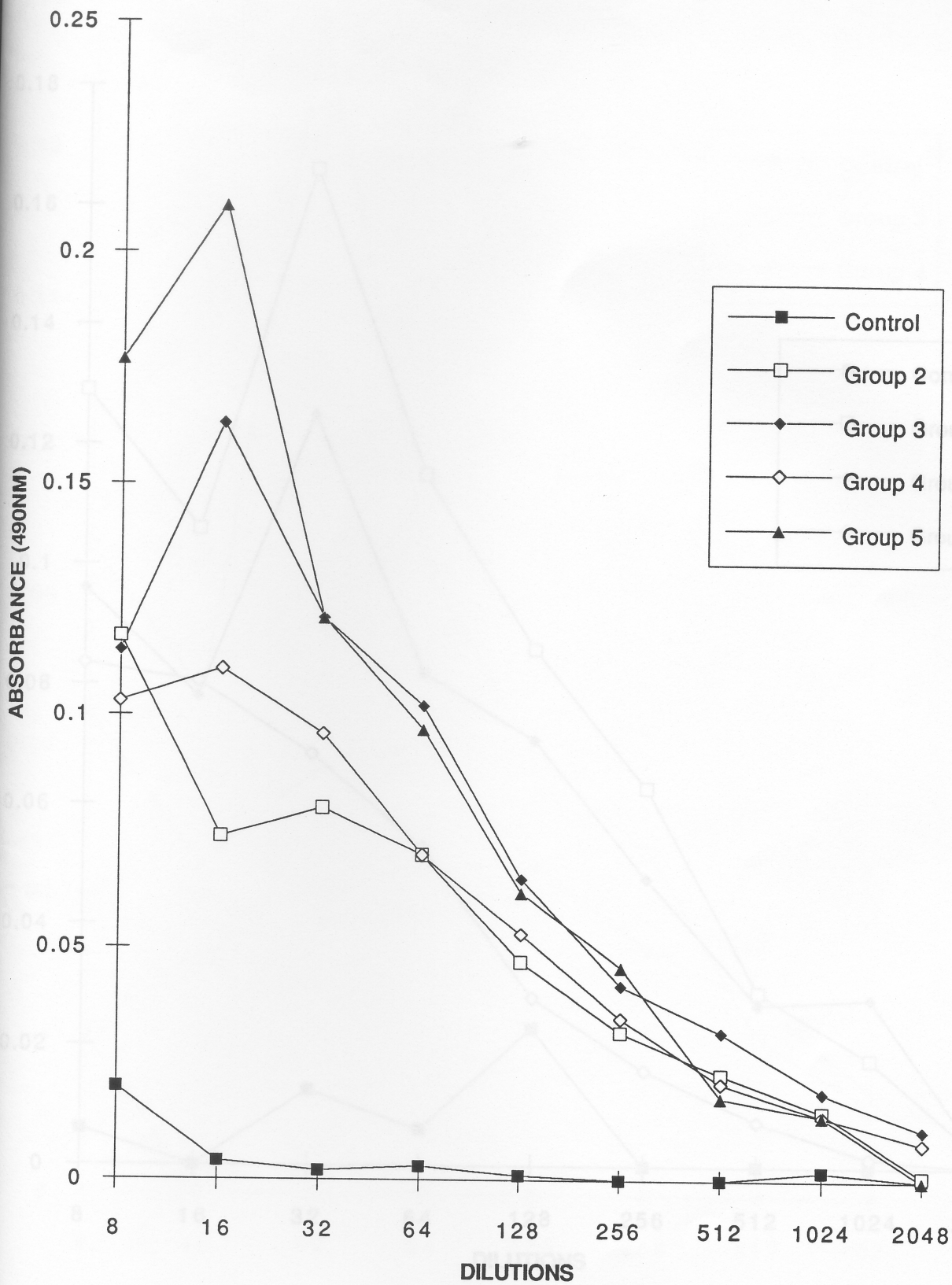

Figure 6. Antibody levels at week 7 post infection. Data represents absorbance values from ELISA. Control, group 2-2ppm, group 3-4ppm, group 4-8ppm, and group 5-16ppm.

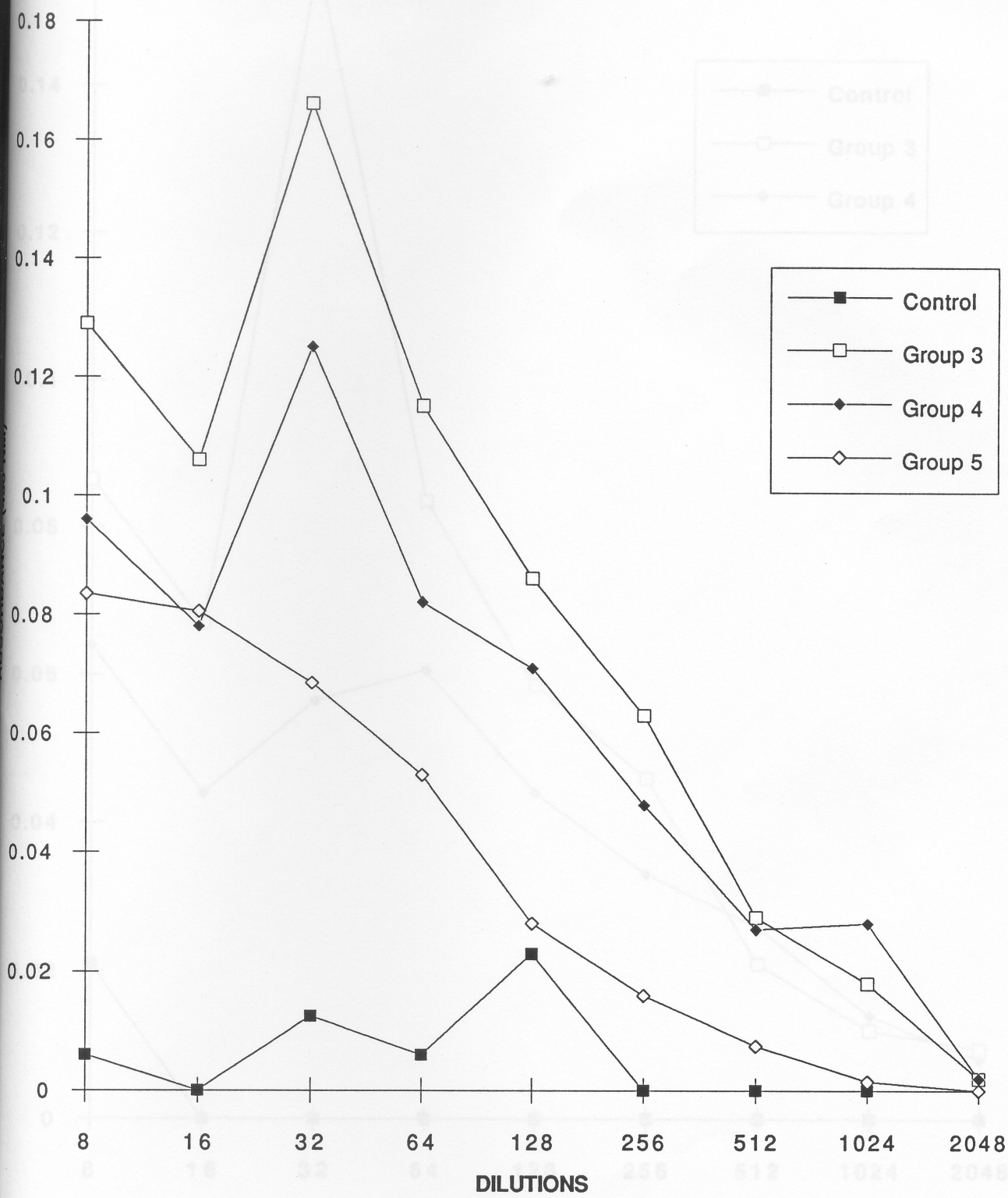

Figure 7. Antibody levels at week 8 post infection. Data represents absorbance values from ELISA. Control, group 3-4ppm, group 4-8ppm, and group 5-16ppm.

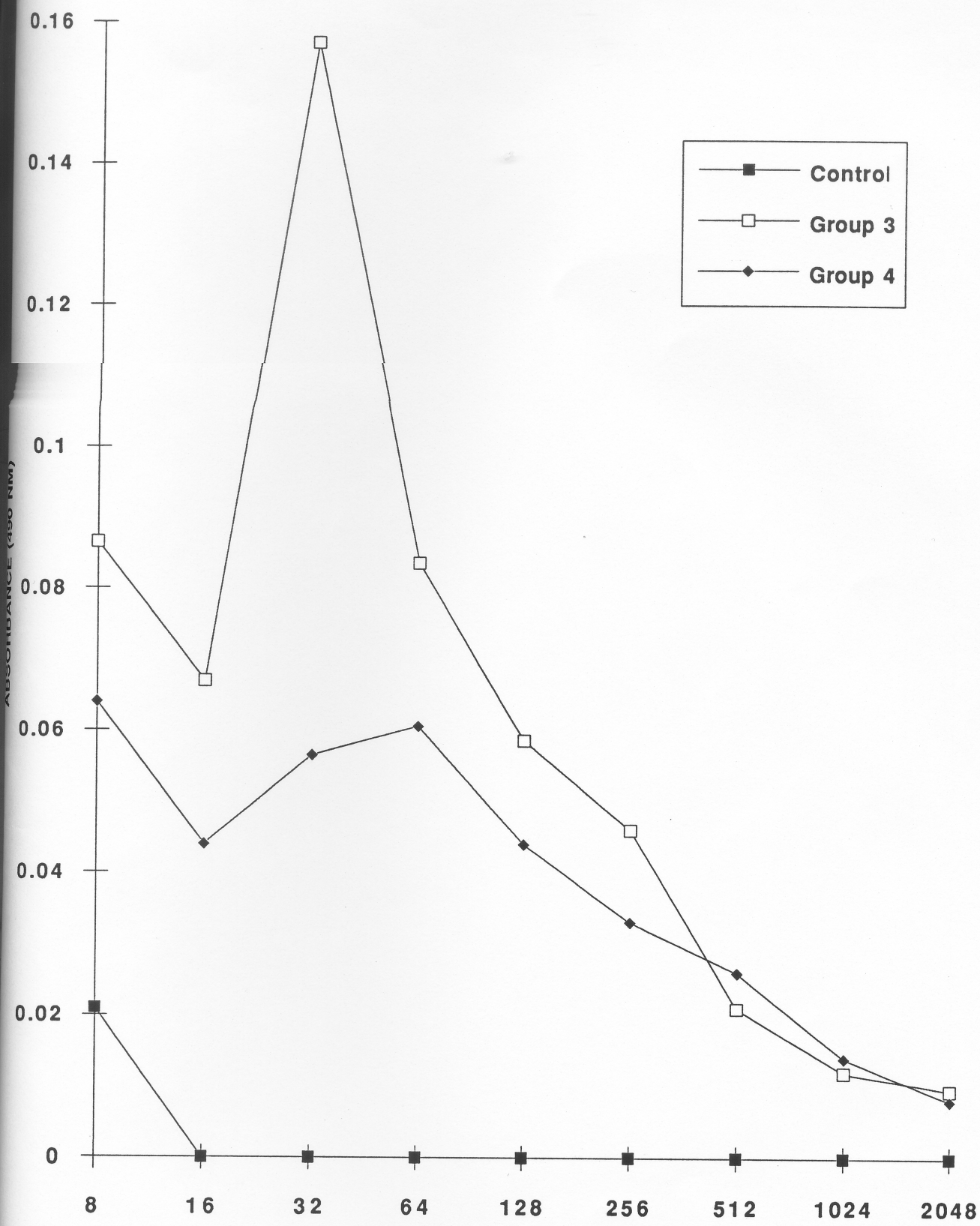

Figure 8. Antibody levels at week 9 post infection. Data represents absorbance values from ELISA. Control, group 3-4ppm, and group 4-8ppm.