PROTECTIVE EFFECT OF MAGNESIUM DEFICIENCY AGAINST BABESIOSIS IN THE RAT

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in partial fulfillment of the requirements
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July, 1970

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PREFACE

This study of the interrelationships between hemotropic parasitism and magnesium deficiency was initially planned to use anaplasmosis in the calf as the disease model. When Dr. A. D. Tillman left Oklahoma State University for a position with the Ford Foundation it became necessary to revise the scope of the study. The disease model of babesiosis in the rat was selected for its similarities to anaplasmosis, for the availability of animal facilities, and because of economic considerations. The interrelationships between Babesia rodhaini infection and dietary magnesium levels were studied in the rat.

E. W. Jones, M.R.C.V.S., Ph.D. and Allen D. Tillman, Ph.D., assisted the author in obtaining fellowship support for this study. Their assistance is greatly appreciated; without it this work would not have been possible.

Gratitude is expressed to the author's committee, Robert Totusek, Ph.D. (Chairman), Don Wagner, Ph.D., E. Wynn Jones, M.R.C.V.S., Ph.D., and W. E. Brock, D.V.M., Ph.D., for their guidance and help. Ron R. Johnson, Ph.D., provided valuable counsel with the experimental design as well as some laboratory support. Robert Morrison, Ph.D., assisted in the selection of appropriate statistical analytical procedures. The work of these men is appreciated.

Without the technicians of the Clinical Research Laboratory, Sandy Ramsey, Wanda Edwards, and Jane Marks, this work would have been most difficult. Thanks is extended to them. Richard Browne, M.S., of the

Statistical Laboratory was most helpful to the author with the computer processing of data for statistical analysis.

The author wishes to extend special thanks to three men: Professor Allen D. Tillman, for encouraging the author to work in the field of nutrition; and to Professor E. W. Jones and Dean W. E. Brock for their continued long term support, moral and otherwise, of his work with hemotropic parasitism.

The proofreading done by my sister, Miss O. L. Norman, C.P.S., is acknowledged and appreciated. My typist, Miss Majel Stites, is extended thanks for her patience and efforts on my behalf.

The author is indebted to his wife, Catalina, and his children for their sacrifices. His wife's cheerful support made the work much less difficult.

This work was done while the author was a Post-Doctoral Fellow (FO2-AI-40-748-02) of the National Institute of Allergies and Infectious Diseases, United States Public Health Service. The author is grateful to the Institute for making this support available.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

In studies of the interrelationships between infection and nutrition, two broad categories emerge: synergism and antagonism. Malnutrition may lower host resistance to infectious disease. Infection may increase the severity of pre-existing malnutrition. When the interaction causes a disease process more severe than either infection or malnutrition alone, the interaction is synergistic. Under some circumstances, malnutrition may do more to inhibit the multiplication of the disease agent than to the lowering of host resistance. In this case, the infection-malnutrition interaction is antagonistic (1).

The observations that more than one type of deficient diet may be synergistic to a disease process raise the question of whether the same mechanism is affected by several types of deficiencies or whether there is more than one type of deficiency mechanism. It has been suggested that besides humoral defenses there are local defenses in the cell which may be influenced by the nutritional status of the host (2).

In the case of antagonism, Scrimshaw and coworkers (3) have presented the following generalizations: a) specific nutrition deficiencies more frequently cause antagonistic responses than do generalized deficiencies, b) antagonistic interactions are usually due to a lack of one or more particular nutrients for which the infectious organism has more dependence than the host, c) antagonism is more prevalent when

infectious agents are highly dependent on host metabolism and, d) antagonism seldom occurs through alteration of tissues which physically interfere with invasiveness or spread of a disease-causing agent.

Experiments can be cited in which nutrient intake in excess of physiologic requirements has altered host response to infectious diseases (4). Both synergistic responses (5,6) and antagonistic responses (7,8) have been observed. An excess of one nutrient may alter the requirements for other nutrients, such as has been described for the relationship between certain amino acids (as with methionine and tryptophan) (4), and for the relationship between excess zinc levels and calcium, phosphorus and magnesium metabolism of young rats (9).

Numerous workers have investigated the interactions of nutrition and disease processes (10). Gieman (11) and Jacobs (12) have reviewed dietary factors in relation to hemoprotozoan infections. These factors included ascorbic acid, blood sugar, thiamine, riboflavin, pantothenate, pyridoxine, vitamin A and lysine. The absence of P-aminobenzoic acid in milk has been shown to suppress development of malarial protozoan parasites in rodent, simian and avian species (12, 13). Gieman concluded that parasites respond selectively to the hosts' dietary deficiencies. Zuckerman (13) inferred that parasitism may be defined in terms of biochemical and biophysical deficiencies of the parasite for which the host is compensating, and when the compensatory capabilities of the host are impaired as in dietary deficiencies, the host becomes an altered milieu.

Nutritionists and protozoologists using avian malaria models have demonstrated nutritional interactions in the following experiments: a) with ducks and chickens it has been shown that biotin deficiency

lowered resistance to Plasmodium lophurae and Plasmodium cathemerium, measured by the degree of parasitemia. This response occurred prior to outward signs of host deficiency (14,15); b) riboflavin deficiency increased resistance of chickens to Plasmodium lophurae (16); and c) Roos and coworkers (17) detected no interaction from thiamine deficiency, a questionable one from choline deficiency, a lowered resistance from niacin deficiency, and an apparent increase in resistance from vitamin A deficiency.

Brock (18) detected a slight difference in response to infection with the hematropic parasite, Anaplasma marginale in splenectomized calves fed at different energy levels.

Bedell and Miller (19) have presented data (see Appendix C) indicating the possible antagonism of magnesium deficiency to development of Anaplasma marginale infection in the young dairy calf. They observed antagonism in calves with serum magnesium levels between 1.0 and 0.8 mg percent. Levels below 0.8 mg percent caused death due to deficiency disease. With levels above 1.0 mg percent, clinical anaplasmosis occurred with its resultant severe anemia. The subclinical state was maintained for longer than 4 months. Circulating blood parasites were not observed during this period, but the blood was infective when subinoculated into splenectomized calves. When changed from the purified casein-starch-corn oil magnesium deficient diet to commercial calf rations, calves with subclinical disease usually died within a week to 10 days from anaplasmosis.

Anaplasmosis has disease aspects resembling babesiosis (20). The knowledge that species of anaplasmata and babesia may exist as mixed infections in the same host dates back to the classical studies of Smith

and Kilborne (21) published in 1893. Species of anaplasmata have not been identified in the rat, but there is literature available on the pathogenesis of Babesia rodhaini (Van den Berghe, et al, 1950) in intact and splenectomized rats (22,23,24).

There is extensive information concerning magnesium metabolism in the rat. Controlled studies of magnesium deficiency in rats date back to the 1930's (25,26,27). Present day studies on this subject are numerous (28,29,30,31,32). McAleese and Forbes (33), feeding a diet containing 20 p.p.m., observed marked hyperemia (reddening) of the ears after 3 to 5 days on the diet. Edema of the ears followed. Later hyperemic and edematous changes of the nose and paws were observed. The deficient animals became hyperexcitable. The hyperemia faded after 7 to 9 days and was replaced by a blanched appearance. Later hyperemia reappeared and persisted. Occasional fatal clonic convulsions were observed. Growth was severely retarded when compared to controls on 200 p.p.m. dietary magnesium. Spotty loss of hair (25) and skin lesions appear later in the deficiency (34).

Young rats fed magnesium deficient diets (less than 100 p.p.m.) had degenerative changes of hepatic tissue, of the myocardium, of the Purkinje cells of cerebellar tissue, and degeneration and calcification of renal tissue (35). In the rat, mitochondrial swelling, followed by the appearance of lipid droplets in cells of the proximal convoluted tubules was reported (35) occurring very early (sic, first few days) in the deficiency syndrome. After prolonged depletion the total body magnesium was reduced by 33 per cent, and total body calcium was 230 per cent of normal levels. Factors disturbing calcium metabolism might be expected to exert the same effect on magnesium deficiency (35).

Many experiments have shown the need and uses of magnesium in immunological systems (36,37,38,39). The presence of magnesium is essential to the action of properdin with complement (40). It has been reported that divalent cations are required for phagocytosis (41). However, Bryan (37) has presented data highly suggestive that ingestion per se, by a phagocyte, is not a cation dependent process. Henon and Delaunay (42) presented evidence for the necessity of divalent cations for the maintenance of chemotactic sensitivity of polymorphonuclear leukocytes. Their data show magnesium ions to be more effective than calcium ions. Opsonization is a magnesium-supported process (37).

Magnesium (Mg) is required by many biochemical systems (43,44). It is well known that Mg plays a key part in oxidative phosphorylation and has regulatory properties in specific enzyme systems (45,46,47,48,49,50, 51). In vitro studies indicate that the integrity of mitochondria and the maintenance of oxidative phosphorylation may depend on the presence of a Mg-ATP complex. This complex is dissolved by increased calcium ion concentration. Dissolution of this complex leads to disruption of the energy producing systems due to coenzymes. Similar mitochondrial changes can be shown in vitro by suspension of mitochondria in a medium free of magnesium ions (35).

The extensive W.H.O. review (10) previously cited covers almost 500 selected references to nutrition interactions with infection. Only one study was listed in which the effects of a magnesium (Mg) deficiency upon an infectious agent were investigated. A magnesium deficient diet had no effect on the percentage of mice paralyzed after infection with Theiler's mouse encephalomyelitis virus (52).

Bienvenu and coworkers (39) reported a direct relationship between

the loss of bactericidal (to Brucella spp.) activity of serum and decreased serum Mg levels. Added Mg restored the bactericidal activity. Data suggested that high serum gonadotropin levels reduced the magnesium available for participation in the properdin system of the bovine animal.

McCreary et al, (53) have reported the protective effect of magnesium deficiency on experimental allergic encephalomyelitis (EAE) in the rat. This deficiency was accompanied by a markedly increased tolerance to the EAE antigenic stimulus. Impairment of the ability of the magnesium deficient rats to produce antibody against a specific antigen (pertussis vaccine) was not detected. The degree of EAE was inversely proportional to the severity of the Mg deficiency myopathy.

In view of the possible role of magnesium in the development of hemotropic parasites (19) and the scarcity of studies in this field (10), it was decided to explore the influence of dietary magnesium levels upon the pathogenesis of Babesia rodhaini in the rat. Since these two areas of study are somewhat well defined in the rat, it should be possible under controlled conditions to detect synergistic or antagonistic interactions between babesiosis and magnesium deficiency. Concurrent manipulation of zinc levels was considered for its possible effect on magnesium retention (9).

CHAPTER II

MATERIALS AND METHODS

Experimental Animal

Random bred white male rats were used as the experimental animal. The rats were shipped from the breeding laboratory[1] via Air Express[2] and were in transit for 48 to 72 hours. The animals were 28 to 32 days old when shipped and weighed between 80 and 100 Gm. when received. Upon arrival, the rats were immediately caged, 2 animals per cage, and given access to feed and distilled water. Within the next 3 to 5 days they were ear marked by scissor cropping either the left or right ear. Permanent identification was assigned each animal by using the cage number and suffixing either L (left crop) or R (right crop). Animals were weighed on a twice per week or weekly basis.

Housing

The cages were made of stainless steel. They measured 20 x 20 x 25 cm. with the floor mesh having 1 cm. square openings. Prior to use, each cage was washed with a commercial cage washer[3] which included a 10 minute distilled water final rinse. After washing, cages were subjected to live steam for at least one hour in a low pressure steam cabinet. The stainless steel racks held 30 cages per bank. The animal room was maintained at about 22 degrees Centigrade by central air conditioning.

The windowless room had fluorescent lighting automatically controlled to 12 hour light and dark cycles.

Feeding

Animals were fed ad libitum, with the feed being replenished at least once each 24 hours. Two porcelain feeders[4] were placed in each cage. Feeders were washed with a commercial glassware washer[5] including a distilled water final rinse. Before use, the feeders were autoclaved for 15 minutes at 15 p.s.i. Clean feeders were used with each change in ration.

Watering

Animals were watered ad libitum with water being replenished at least once every 24 hours. Pint (250 ml.) milk bottles were used with No. 6½ black rubber stoppers perforated by curved stainless steel drinking tubes. Bottles were washed with a commercial glassware washer[6] including a distilled water final rinse, then autoclaved at 15 p.s.i. for 15 minutes. Distilled water was re-glass distilled and used as the sole source of drinking water. Bottles were changed weekly and with each ration change to control water contamination by rat mouth parts. All bottles were marked with cage numbers to control accidental interchange between animals.

Diet

Diet ingredients were measured on a Mettler scale[7] or Toledo scale[8], depending upon the amount to be weighed. Ingredients for Experiment I were mixed in a Hobart mixer.[9] For Experiments II and III,

dry ingredients (exclusive of the zinc and magnesium supplements) were batch mixed in a large volume Marion mixer[10]. The mixture was then subdivided by weight and the appropriate zinc and magnesium supplements added and mixed for 30 minutes in a smaller mixer.[11] The appropriate amount of corn oil was then added and mixing completed. Dextrose was used to replace the magnesium and zinc compounds in the deficient diets. Premixing of the trace minerals, vitamin, calcium, potassium and sodium sources was practiced to improve mixing characteristics and ingredient distribution.

Diets were placed in marked plastic bags, closed tightly and stored at 4 degrees C. for future use. Daily feeding was from closed double plastic bags containing about 10 to 15 days supply which were maintained at room temperature in the animal room. For Experiments II and III feed consumption was measured by periodic weighing of feed bags kept in the animal room.

Daily Blood Collection

Between 6:00 and 9:00 a.m. tail blood was collected from unanesthetized rats by clipping off 1 to 3 mm of tail at each sampling with sharp surgical scissors. Light digital pressure was applied at the base of the tail until a drop of blood welled up on the cut stump. Micropipettes and capillary tubes were filled directly from this drop. Care was taken to avoid rough handling of the tail. Coagulation was not a problem except in moribund anemic animals. Only on 6 animals was it necessary to collect blood through the medial canthus of the eye by insertion of a heparinized capillary tube into the retroorbital area.

Final Blood Collection

Lightly etherized rats were bled out by inserting a 20 ga. x 1½ in. needle on a heparinized syringe into the posterior abdominal aorta and slowly (over a period of 10 to 30 seconds) withdrawing as much blood as easily obtainable (usually 9 to 12 ml for 300 Gm. rats). To do this, the abdominal cavity was opened from the pubis to the xyphoid along the ventral midline with strong surgical scissors. Secondary scissor cuts were then made in each side from the xyphoid to the region of the lateral lumbar processes. This "X" excision provided excellent visualization of the abdominal aorta. The needle was then inserted with the bevel against the artery in a cranial direction, penetration of the vessel wall taking place at the level of caudal pole of the kidneys. Leakage was not a problem as long as the needle remained threaded at least one centimeter within the aorta. After collection the hypodermic needle was used to pith the rat prior to disposal of the carcass.

Splenectomy

Splenectomy was performed on lightly etherized rats by making a 1 to 2 cm transverse scissor cut through the loose skin over the dorsal midline at the junction of the thoracic and lumbar vertebrae. The skin opening was moved over the left costal notch and a blunt stab perforation of the abdominal wall was executed with a pair of hemostats.[12] The jaws of the hemostats were spread and the tips of a pair of Rochester-Ochsner forceps[13] were passed into the abdominal cavity. The spleen was observed as a dark purplish glistening surface in the opening maintained by the forceps. The spleen was grasped lightly with

the forceps and withdrawn.

The pedical was tied off with triple "0" type C chromic cat gut[14] approximately one centimeter from the base of the spleen. The spleen was freed by cutting between the ligature and its base. The pedicle and any exposed omentum was replaced into the abdominal cavity. The skin incision was allowed to slide back to its original dorsal midline position. One drop of liquid Furacin[15] was placed in the incision which was allowed to heal by granulation. Death loss was less than 2% and was limited to eviceration or internal hemorrhage, both of which occurred within the first 12 hour postoperative period.

Hematological Techniques

Packed Cell Volume: The microhematocrit method was used to determine the packed cell volume with an International microhematocrit centrifuge.[16] Standard heparinized capillary tubes[17] were used to directly collect each tail blood sample. The tubes were sealed by inserting them in a numbered tray containing a plastic putty.[18] This also served to maintain tube order and identification.

Erythrocyte Count: Five lambda micropipettes[19] were heparinized by drawing through a few drops of distilled water containing 60 units of heparin[20] per ml, then air drying by water suction pump vacuum for 1 to 2 hours. Five lambda of blood was taken directly from the cut stump into the pipette. The outside wall of the pipette was wiped clean with disposable paper tissue. The blood was then gently expelled into 10 ml. of special saline solution.[21] Cells were counted on a Coulter Particle Counter, Model B,[23] using standard procedures (56). A lower threshold

setting of 5 was used. Erythrocyte indices were calculated in a routine manner (57).

Parasite Counts

The percentage of parasitized erythrocytes was determined using methods described by Schroeder and coworkers (24). It was necessary to keep the methyl alcohol base stain in a tightly capped container between uses to prevent hydration from atmospheric moisture.

Mineral Analysis

Mineral values were determined by use of an atomic absorption spectrophotometer.[24] Routine analytical procedures recommended by the maker of the machine (58) were used to obtain zinc and magnesium values of feed and blood samples.

Babesia rodhaini

The strain of Babesia rodhaini used in these studies was obtained from Parke-Davis Laboratories, Ann Arbor, Michigan. It was maintained by serial passage in retired-breeder female white mice. Between 0.02 and 0.05 ml. of tail blood was collected from clinically ill mice and passed by intraperitoneal injection. At the end of each rat experiment, infected rat blood from at least 6 rats was pooled and inoculated into mice, when this culture was established, it was maintained in mice for future use. The previous culture was then abandoned.

To obtain sufficient parasitemic blood to inoculate rats on experiment, 6 to 10 splenectomized 150 gm. white male rats of the same strain as used in the experiment were inoculated with 0.05 ml of infected mouse

blood. Blood values were followed daily; when two or more splenectomized rats reached a minimum of 25% parasitemia they were anesthetized with ether and bled out. Blood was collected in cold (4^{o} Centigrade) heparinized saline (6 units per ml.) solution at approximately a 1:1 dilution. The blood was then diluted with the same solution to contain 100×10^{6} infected erythrocytes per 0.2 ml. dose, for Experiment I, and 50×10^{6} infected erythrocytes per 0.2 ml. dose for Experiments II and III. Rats on experiment were inoculated by intraperitoneal injection with a tuberculin syringe with a 25 ga. 3/4 inch needle within two hours of having bled out the splenectomized culture rats.

Computerized Curve Fitting

A computerized program for fitting exponential curves was developed using least squares fitting theory (See Appendix D). For computational purposes the maximum and minimum observed values of Y were used to set the limits of Y over the range of X under study. Sequential varying of the value of C, (X at infinity) was performed to obtain the smallest "error" term. Since the theoretical statistical validity of this procedure is beyond the scope of this study, plots using this method are presented simply as an alternative to french curve fitting procedure. No probability statements are inferred from the derived equations.

Statistical Design and Analysis

In Experiment I a completely randomized design was used: 18 rats, with 3 treatments, 3 cages per treatment, and 2 rats per cage. Cage was the experimental unit. The treatments were uninfected-normal magnesium (mg), infected-normal Mg, and infected-low Mg rats. In

Experiment II a 3x5 factorial of treatments was set up in a randomized block design having 3 replicates. There were 3 treatments with 5 mineral levels per treatment. The treatments were: uninfected-intact, infected-intact, and infected-splenectomized rats. Magnesium and zinc levels were confounded. The experimental unit was a cage containing 2 rats. This gave a total of 90 rats used in the experiment. Experiment III had 120 rats and was set up in the same manner except there were 4 replicates. Two of these replicates were near the wall and two were near the aisle and work table where there was greater human activity. Treatments were 5 levels of mangesium, each containing 3 levels of zinc. All animals were intact and were infected. The experimental unit was the average of 2 cages each containing 2 rats.

Statistical significance of results was determined using standard analysis of variance procedure (59). Computation was performed using off-the-shelf computer programs[25] for factorial analysis including polynomial significance tests. Dunnet's method of comparing treatment means against a control mean was used (6) in Experiments I and II.

Figure 1 provides the experimental schedule followed for each of the 3 experiments. Slight differences in timing are indicated in its text.

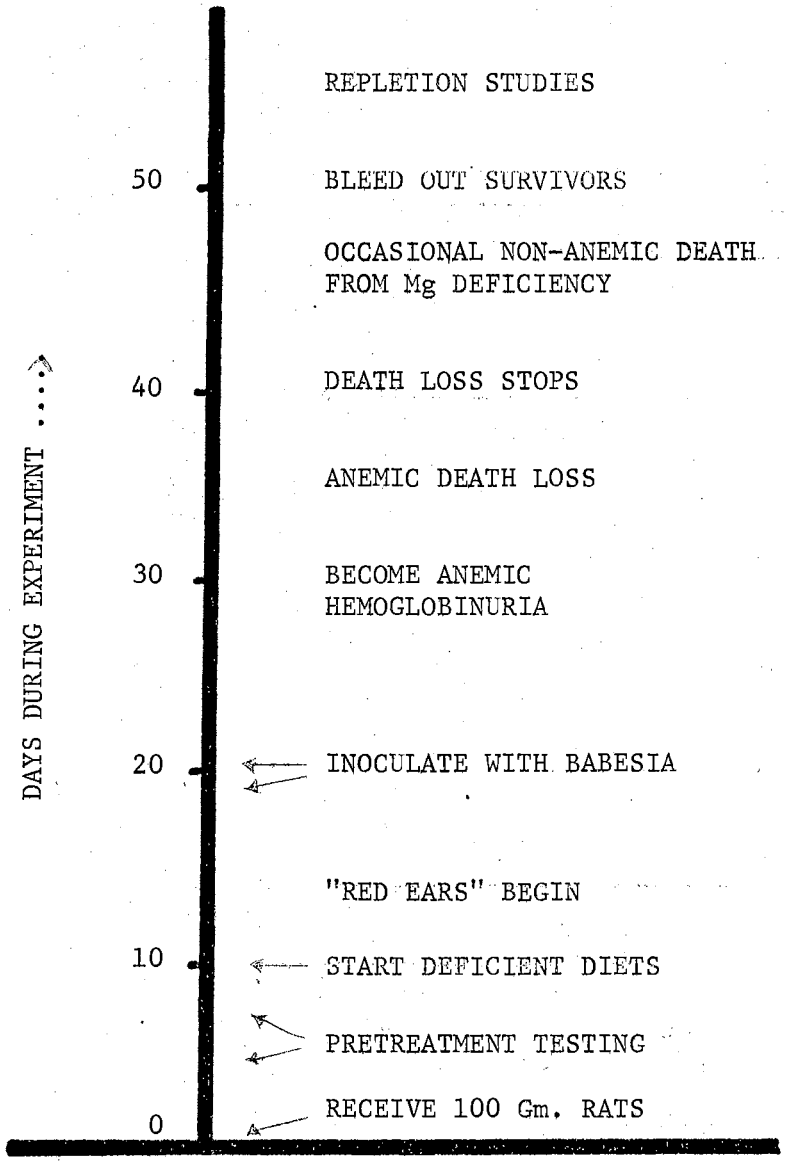

Figure 1. Treatment schedule for the experiments. Bleed out surviving rats: Experiment I, 30 days, postinoculation; II, 22 days, postinoculation (uninfected animals only; others continued on repletion studies for 17 additional days); and, III, 33 days postinoculation.

FOOTNOTES

[1]Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

[2]Air Express, Suit 825, 1000 Connecticutt Avenue N.W., Washington, D.C.

[3]Heinike Animal Cage Washer, Heinike Instruments Co., Hollywood, Florida.

[4]Animal Feeding Jar. Fisher Scientific Company, 600 Avenue H. East, Arlington, Texas, 76010.

[5]Heinike Glassware Washer, Heinike Instruments Co., Hollywood, Florida.

[6]Ibid.

[7]Mettler, 100 Gm Electronic Balance, Mettler Instrument Corporation, Hightstown, New Jersey.

[8]Toledo Scale, Toledo Scale Company, Toledo, Ohio.

[9]Beater Mixer, Hobart Manufacturing Company, Troy, Ohio.

[10]Marion Mixer, No. 6545. Rapids Machinery Company, Marion, Iowa.

[11]Beater Mixer, Model A-200, Hobart Manufacturing Company, Troy, Ohio.

[12]Kelley, 5½ inch curved stainless steel forceps. Scientific Products, 1210 Leon Place, Evanston, Illinois, 60201.

[13]Rochester-Ochsner, 8-inch straight stainless steel forceps. Scientific Products, 1210 Leon Place, Evanston, Illinois, 60201.

[14]3-0 Medium Chromic gut. Vet-Co Division, Johnson & Johnson, New Brunswick, New Jersey.

[15]Furacin Solution, Veterinary. Eaton Laboratories, 17 Eaton Avenue, Norwich, New York, 13815.

[16]Micro-Hematocrit Centrifuge (IEC 3411), Scientific Procucts, 1210 Leon Place, Evanston, Illinois, 60201.

[17]Micro-Hematocrit Capillary Tube (Heparinized), Ibid.

[18]Seal-Ease Capillary Tube Holder, Ibid.

[19]Micropipet, Kirk Design, Ibid.

[20]Heparin 1% Solution (Ammonium Salt), Ibid.

[21]S/P Saline for Coulter Counter in Handy-Bag Dispenser, Ibid.

[22]Coulter Dual Diluter, Ibid.

[23]Coulter Counter, Research Model B with 100 Micron Aperture Tube, Ibid.

[24]Perkin-Elmer Atomic Absorption Spectrophotometer, Model 303, Perkin-Elmer, Norwalk, Connecticutt.

[25]Statistical Laboratory, Oklahoma State University, Stillwater, Oklahoma.

CHAPTER III

RESULTS

Statistical tables are presented with mean square values for each of the 3 experiments. Treatment means from which the analyses are derived are presented in table form in Appendix B. The common convention is followed of indicating statistically significant results with asterisks (*, $P<.05$; **, $P<.01$; non-significant, n.s., $P>.05$). The figures presented with standard deviation or error mean square information are graphic combinations of statistical tables and treatment mean tables. An interval encompassing the mean plus or minus one standard deviation of a mean is indicated on most graphs.[1] Some graphs are presented for illustrative purposes without statistical descriptions. Equations for the machine plotted graphs are presented in Appendix D.

Experiment I

From Table I, it can be seen that rats fed a magnesium (Mg) deficient diet for 10 days and then infected with Babesia rodhaini experienced a milder form of the disease than did those infected rats which consumed a ration more nearly adequate in magnesium. When uninfected rats receiving adequate magnesium were used as a control baseline, statistically significant differences were not detected ($P>.05$) between the control values and the Mg deficient-infected rats for any of the parameters under study. The infected rats on the higher

TABLE I

EXPERIMENT I, ANALYSIS OF VARIANCE: SUMMARY OF MEAN SQUARE VALUES

Source	df	Lowest Observed Packed Cell Volume (P.C.V.)	Lowest Observed Erythrocyte Count	Highest Observed % Parasitemia	Interval from Inoculation to Lowest Observed P.C.V.	Days Survived During 30-Day Observation
Treatments	2	594.54*	10.35	4454.34*	62.72	141.56*
Error	6	113.36	2.20	330.58	48.67	20.94
C.V., %	-	37.8	38.1	82.1	38.6	17.5
N =	-	18	18	18	18	18
		T R E A T M E N T M E A N S				
Uninfected, Complete Ration (Control)		37.6	5.20	00.0	20.2	30.0
Infected, Magnesium Deficient Ration		29.2	3.19	13.8	19.7	27.7
Infected, Complete Ration		17.8+	2.57+	52.6+	14.3	20.7+

* Significant, P<.05; F test. C.V. = Coefficient of Variation.
\+ Significantly different from Control Mean, P<.05; Dunnett's one-tailed t' test.

Mg levels were significantly different from controls, considering: days survived (.05>P>.01) during the 30-day period (Figure 2); highest observed percent parasitemia (P<.01) (Figure 3); lowest observed erythrocyte count (.05>P>.01)(Figure 4); and lowest observed packed cell volume (P.C.V.) (.05>P>.01) (Figure 5). No significant difference (P>.05) was detected between the control and the other two groups for the interval between the day of babesia inoculation and the day of lowest observed P.C.V. (Figure 6).

Growth of the experimental animals was below expectation (55). Terminal weights of the control uninfected animals on the higher Mg level averaged only 189 Gm/rat (range, 164 to 213, N = 6); the Mg deficient-infected group on the higher level of Mg averaged 168 Gm/rat (range, 158 to 178, N = 2). A group of rats from the same shipment as the experimental animals was maintained on distilled water and commercial laboratory rat cubes.[2] They were neither bled nor subjected to experimental procedures. They averaged 390 Gm/rat (range, 360 to 442, N = 6) at the time the experimental group was weighed. In view of these results the vitamin mixture used in Experiment I was discarded.

All of the Mg deficient rats exhibited erythema of the ears and feet between the third and fifth day on their diet. This persisted for 4 to 6 days. Spotty, hairless areas were observed 10 to 14 days after starting the deficient diet. Some of these areas became completely denuded of hair and developed a moist dermatitis by the fifteenth to twentieth day on the diet. Two Mg deficient rats experienced convulsive seizures 21 and 28 days after starting the deficient diets.

Hemoglobinuria was usually observed 2 to 3 days prior to anemic crisis. The animals were noticeably anemic usually by the second day

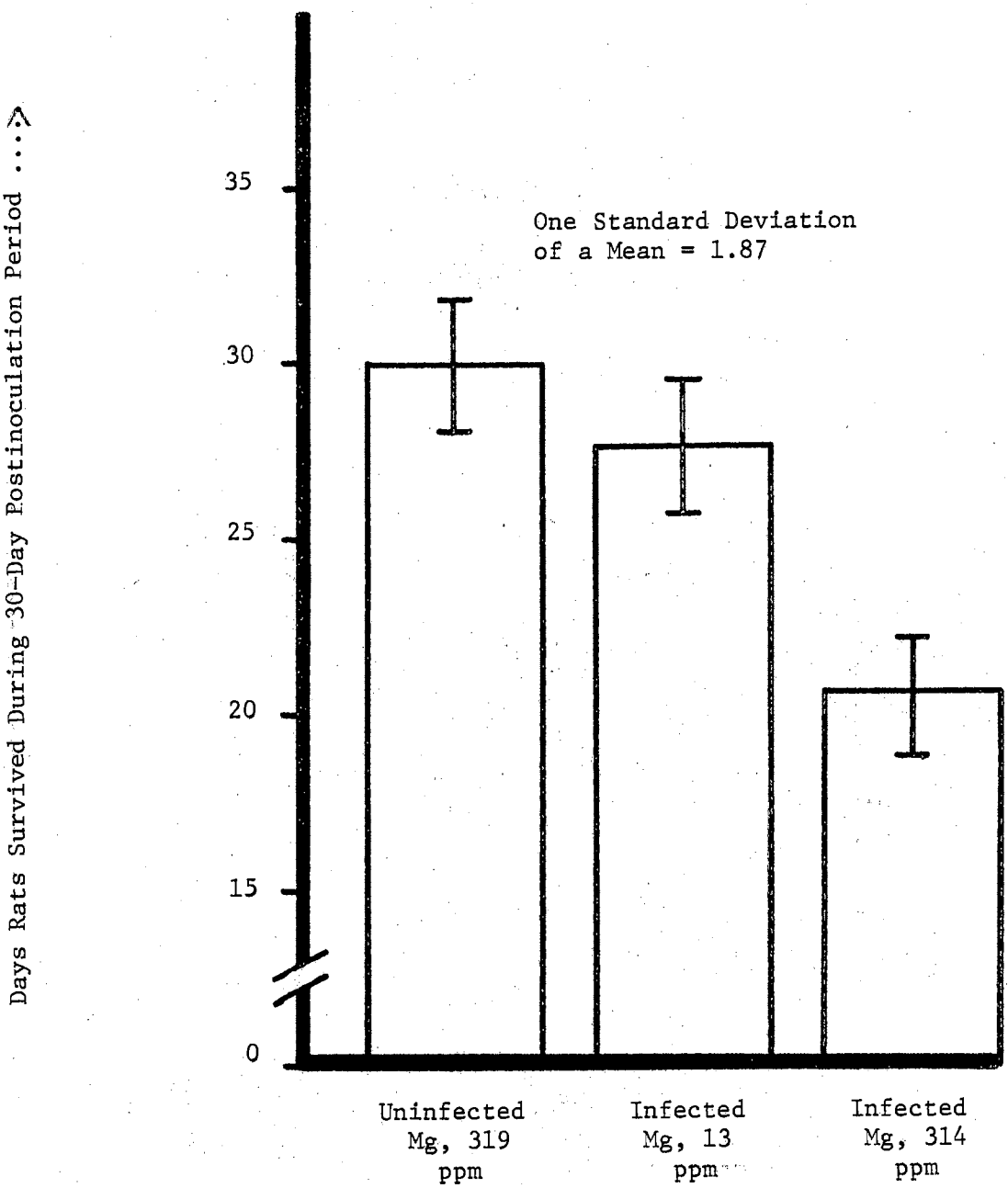

Figure 2. Effects of dietary magnesium (Mg) level on survival of rats for 30 days after inoculation of two treatments with Babesia rodhaini. N = 6 rats per treatment.

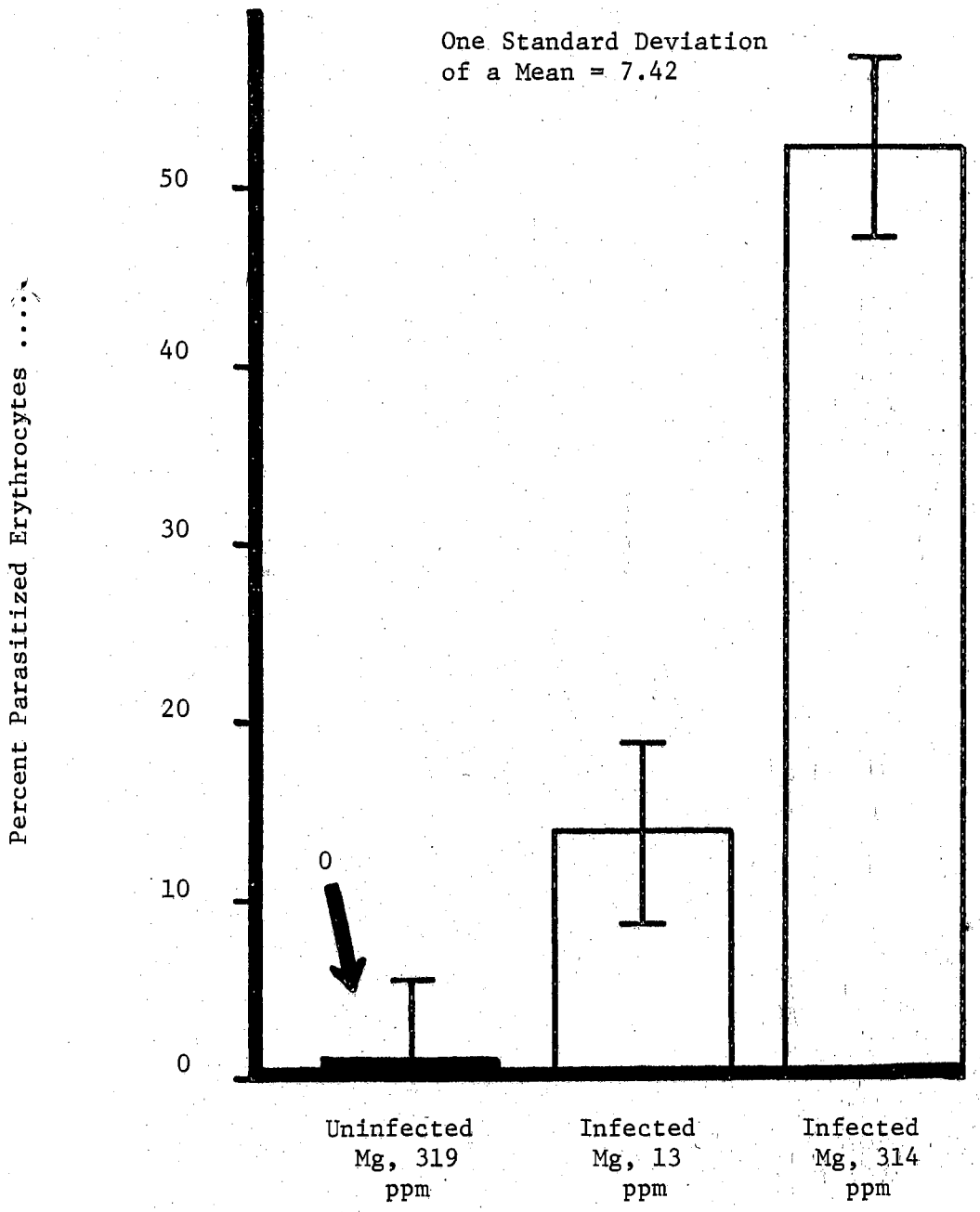

Figure 3. Effects of dietary magnesium levels on highest percentage of erythrocytes observed infected with Babesia rodhaini during 30-day postinoculation period. Magnesium given in parts per million (ppm). N = 6 rats per treatment. (See treatment means, Appendix B, Table XV).

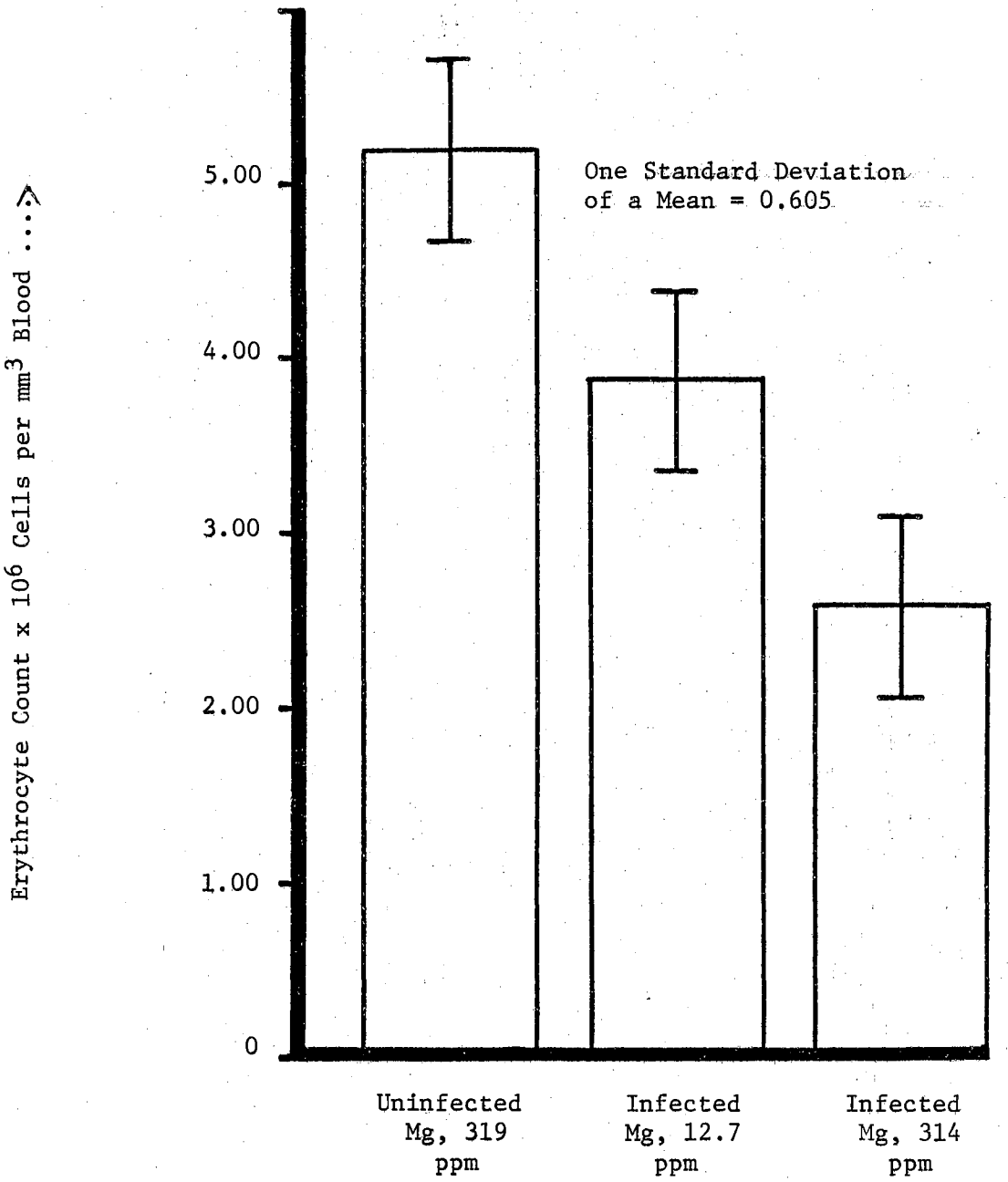

Figure 4. Effects of dietary magnesium levels on lowest erythrocyte counts observed for 30 days after inoculation of 2 treatments with Babesia rodhaini. Magnesium (Mg) in parts per million (ppm). N = 6 rats per treatment.

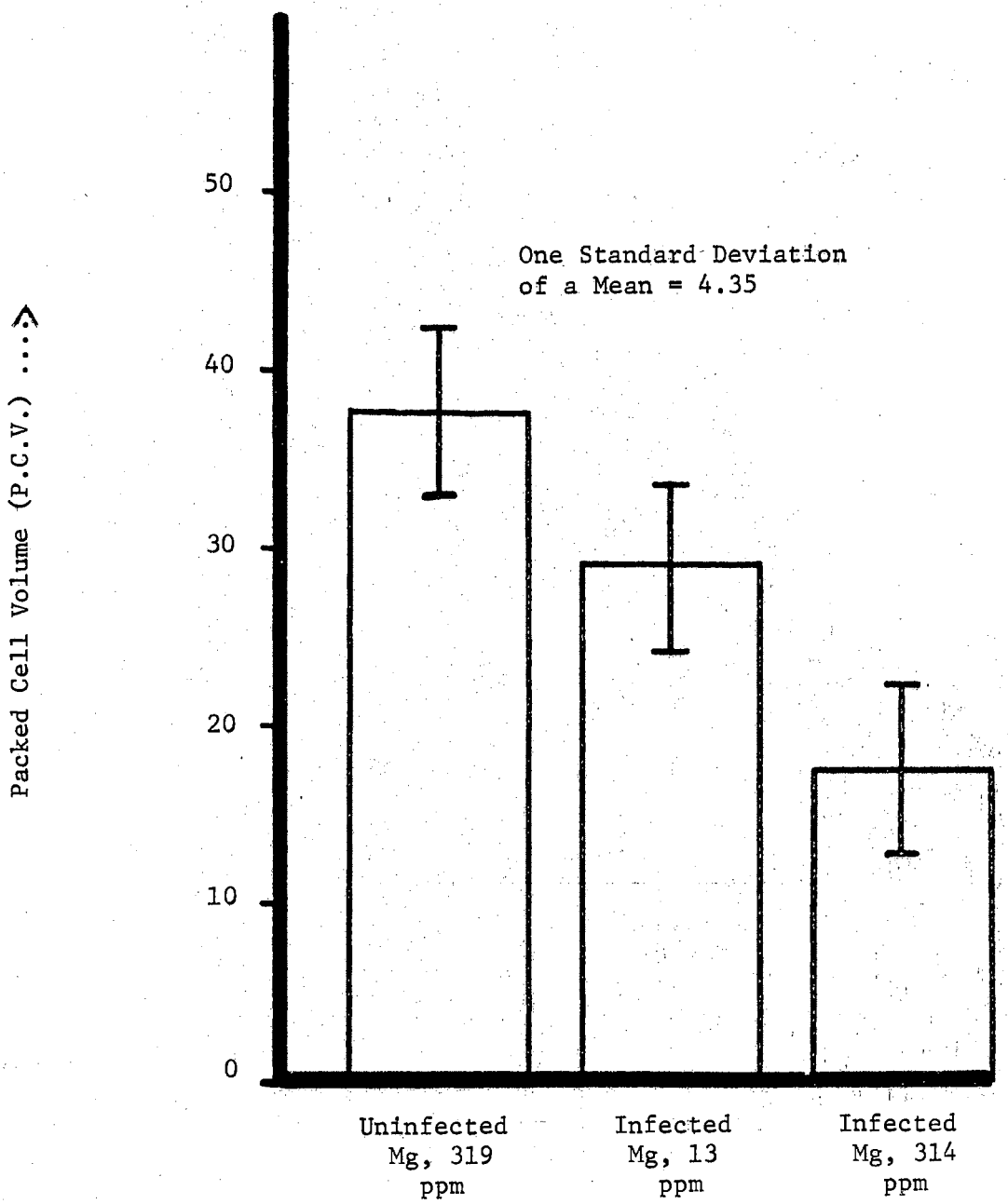

Figure 5. Effect of dietary magnesium (Mg) levels upon the lowest Packed Cell Volume observed during a 30-day period after inoculation of 2 treatments with Babesia rodhaini. N = 6 rats per treatment.

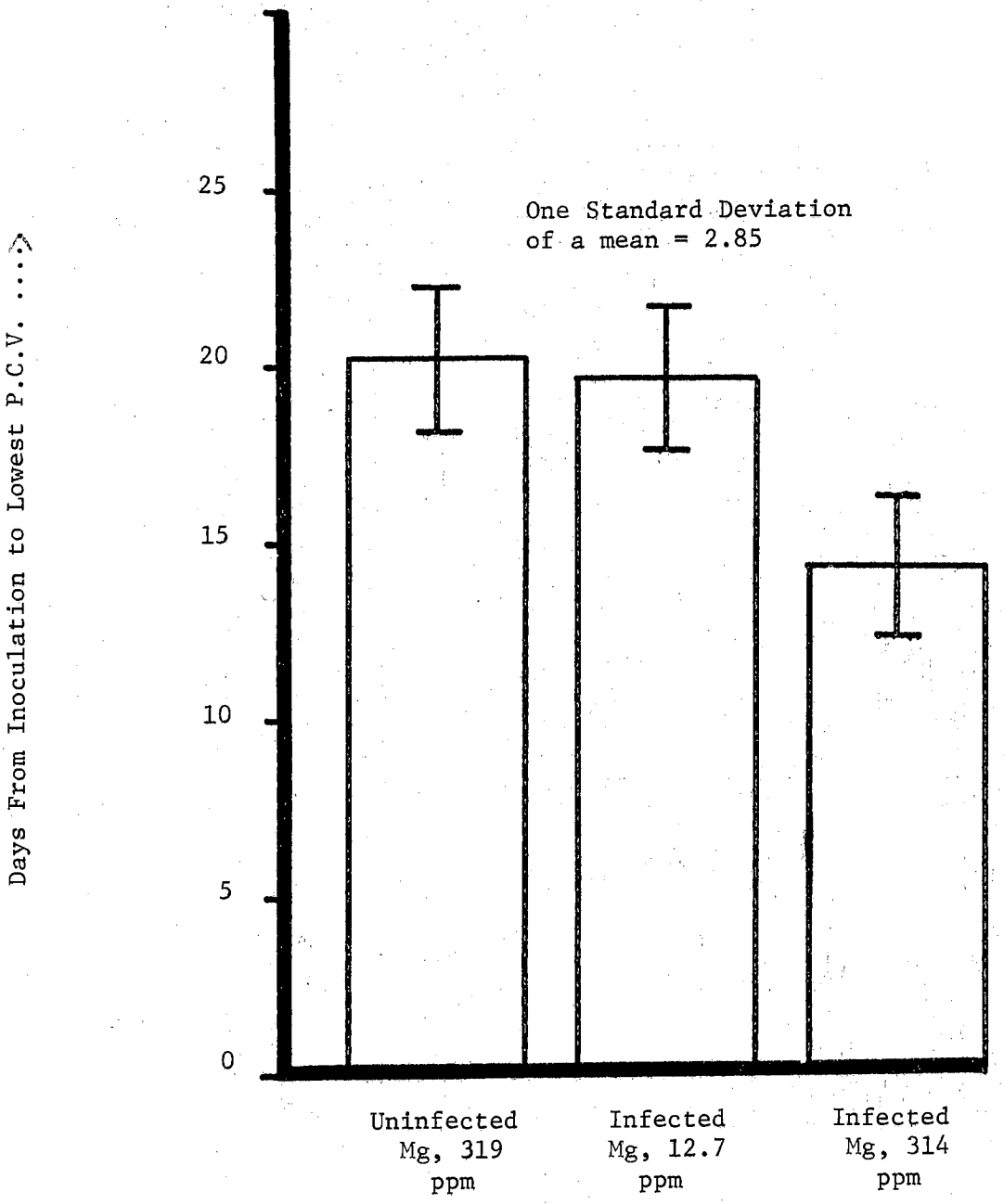

Figure 6. Effects of dietary magnesium levels on time interval between Babesia rodhaini inoculation of two treatments and lowest observed packed cell volume during 30-day postinoculation period. Magnesium (Mg) given in parts per million (ppm). N = 6 rats per treatment.

of hemoglobinuria. Cannibalism of the dead rats by their cage mates was a problem. Cages were checked 4 to 6 times per day during the period of highest death loss.

By inspection of Figures 1, 2, 3, and 4 it can be seen that babesiosis did influence both infected groups. However, the Mg deficient infected group was significantly protected ($P<.05$) from the full weight of the disease.

Experiment II

In an attempt to improve rate of growth, a different vitamin preparation (see Appendix A) was used in Experiment II rations. Otherwise the basic formulation remained unchanged. Terminal weights were essentially the same for rats on Ration 5 ($371.5, \pm 21$[1] Gm/rat) as for terminal weights of rats on the commercial diet in Experiment I, even though the Experiment II rats were bled daily, and their terminal weights reflected an approximately 8-day shorter growing period. Growth rate was very satisfactory.

Fitted growth curves in Figure 7 very closely approximate the actual treatment mean data points as can be seen by comparing these curves with the values in Table II. By inspection, it can be noted that the 5 growth curves fall into 2 sets--the lower 2 mineral levels into the slower growth rate set and the upper 3 curves into the faster growth rate set. Analysis of variance (Table III) of the first 3 growth periods indicate that significant growth difference ($P<.01$) due to mineral levels began during the second week on the experimental rations. Significant treatment differences ($P<.01$) were observed during these periods for low body weights of the splenectomized group. Table III

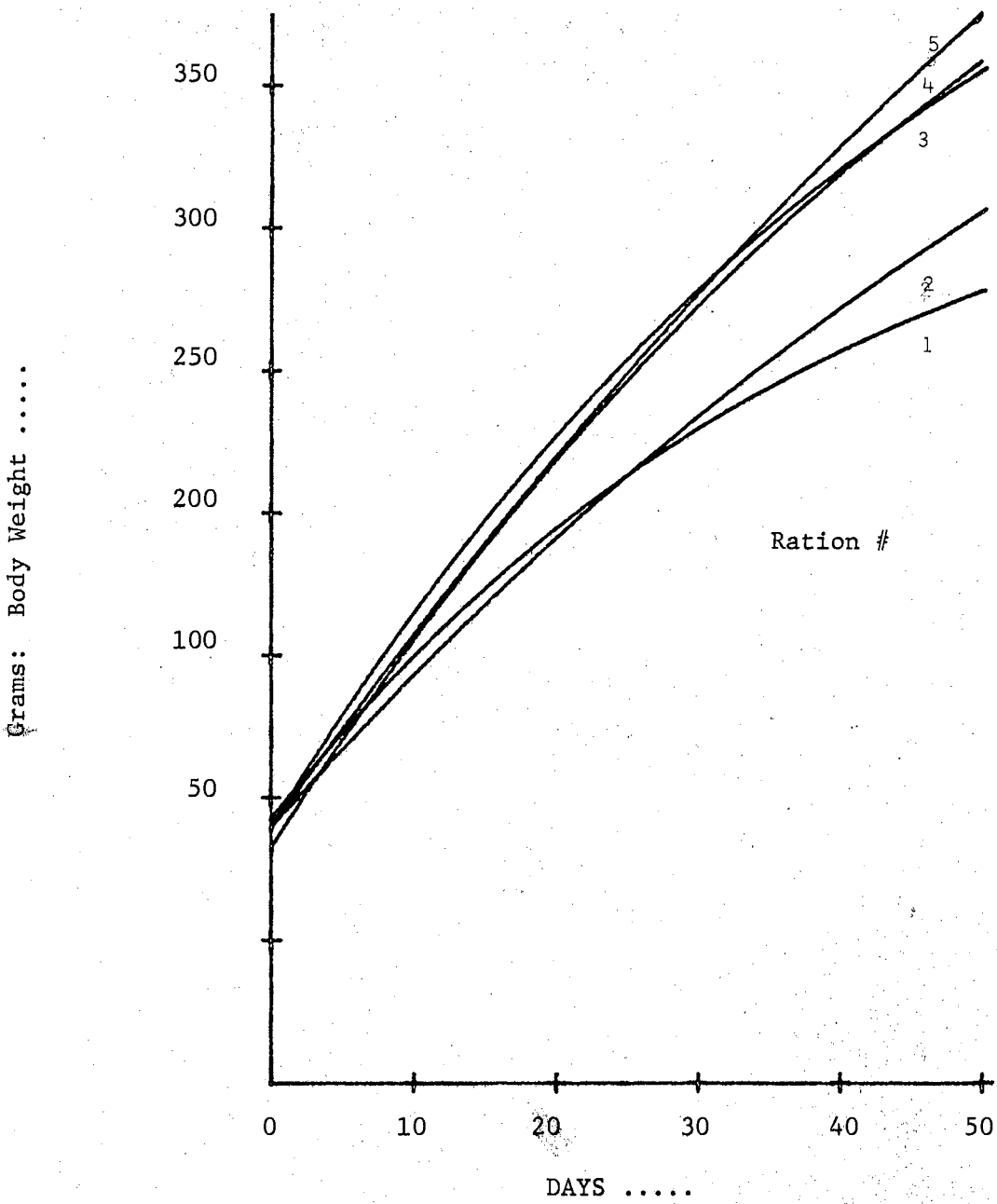

Figure 7. Growth rate of intact uninfected rats on various mineral levels of magnesium (Mg) and zinc (Zn): Ration #1, Mg 35.5, Zn 13.0; #2, Mg 65.1, Zn 14.2; #3, Mg 97.7, Zn 15.3; #4, Mg 172.6, Zn 20.0; #5, Mg 363.5, Zn 26.0. (For curve equations see Appendix D, Table XXXIX). Fitted from data in Table II. Machine plotted graph.[2]

TABLE II

GROWTH DATA: BODY WEIGHT (GRAMS) VS. TIME
FOR UNINFECTED INTACT RATS

Ration	1	2	3	4	5	$\bar{x}$ for Time
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	
N	6	6	6	6	6	30
Day						
1*	90.1	89.1	94.9	88.3	92.5	90.9
7*	141.1	131.1	144.6	132.1	138.1	137.4
14	183.8	174.9	200.4	191.8	190.9	188.3
21	196.3	189.8	226.1	219.8	218.3	210.0
24	206.8	203.4	243.3	238.4	243.3	227.0
28	219.8	222.6	267.6	260.5	265.0	247.1
31	223.0	235.3	280.6	275.5	280.5	259.0
35	238.6	253.5	302.3	297.0	306.3	279.5
38	253.3	266.8	315.9	311.1	311.0	291.6
42	267.4	282.1	333.1	329.4	342.4	310.0
45	270.8	289.8	337.4	339.8	353.5	318.2
49	279.1	302.4	351.1	356.0	371.5	332.0
$\bar{x}$ for Rations	214.2	220.5	258.1	253.3	259.4	$\bar{X}_{\bar{x}}$=241.0

Column and row means include rounding error.

*All rats on complete basal diet, 353.5 ppm Mg, 13.0 ppm Zn, first week of arrival.

TABLE III

EXPERIMENT II, ANALYSIS OF VARIANCE: SUMMARY OF MEAN SQUARES

Source	df	Body Weight in Grams During 3 Consecutive Growth Periods		
		After 7 Days on Basal Diet+	After 7 Days on Experimental Diet	On Diet 14 Days, Infected 6 Days
Mineral Levels (M)	4	37.21	388.0	2,412.4**
Linear++	1	-	-	3,126.0*
Quadratic++	1	-	-	5,194.1**
Cubic++	1	-	-	974.8
Treatment (T)	2	1,103.5**	2,010.83**	1,841.2
T x M	8	198.0	386.8	530.1
Error	28	178.7	309.8	448.0
C.V.		9.7	9.3	10.0
N		90	90	90
Treatment Means+++:				
Intact, Infected++++		142.0	197.1	218.6
Intact, Not Infected		137.8	189.0	212.0
Splenectomized, Infected		130.1**	180.7**	203.0**

+ Only treatment applied: Splenectomy; all rats on same diet.
++ Significance: *, P<.05; **, P<.01; Based on Magnesium values.
+++ Dunnet's one-tailed t' test used.
++++ Considered as "control" or base line treatment.
C.V. = Coefficient of Variation.

is graphically represented by Figure 8.

Erythema of ears, feet and tail occurred in the same sequence as in Experiment I. Hemoglobinuria tended to start closer to the anemic crisis (1-2 days) than in Experiment I (2-3 days). No difference was noted between the 2 experiments for the dermatitis and the spotty hair. No animals were observed with convulsive seizures during the 22-day observation period. There was a clinical impression that the animals in this experiment died faster after becoming clinically ill (general malaise, pale pink eyes, white or icteric skin) than did the rats in the first experiment.

Analysis of the hematologic data is presented in Table IV. In the column for Pretreatment P.C.V. it is noted that there was a significant block effect ($P<.01$) for the two different cage racks. This initial effect was not detected as the experiment progressed. One rack had a slightly more open mesh type front cage wall which would permit these rats a better view of the work area. Room position of racks was varied daily to facilitate bleeding animals and cleaning of the cages and floors.

Highly significant mineral level effects ($P<.01$) were indicated for survival days, interval between inoculation and anemic crisis and parasitemia. When the analysis of linear and polynomial effects was performed on the basis of magnesium levels, significant effects were detected for: survival days (linear: $P<.01$; quadratic: $P<.01$), interval (linear: $P<.01$; quadratic: $P<.01$), and M.C.V. (linear: $P<.01$).

Good evidence for treatment effects was not detected ($P<.05$) for: interval, pretreatment P.C.V., or M.C.V. Although splenectomy did not influence P.C.V. during this same period. Highly significant treatment

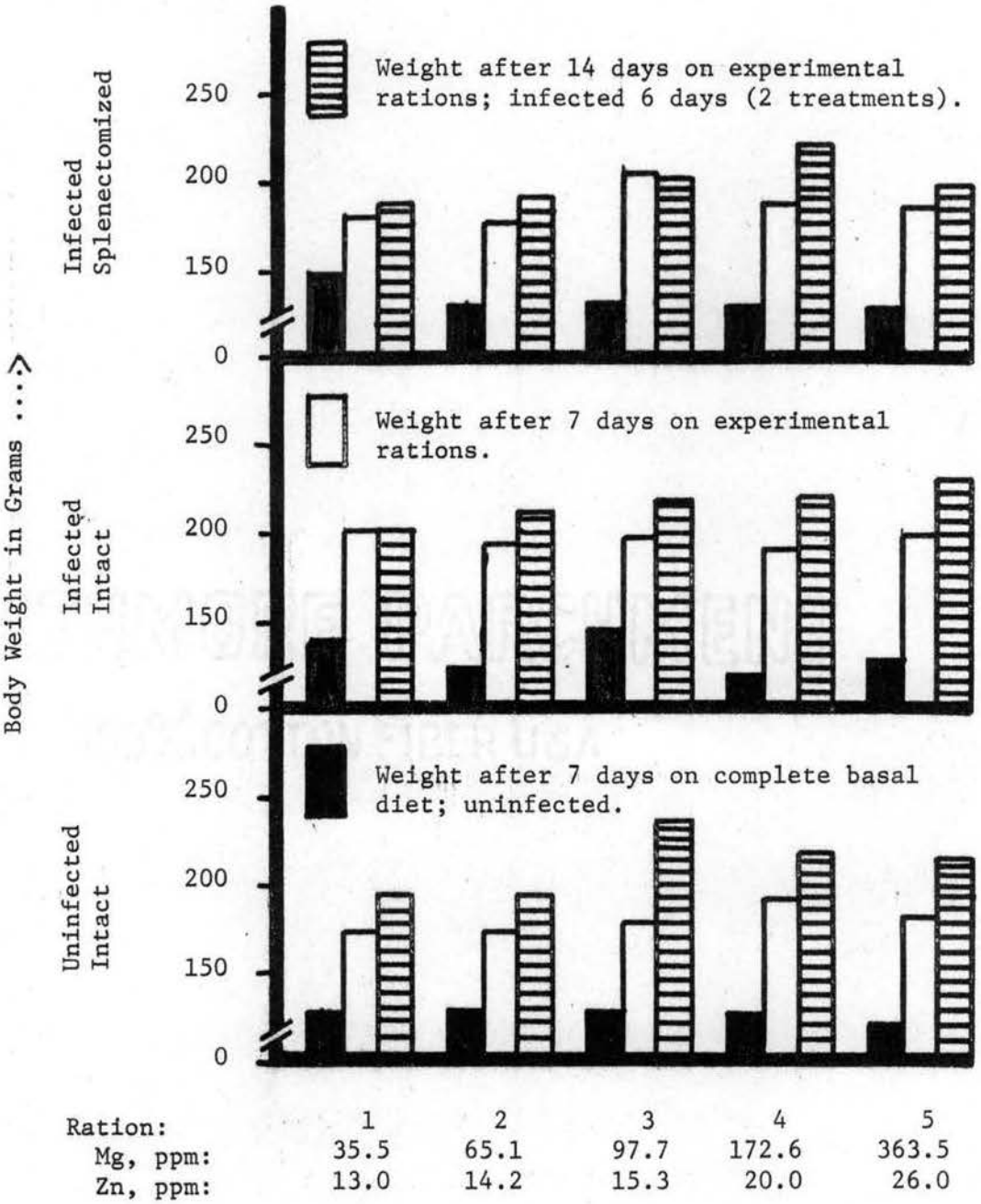

Figure 8. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on weight gain. N = 6 rats per treatment mean. Infected with Babesia rodhaini.

TABLE IV

EXPERIMENT II, ANALYSIS OF VARIANCE: SUMMARY OF MEAN SQUARES

5 x 3 Factorial: Source:	df	Days Survived	Interval: Day of Inoculation to Crisis	Lowest Observed P.C.V.	Percent Parasitized Erythrocytes	Pretreatment P.C.V.	Lowest Observed Erythrocyte Count (R.B.C.)	M.C.V., day low R.B.C.
Mineral Levels (M)	4	1,242.1**	146.7**	100.32	3,255.4**	8.67	0.55	5,162.5
Linear+	1	1,001.6**	459.1**	1.60	-	-	-	18,387.7**
Quadratic+	1	193.1**	115.0**	37.93	-	-	-	1,604.5
Cubic+	1	21.2	2.7	174.24	-	-	-	76.8
Treatment (T)	2	367.7**	41.4	2,374.34**	23,915.9**	4.04	17.57**	6,352.9
T x M	8	85.0**	35.5	80.11	795.0	10.00	1.17	2,818.7
Replicates (R)	2	-	-	-	121.3	-	-	-
R wall vs R aisle	1	2.2	0.4	120.87	-	17.92	0.33	4,884.7
R $rack_1$ vs R $rack_2$	1	0.1	24.1	28.7	-	93.88**	0.20	158.4
Error	28	9.4	16.2	62.60	239.5	7.05	1.21	2,248.9
C.V. %		16.9	36.4	26.8	47.8	6.8	40.0	32.9
N =		90	90	90	90	90	90	90
				TREATMENT MEANS++				
Intact, Infected+++	$\bar{x}$	17.9	9.7	25.9	36.8	39.3	2.34	153.3
Intact, Not infected	$\bar{x}$	21.7**	11.7	40.0**	2.16++++	39.0	3.62	127.1*
Splenectomized, Infected	$\bar{x}$	14.6**	11.8	23.9	58.10**	38.6	2.45	151.1

* Significant, P<.05; ** Significant, P<.01.
\+ Based on magnesium levels.
++ Dunnet's t': Other treatments vs. the control, marked means show significant difference from "control".
+++ Considered as "control" treatment since the deficient, Babesia infected animals were under study.
++++ Due to one animal having accidental infection.
C.V. = Coefficient of Variation.
P.C.V. = Packed Cell Volume.
R.B.C. = Erythrocytes x 10^6 per mm^3 of blood.
M.C.V. = Mean Corpuscular Volume, in cubic microns.

effects ($P<.01$) were noted for survival days, lowest P.C.V., parasitemia, and lowest R.B.C. Application of Dunnett's t' test using the intact infected treatment as a base line or control indicates significant difference between this group and the other two treatments for survival days ($P<.01$) and between the control group and uninfected-intact group for lowest P.C.V. ($P<.01$) and M.C.V. ($P<.05$).

Figure 9 presents the survival data for all 15 mineral level treatment groups. One animal died of babesiosis in the uninfected-intact treatment group. This was an accidental infection due to unintentional inoculation or fomite transfer. The splenectomized animals were protected but not so much as the intact group. Figure 10 sums the same responses across treatments where it can be seen that the two lower mineral levels offered the greatest degree of protection against death.

The data presented in Figures 11 and 12 (and Table V) represent the interval in days from the day of inoculation with Babesia rodhaini to the day of lowest observed packed cell volume during the 22-day observation period. Figure 11 shows a trend for the higher mineral levels to have shorter intervals, which can be explained for the infected groups but not for the uninfected-intact treatment. The trend is more pronounced in Figure 12 when the results were summed over all treatments. Table V shows that those groups severely affected by babesiosis tended to have about half the variability for interval when compared to groups not severely affected by babesiosis.

Figure 13 shows that all groups started with about the same P.C.V. levels. From Figures 14 and 15 it can be seen that the lower P.C.V. values did not entirely correspond to the survival data presented in Figures 9 and 10. In both the intact-infected and splenectomized-

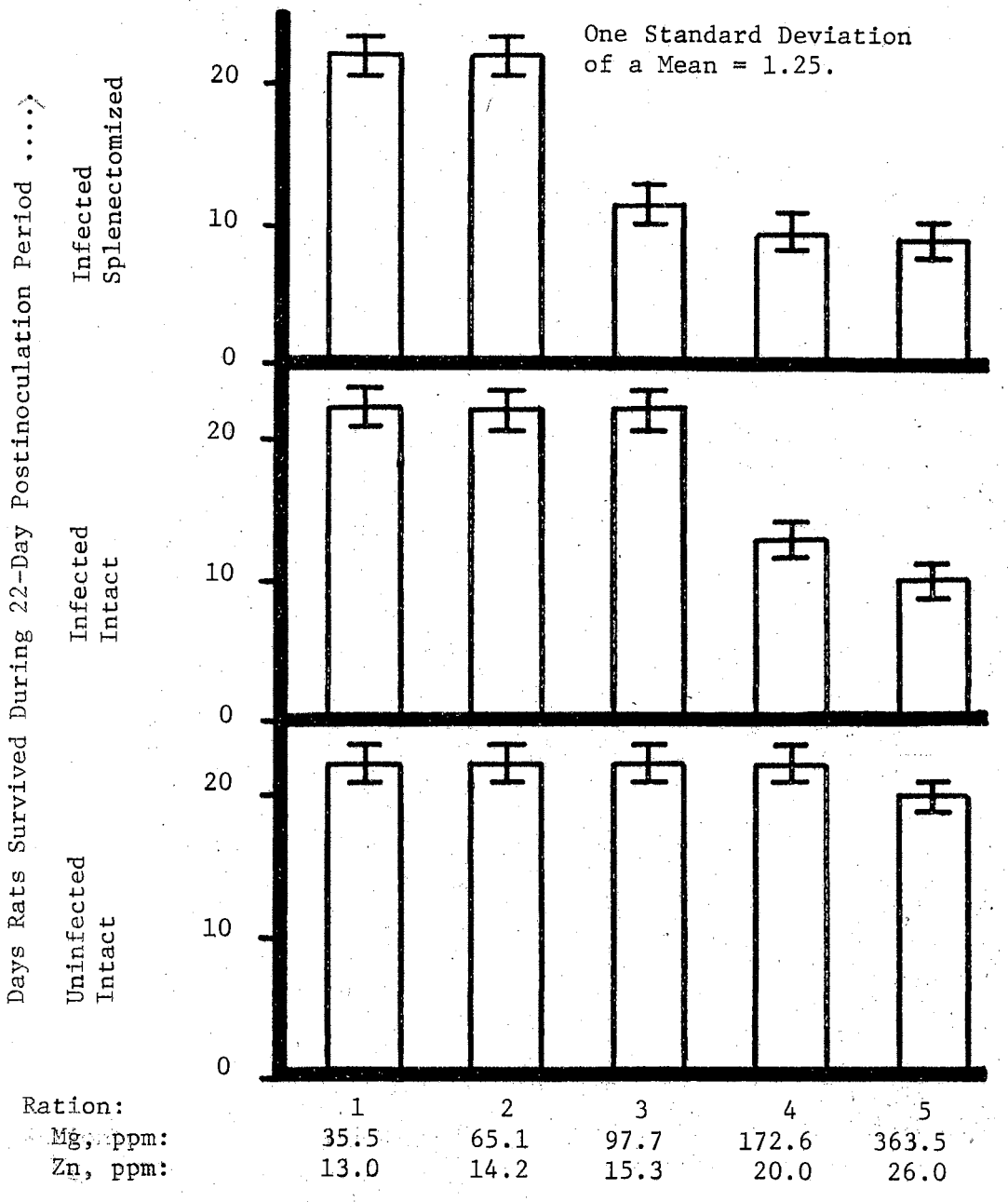

Figure 9. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on days survived during 22-day observation period after inoculation with Babesia rodhaini. N = 6 rats per treatment mean.

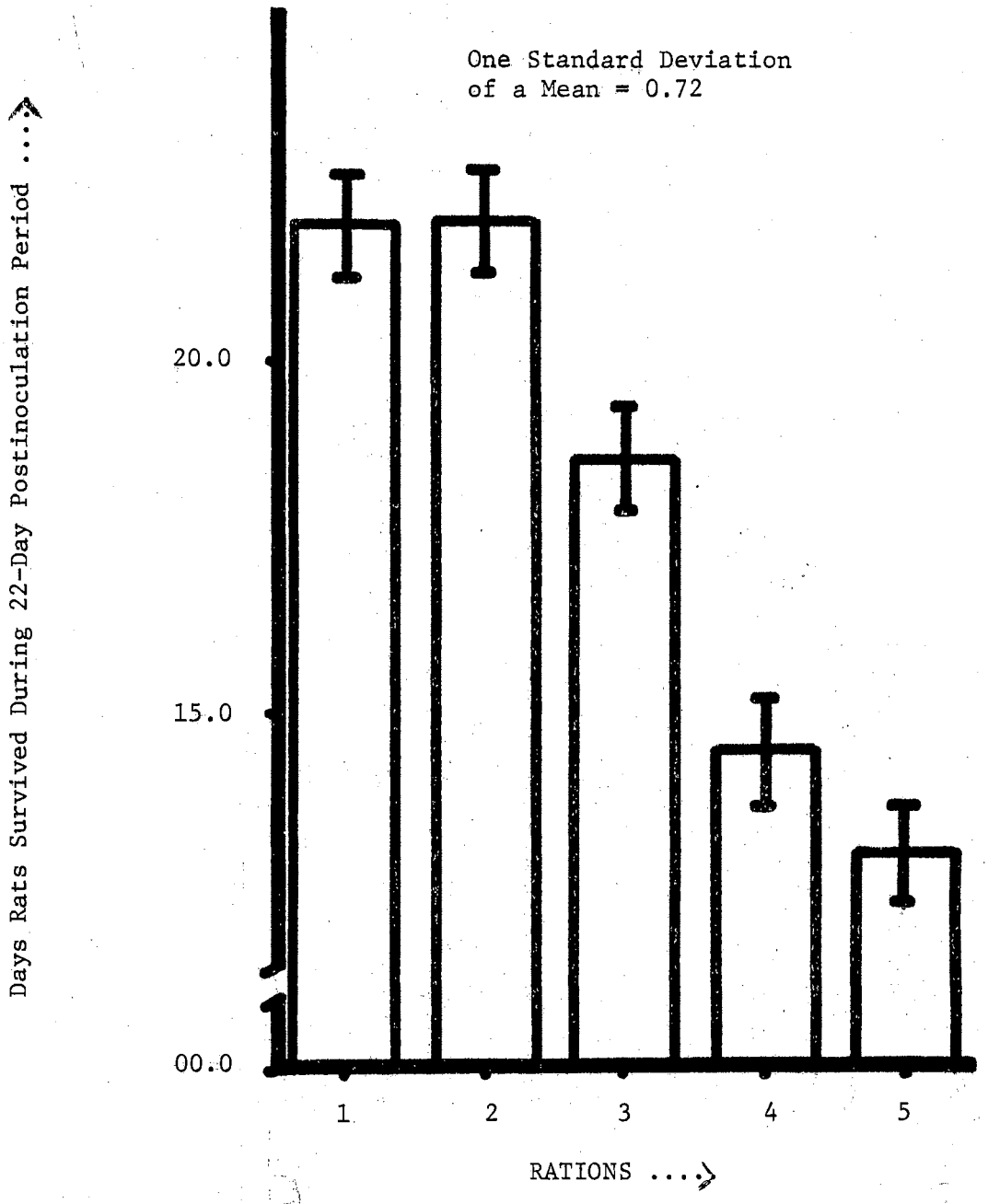

Figure 10. Effect of various dietary magnesium (Mg) and zinc (Zn) levels upon ability of the rat to survive inoculation with Babesia rodhaini. Mineral levels, p.p.m. of diet: Ration #1, Mg 35.5, Zn 13.0; #2, Mg 65.1, Zn 14.2; #3, Mg 97.7, Zn 15.3; #4, Mg 172.6, Zn 20.0; #5, Mg 363.5, Zn 26.0. Summed over treatments. Significant (P .01). Treatment x mineral interaction present, see Table IV.

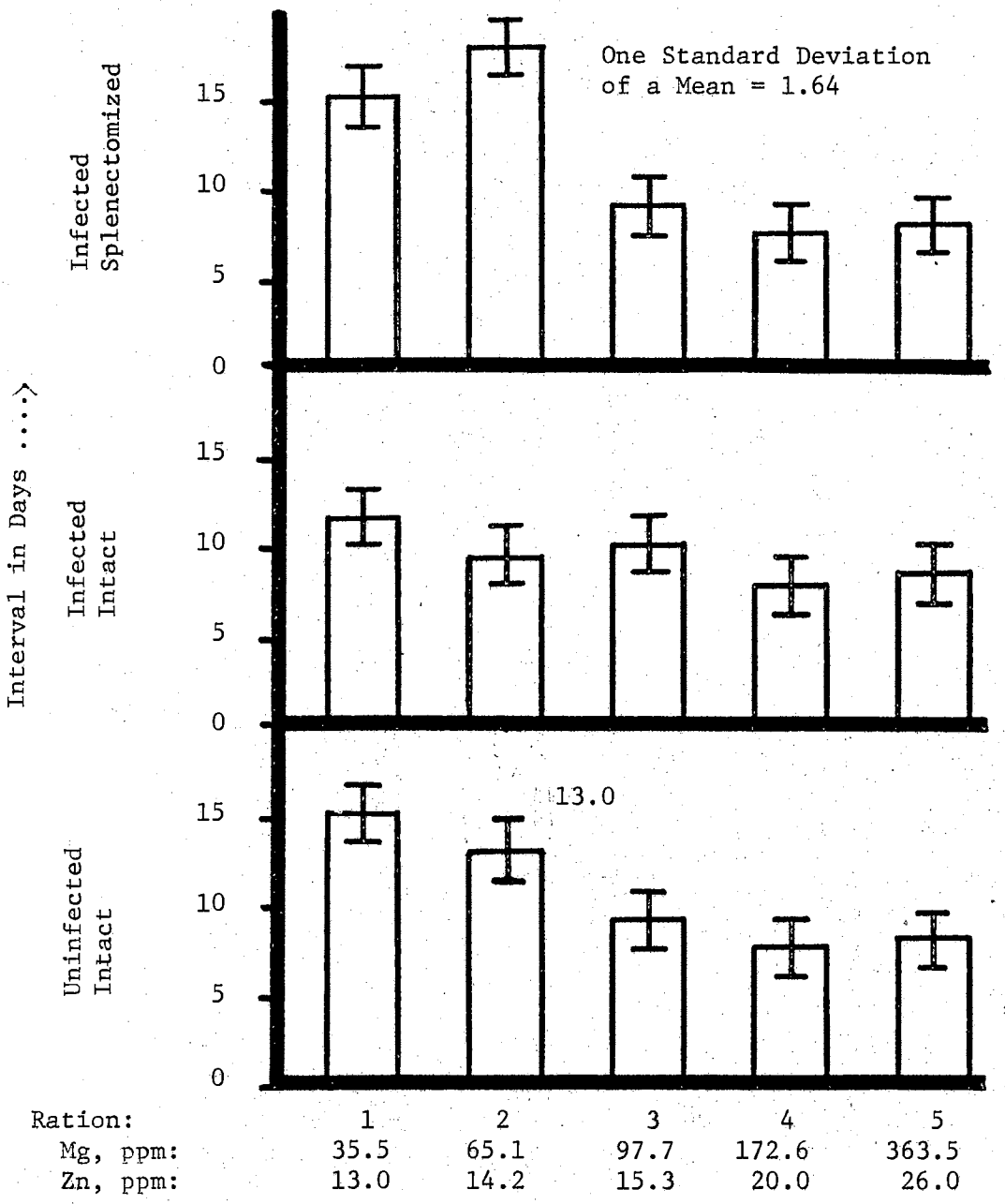

Figure 11. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) the interval in days between inoculation with Babesia rodhaini and the day the lowest packed cell volume (P.C.V.) during 22-day postinoculation period. N = 6 rats per treatment mean.

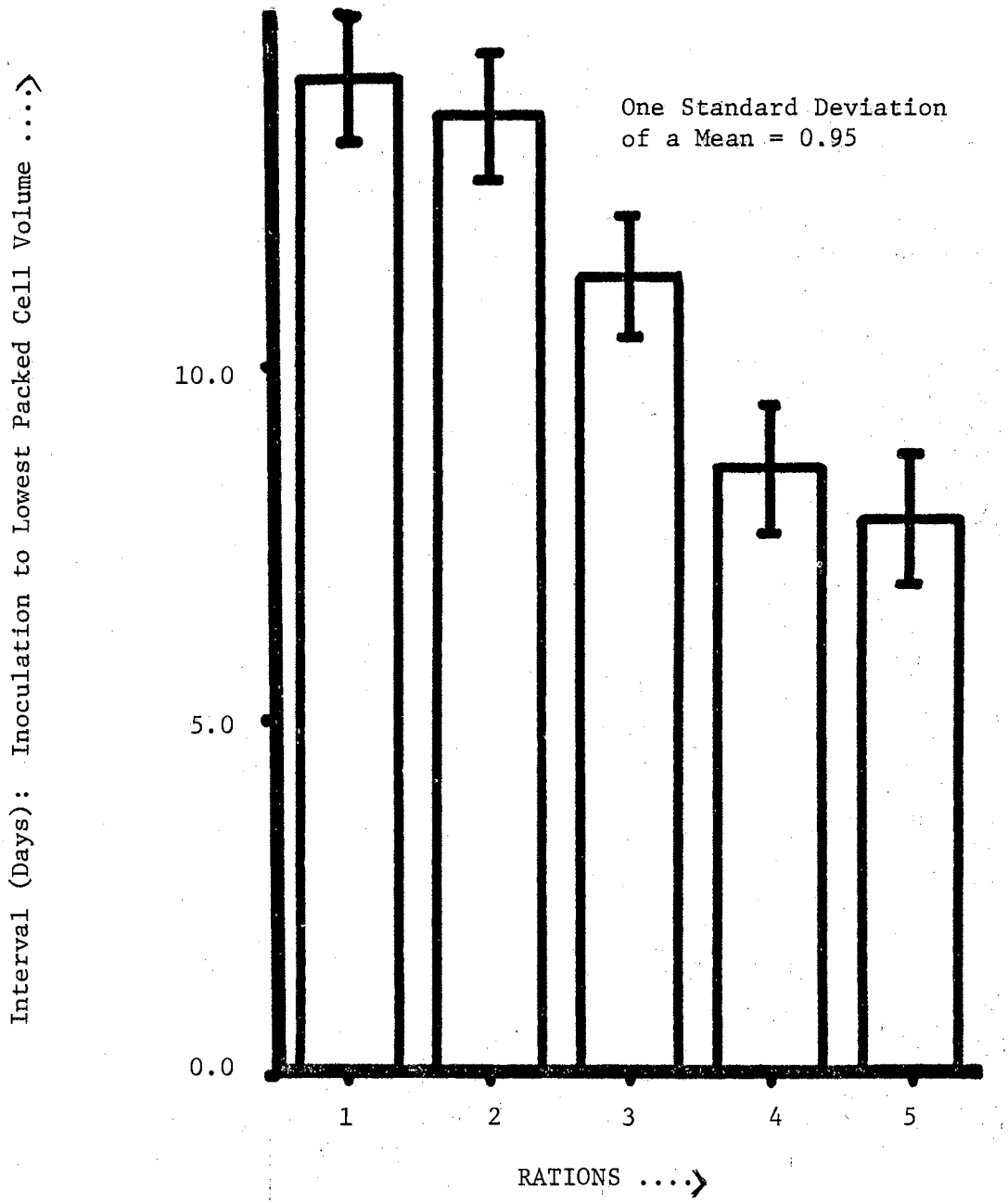

Figure 12. Effect of various dietary magnesium (Mg) and zinc (Zn) levels upon the time interval between the day of inoculation with Babesia rodhaini and the day of the lowest observed packed cell volume during 22-day postinoculation period. Mineral levels, ppp.m. of diet: Ration #1, Mg 35.5, Zn 13.0; #2, Mg 65.1, Zn 14.2; #3, Mg 97.7, Zn 15.3; #4, Mg 172.6, Zn 20.0; #5, Mg 363.5, Zn 26.0. Summed over treatments.

TABLE V

RANGE OF INTERVALS BETWEEN DAY OF INOCULATION WITH BABESIA RODHAINI AND THE DAY OF LOWEST OBSERVED PACKED CELL VOLUME DURING 22-DAY POSTINOCULATION PERIOD

Dietary Mineral Level:	1	2	3	4	5
Mg, ppm	35.5	65.1	97.7	172.6	353.5
Zn, ppm	13.0	14.2	15.3	20.0	26.0
Uninfected, Intact, N =	6	6	6	6	6
Interval, $\bar{x}$, days =	15.2	13.0	13.8	10.0	6.5
range, days =	(8-21)	(4-21)	(9-21)	(4-21)	(3-9)
Days covered by range =	13	17	12	17	6
% of 22 days covered by range =	59%	77%	55%	77%	27%
Intact, Infected, N =	6	6	6	6*	6*
Interval, $\bar{x}$, days =	11.8	9.7	10.3	8.0	8.7
range, days =	(9-21)	(4-13)	(9-15)	(6-10)	(7-13)
Days covered by range =	12	11	6	4	5
% of 22 days covered by range =	55%	50%	27%	18%	23%
Splenectomized, Infected, N =	6	6	6*	6*	6*
Interval, $\bar{x}$, days =	15.3	18.2	9.3	7.8	8.2
range, days =	(9-22)	(11-21)	(8-13)	(6-12)	(7-11)
Days covered by range =	13	10	5	6	4
% of 22 days covered by range =	59%	46%	23%	27%	18%

Groups	N	Interval, $\bar{x}$, days	Range Days	Days Covered by Range	% of 22 Days Covered by Range
Not severely affected by Babesiosis:	10	12.4	7.0-18.5	11.5	52%
Severely affected by Babesiosis*:	5	8.4	6.8-11.8	5.0	23%

P.C.V. = Packed Cell Volume.
* Groups showing marked decreased survival time.
Six rats per treatment-mineral mean; 90 rats total.

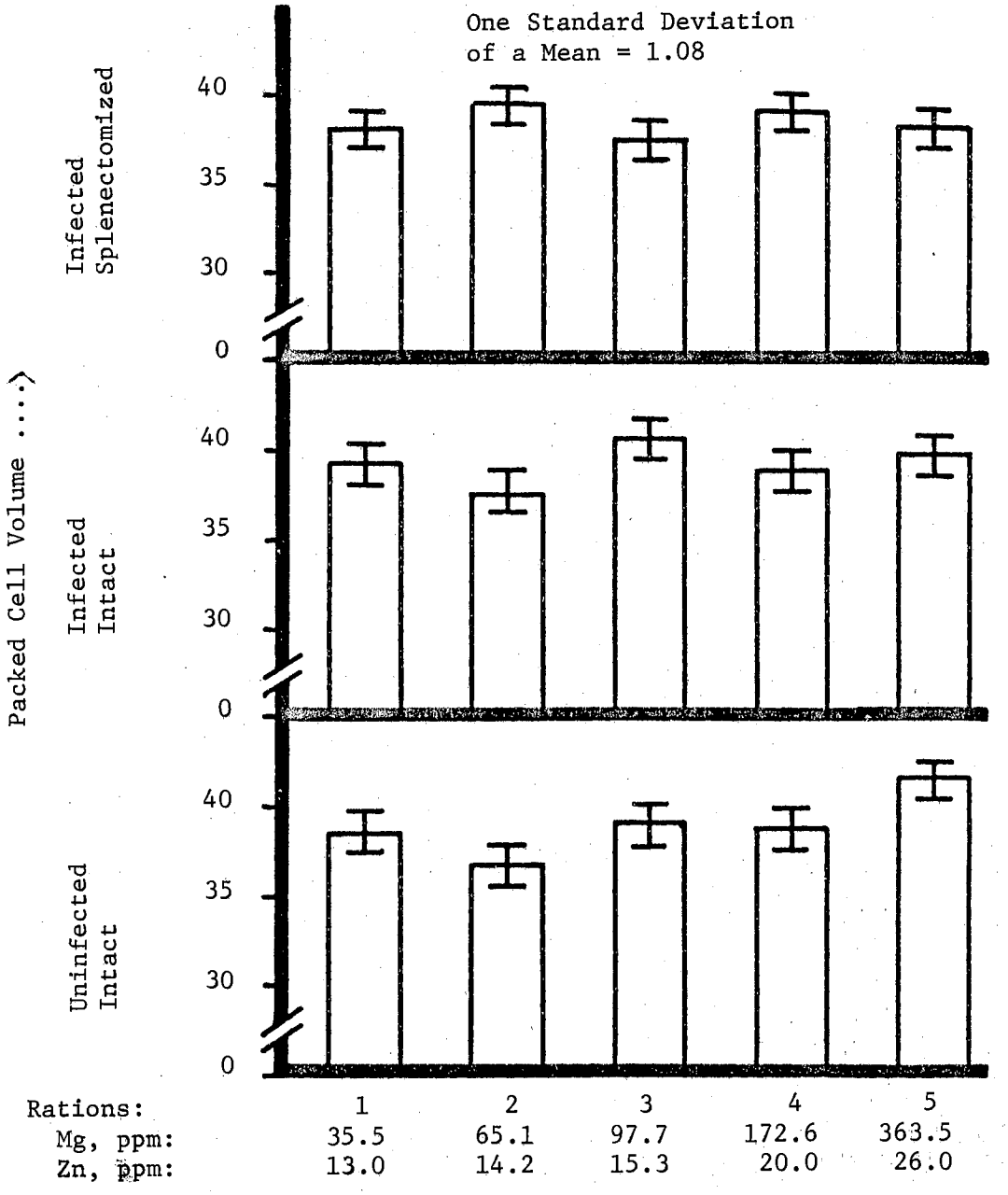

Figure 13. Pretreatment Packed Cell Volumes of rats assigned to various dietary levels of magnesium (Mg) and Zinc (Zn). All animals on basal complete ration; none infected with Babesia rodhaini; splenectomies already performed 4-5 days prior to taking pretreatment samples. N = 6 rats per treatment mean.

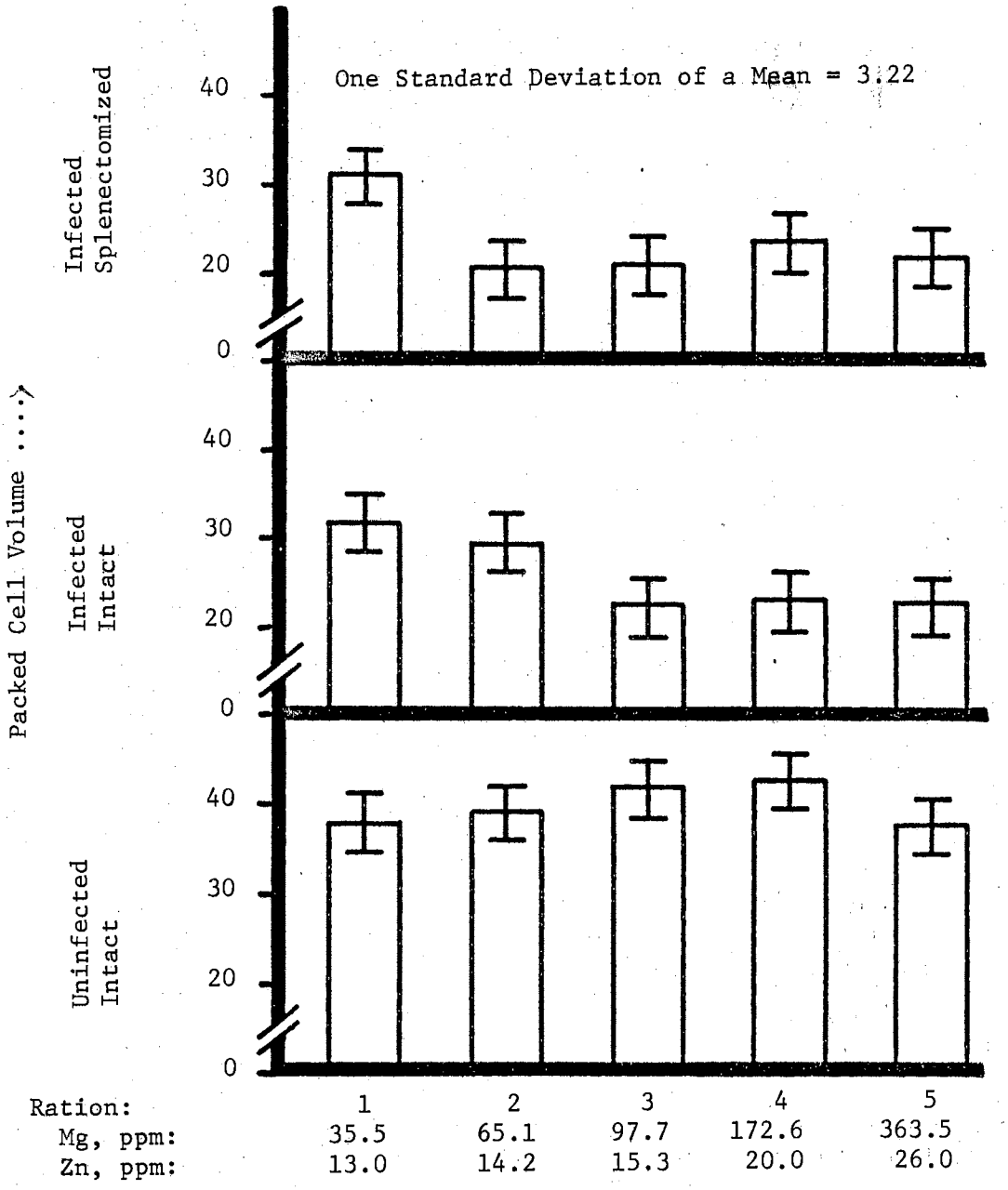

Figure 14. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on lowest observed packed cell volume for 22 days after inoculation with Babesia rodhaini. N = 6 rats per treatment mean.

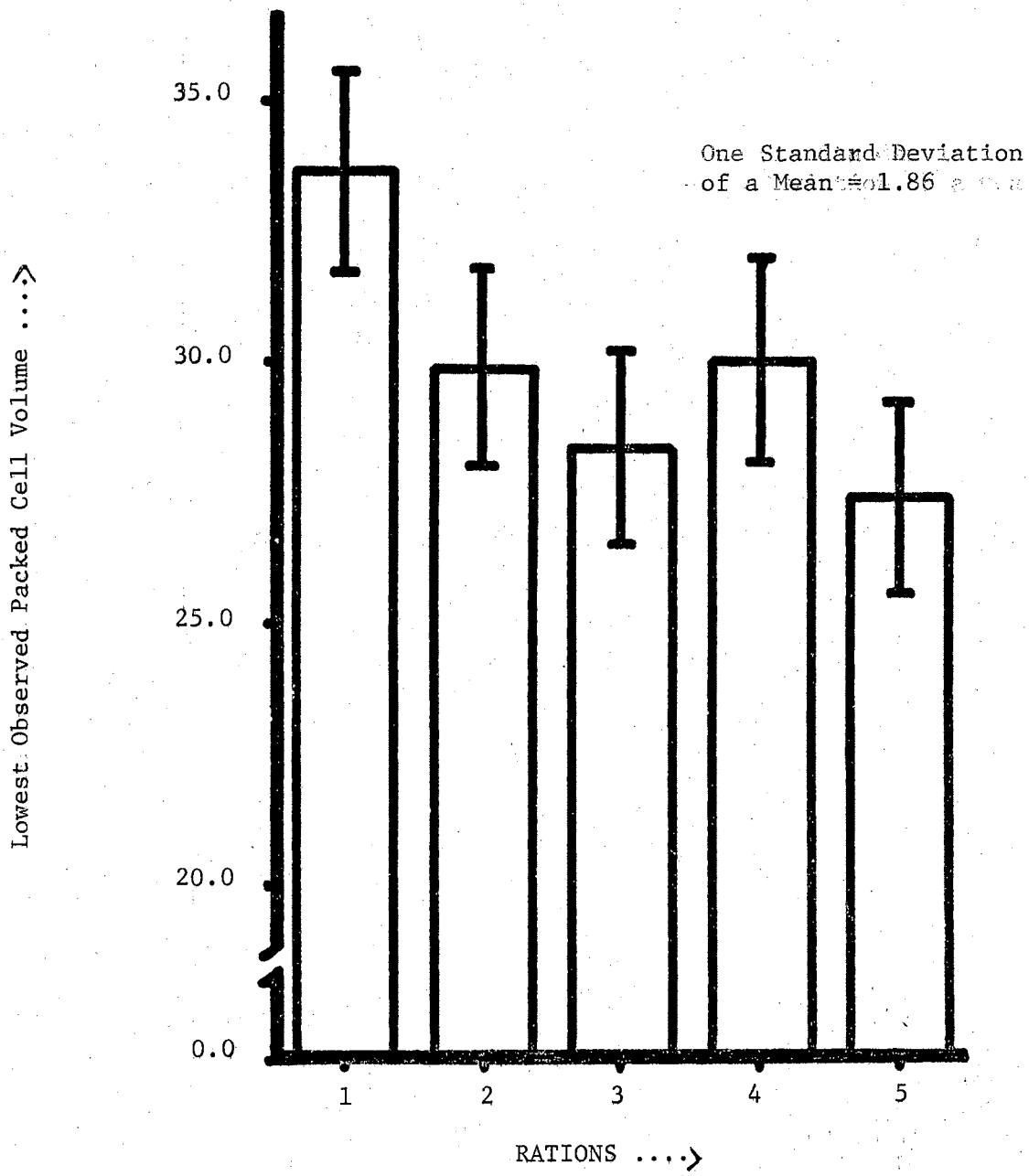

Figure 15. Effect of various mineral levels in the diet upon lowest observed packed cell volume in the rat after inoculation with Babesia rodhaini during 22-day postinoculation period. Minerals in p.p.m.: Ration #1, Mg 35.5, Zn 13.0; #2, Mg 65.1, Zn 14.2; #3, Mg 97.7, Zn 15.3; #4, Mg 172.6, Zn 20.0; #5, Mg 363.5, Zn 26.0. Summed over treatments.

infected animals there was one group of low P.C.V. animals which survived the course of the disease. Figure 15 which represents the data summed over treatments graphically depicts that penultimate mineral level has a higher P.C.V. level than that on either side of it. The slightly more rapid course of the disease from initial hemoglobinuria to death found in this experiment (1½ days) when compared to the first experiment (2 days) may mean that by maintaining the same 24-hour sampling interval we did not observe significantly ($P<.05$) lowered P.C.V. because those animals died before being sampled at the next sample period.

By comparing Figure 16 with Figure 9, it can be seen that those groups developing parasitemia above 50 percent are the same groups which have a marked reduction in survivability. When compared to Figure 15, there was a discrepancy in blood loss and degree of parasitemia in Ration 3 for the intact-infected treatment and in Ration 2 for the splenectomized-infected treatment. Figure 17 presents the response summed over treatments. The parasitemia observed in Ration 5 of the uninfected-intact treatment represented 1 of 30 rats in that treatment which developed babesiosis and subsequently died of the anemia. This was an accidental transfer.

Erythrocyte count (R.B.C.) data, seen in Figure 18, and mean corpuscular volume (M.C.V.) data of Figure 19, when interpreted together indicated the possibility of a lack of bone marrow response in those groups developing severe babesiosis (lower average M.C.V. values for these groups). Higher R.B.C. values are observed in the splenectomized-infected groups on the higher mineral levels. This is a significant ($P<.01$) linear decrease in the average M.C.V. with increased mineral

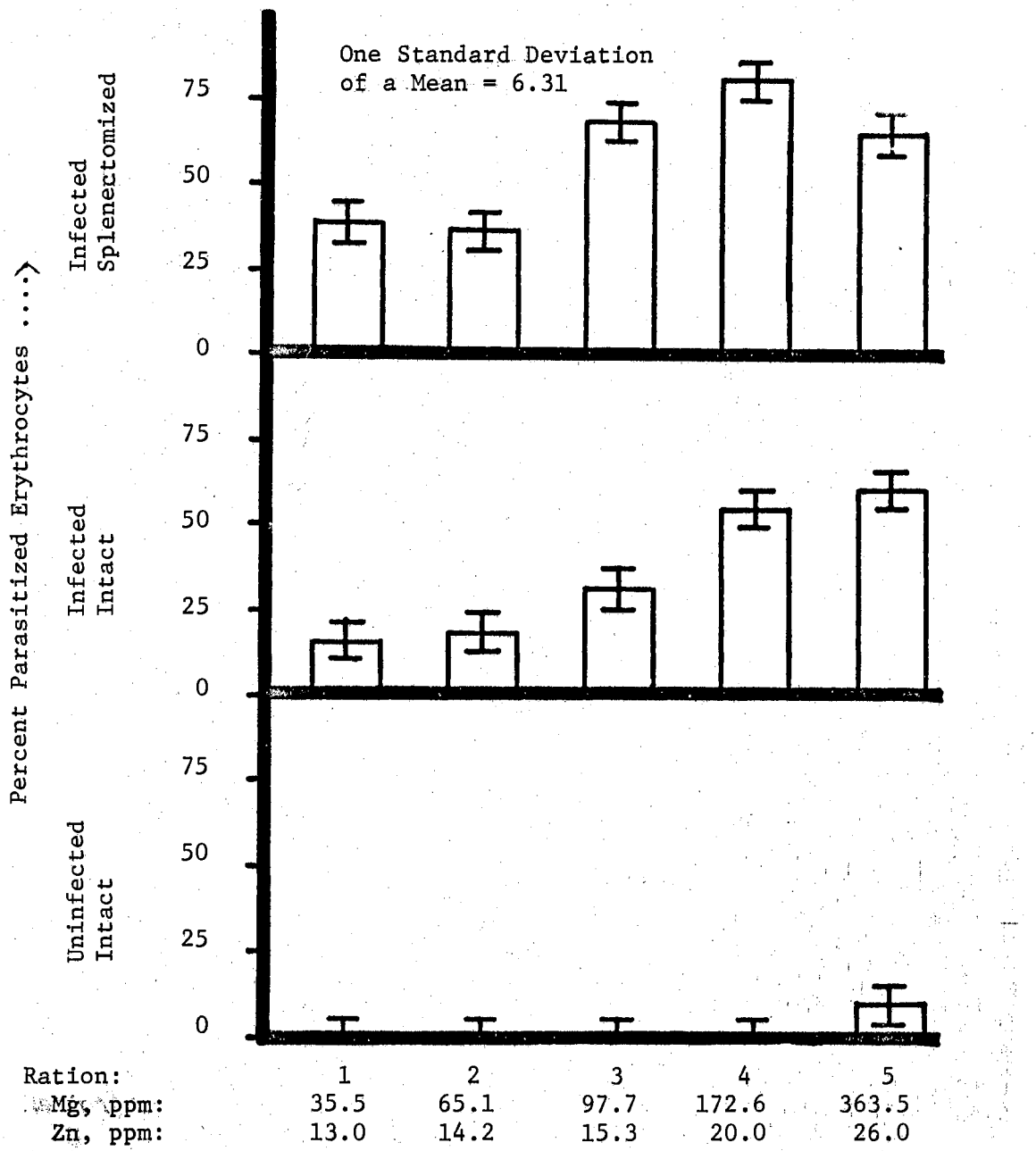

Figure 16. Effects of various dietary levels of magnesium (Mg) and zinc (Zn) on the highest percentage of parasitized erythrocytes observed during 22-day period after inoculation with Babesia rodhaini. N = 6 rats per treatment mean.

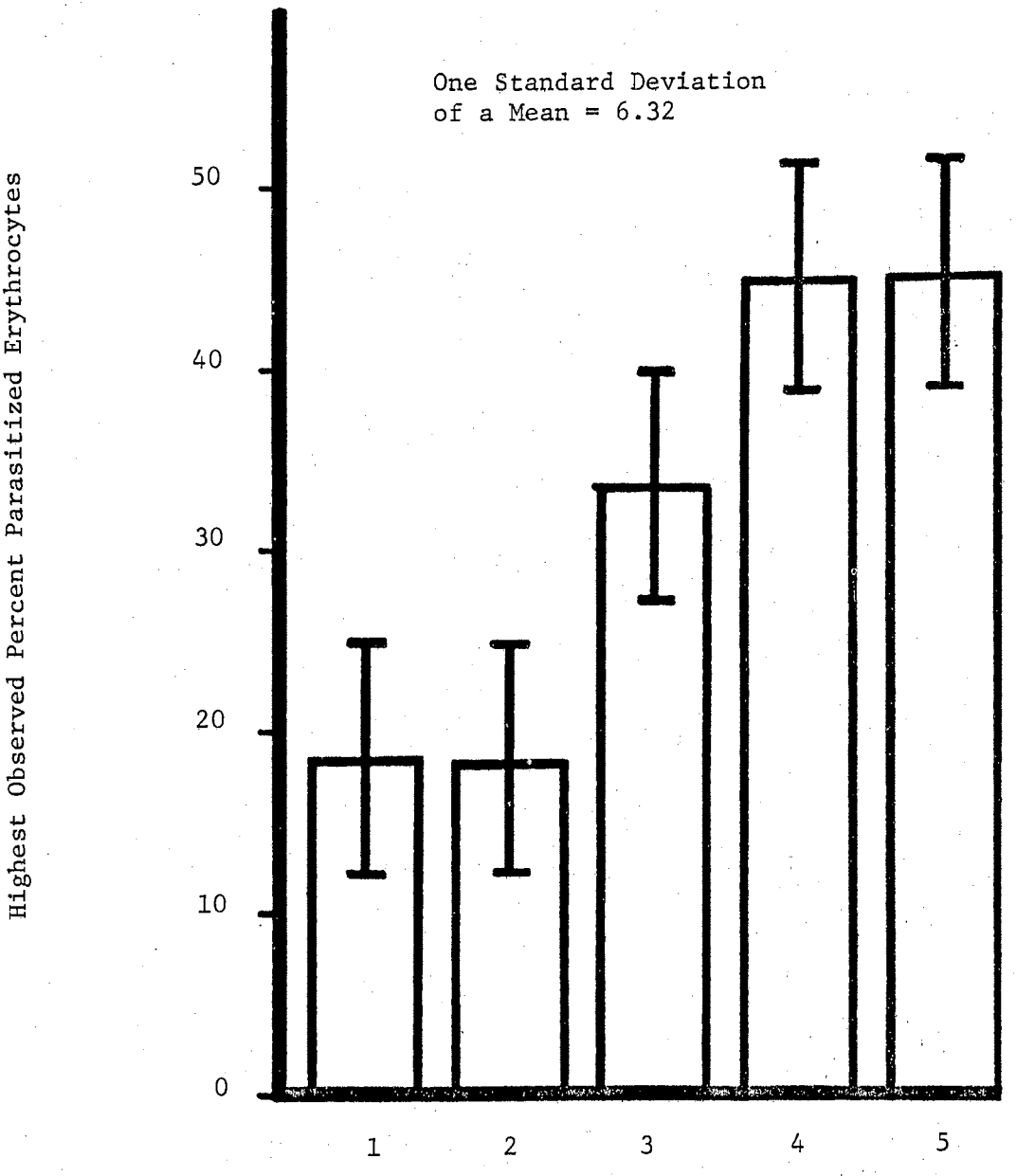

Figure 17. Effect of various mineral levels upon highest percentage of parasitized erythrocytes observed during a 22-day period postinoculation with Babesia rodhaini. Mineral levels in p.p.m.: Ration #1, Mg 35.5, Zn 13.0; #2, Mg 65.1, Zn 14.2; #3, Mg 97.7, Zn 15.3; #4, Mg 172.6, Zn 20.0; #5, Mg 363.5, Zn 26.0. Summed over treatments.

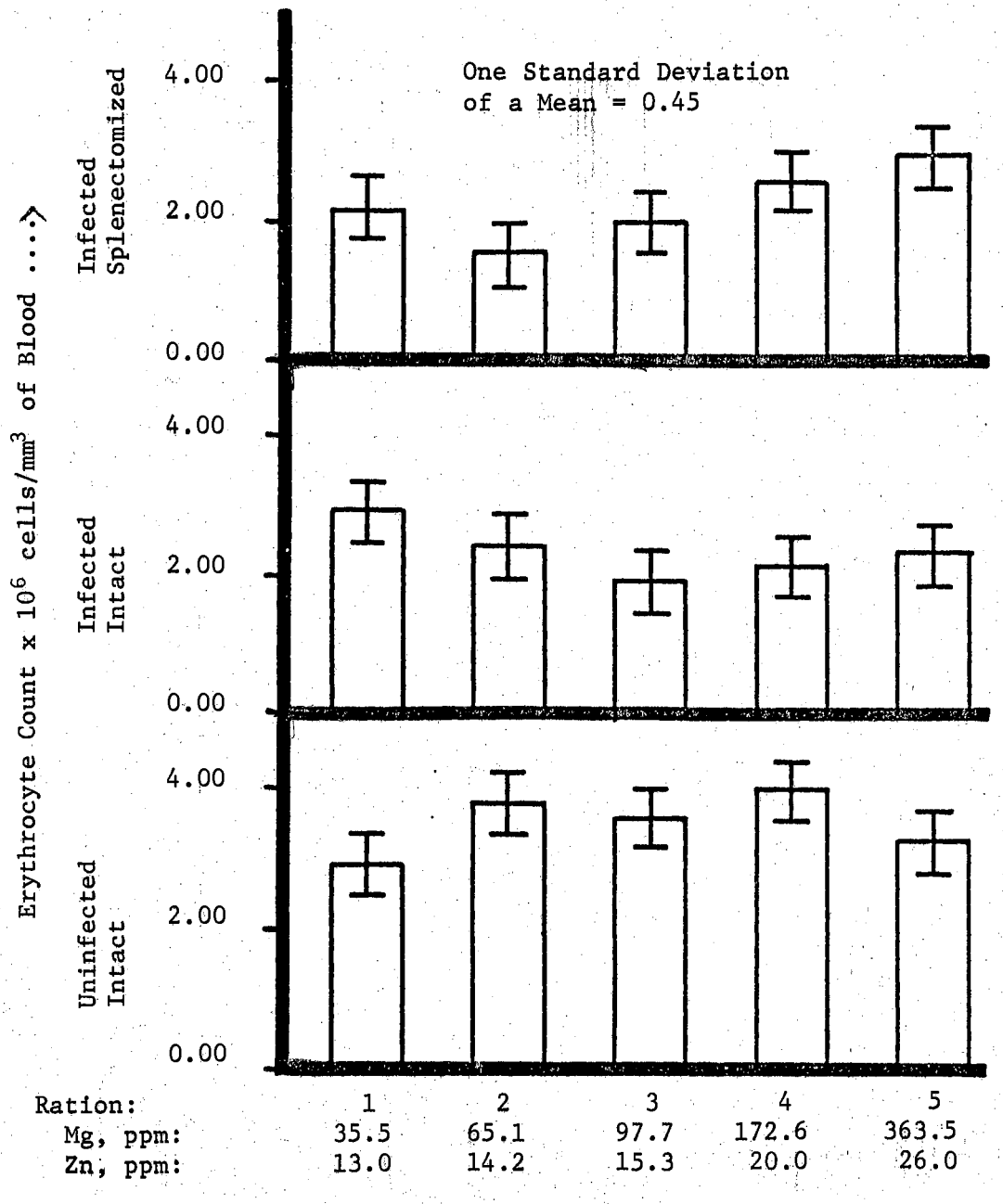

Figure 18. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on the lowest observed erythrocyte count during 22-day period after inoculation with Babesia rodhaini. N = 6 rats per treatment mean.

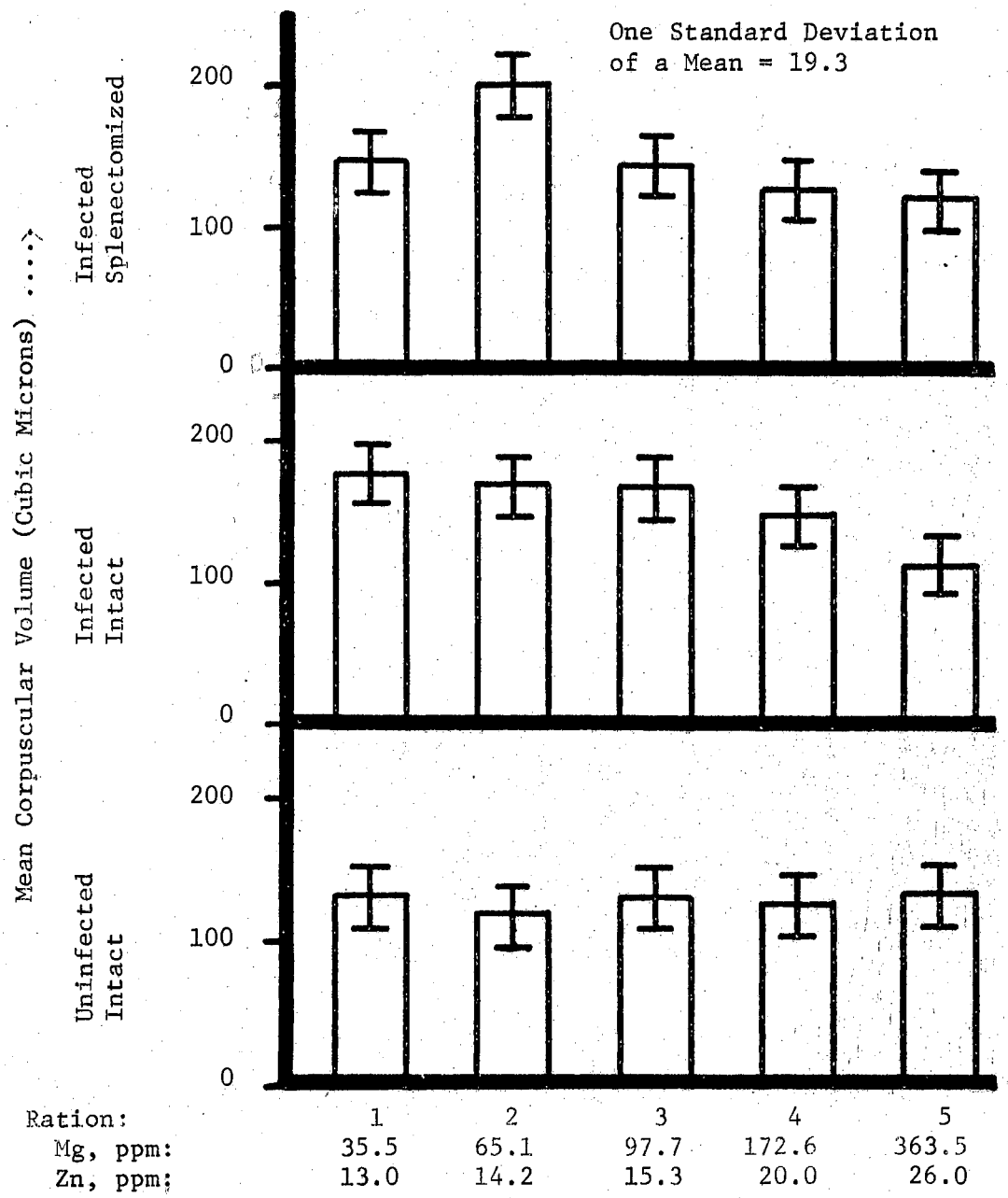

Figure 19. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on mean corpuscular volume of erythrocytes in rats on the day the lowest erythrocyte count was observed during 22-day period after inoculation with Babesia rodhaini. N = 6 rats per treatment mean.

levels (for treatment means for minerals, see Appendix B).

Figure 20 shows the influence of the various dietary mineral level upon the plasma magnesium (Mg) and erythrocyte Mg in the uninfected-intact rats after 22 days of experiment ration consumption. From the graph it can be seen that plasma magnesium levels plateaued at 1.4 mg Mg per 100 ml. Red cell values climbed to 6.4 mg Mg per 100 ml of cells, but on the lower end plateaued at about 3.5 mg percent for Mg.

Attempts to ameliorate the protection of the mineral deficiency by magnesium (Mg) or zinc (Zn) repletion were not considered successful (See Table VI). Of 15 surviving rats shown to have infective blood, one developed patent babesiosis after a repletion regime with oral and injected Mg, and subsequently died with the disease. Of 14 surviving rats shown to have infective blood, none broke or died with the disease after the same repletion regime with Zn. The one animal which died was from the infected-intact group on the lowest mineral level.

Experiment III

From the statistical data presented in Table VII, it can be seen that no significant effects ($P>.05$) were detected for any levels of zinc (Zn). The significant block effect ($P<.01$) for the pretreatment packed cell volumes (P.C.V.) indicated that the block occupying the position against the wall had higher values than did the block on aisle. The wall block was also on the left-hand side of the cage racks. Although a random tier order was used with each day's bleeding, tiers were always bled from left to right. The wall block was always bled before the aisle block in each cage tier or row. Block effect disappeared after the treatments were started.

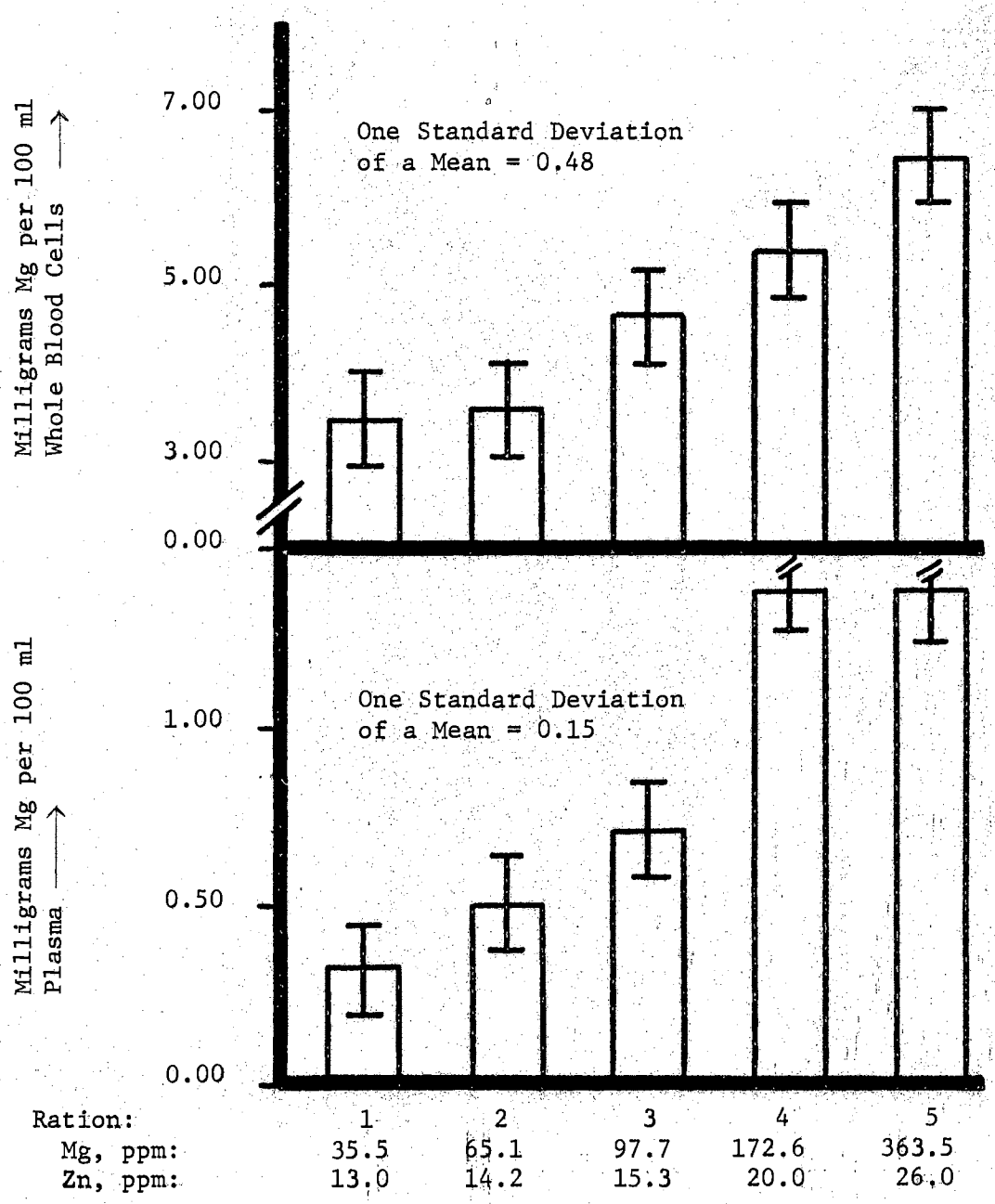

Figure 20. Effect of various dietary levels of magnesium (Mg) and zinc (Zn) on plasma and whole blood cell Mg. Uninfected, intact rats bled out after 22 days on experimental rations. N = 6 rats per treatment mean.

TABLE VI

EXPERIMENT II: ATTEMPT TO AMELIORATE PROTECTION OF MINERAL DEFICIENCY BY REPLETION OF SURVIVING ANIMALS WITH MAGNESIUM CHLORIDE (Mg) OR ZINC CHLORIDE (Zn), PER OS AD LIBITUM, PLUS DAILY INTRAPERITONEAL INJECTIONS FOR SEVENTEEN DAY PERIOD

	Dietary Mineral Status*		Number of Rats on Mineral Repletion		Rats: Babesia Carriers**	Rats: Ill with Babesia	Rats: Babesia Carriers***	Rat Blood Magnesium**** at End of Repletion	
	Mg ppm	Zn ppm	Mg	Zn	Starting Repletion	During Repletion	at End of Repletion	$\bar{x}$ mg% Cells	Plasma
Spleen Intact	30.0	13.6	4	-	3 of 4	1 (Died)	1	6.28	3.00
	30.0	13.6	-	4	3 of 4	0	?	5.14	0.94
	60.1	14.8	4	-	4 of 4	0	1	6.71	2.72
	60.1	14.8	-	4	4 of 4	0	?	5.04	0.94
	91.0	15.9	4	-	3 of 4	0	?	7.42	2.13
	91.0	15.9	-	4	2 of 4	0	1	5.63	1.23
	157.5	20.6	2	-	0 of 2	0	?	7.57	2.46
	157.5	20.6	-	1	1 of 4	0	1	(6.02)	(1.04)
	363.1	37.0	0	-	0 of 0	0	?	-	-
	363.1	27.0	-	1	1 of 1	0	1	(5.83)	(2.09)
Splenectomized	30.1	13.6	3	-	2 of 3	0	1	(7.01)	(4.73)
	30.0	13.6	-	2	2 of 2	0	1	7.82	1.99
	60.1	14.8	3	-	3 of 3	0	2	8.17	4.78
	91.0	15.9	0	-	0 of 0	0	?	-	-
	91.0	15.9	0	1	1 of 1	0	?	(6.00)	(0.88)

* Repletion scheme: Experimental rations continued; magnesium chloride or zinc chloride, 1 normal solution offered ad libitum, 17 days; minimum of 12 mg Mg (as $MgCl_2$) or 0.36 mg Zn (as ZnCl) injected intraperitoneally last 12 days of 17 day repletion period.

** Carrier status identified by subinoculation into at least 2 mice per rat with one or both of the mice dying with Babesia.

*** Severe cannibalism among these animals caused loss of identification of most mice. Results inconclusive.

**** Etherized rats bled out via abdominal aorta with heparinized syringe. Mg determined with Perkin-Elmer Model 303 Atomic Adsorption Spectrophotometer.

( ) Indicates value for only one animal.

TABLE VII

EXPERIMENT III, ANALYSIS OF VARIANCE: SUMMARY OF MEAN SQUARES

5 x 3 x 2 Factorial Source:	df	Days Survived	Interval: Incubation	Lowest Observed P.C.V.+	Percent Parasitized Erythrocytes	Pretreatment P.C.V.+
Magnesium (Mg)						20.49
Linear	1	3973.8**	64.85*	659.77*	22642.3**	-
Quadratic	1	3378.0**	37.28	193.06	19363.2**	-
Cubic	1	627.1	26.00	1857.07**	6083.2**	-
Quartic	1	361.2	47.49	3.17	586.4	-
Zinc (Zn)						18.01
Linear	1	28.0	2.24	112.31	428.3	-
Quadratic	1	58.1	3.00	180.70	582.6	-
Mg x Zn	6	116.5	23.46	83.63	774.3	20.9
Blocks	1	264.0	0.01	38.78	421.1	448.4**
Error	14	174.6	9.00	90.25	662.8	20.75
C.V.%++		80.5	33.0	40.0	51.2	10.4
N =		120	120	120	120	120

* Significant, P<.05; ** Significant, P<.01.
\+ P.C.V. = Packed Cell Volume.
++ C.V. = Coefficient of Variation.

Significant linear effects were detected for: days survived ($P<.01$), interval between inoculation and crisis ($.01<P<.05$) lowest observed P.C.V. ($.05>P>.01$) and parasitemia ($P<.01$). Good evidence was presented for the presence of polynomial effects in: days survived (quadratic: $P<.01$), lowest observed P.C.V. (cubic: $P<.01$), and parasitemia (quadratic: $P<.01$; cubic: $P<.01$).

No significant interactions ($P>.05$) were detected. With no detectable significant zinc response or significant interaction, the rest of the data for Experiment III is presented on the basis of the responses to the various levels of magnesium. The tables of treatment means in Appendix B permit inspection of the data for actual values given by various levels of zinc.

Figures 21 and 22 show typical pairs of rats after only 5 days on the high and low magnesium (Mg) rations. Reddened ears are noticeable on the rats in Figure 22. Reddened feet and tail are less pronounced, but are also present on these animals. This erythematous response is similar to those observed in the two previous experiments.

The marked ability of the lower magnesium levels to increase survival time in rats inoculated with babesia is seen in Figure 23. Figure 24 presents a machine-computed plot of the same data. (The curve equation derived from that plot is found in Appendix D).

Lower dietary magnesium levels also corresponded with lower blood loss associated with patent babesiosis in the rat (Figure 25). A highly significant ($P<.01$) cubic effect was observed in this data, indicating that the high mean at the 157.5 ppm Mg level was probably a significant response. Figure 26 fits an exponential curve to the data and provides an equation for that curve (Appendix D).

Figure 21. Uninfected rats after 5 days on ration containing 343.1 ppm magnesium. [4]

Figure 22. Uninfected rats after 5 days on ration containing 30.0 ppm magnesium. Note color of ears, feet and tails. [5]

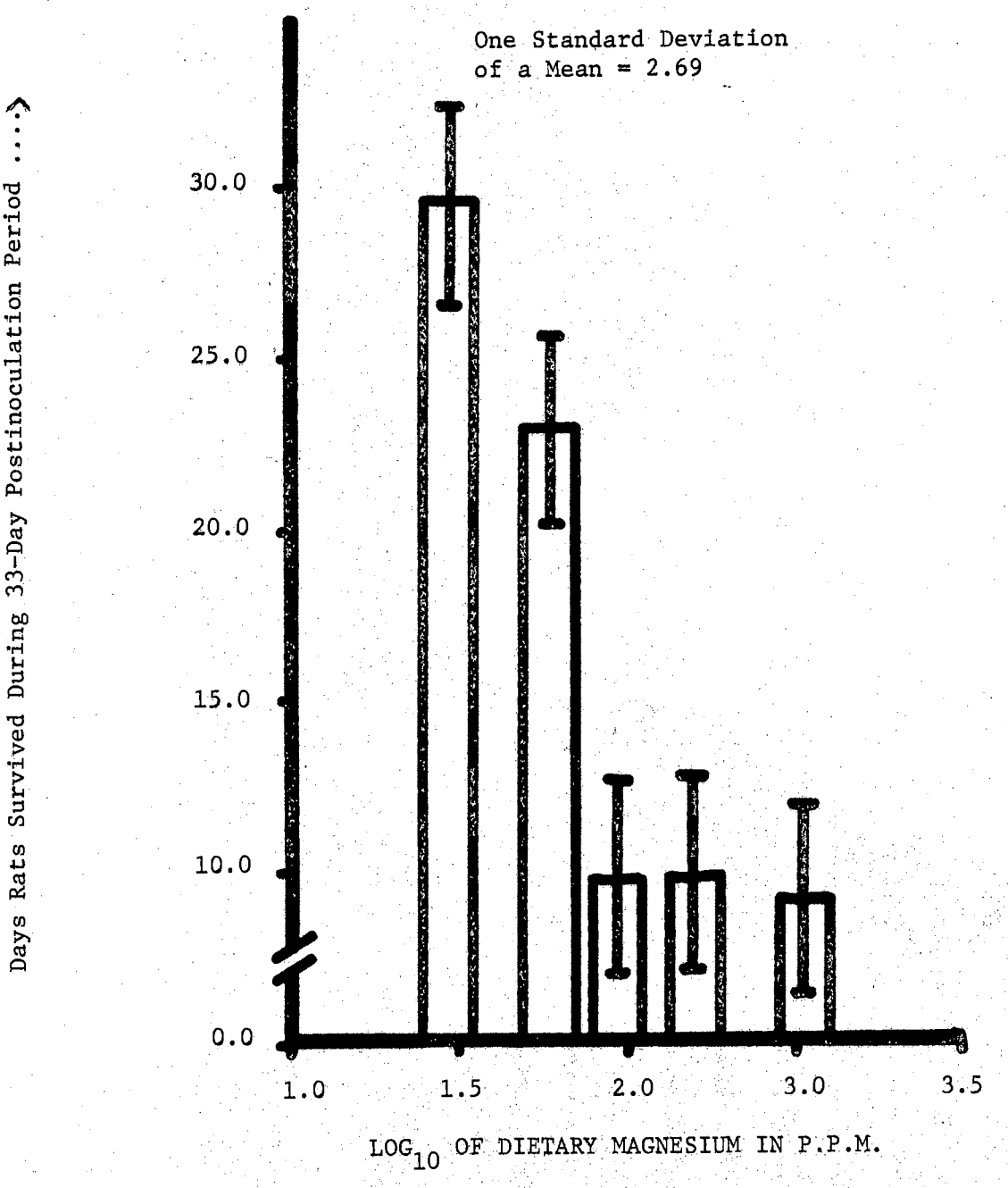

Figure 23. Effect of dietary magnesium (Mg) levels upon the rat's ability to survive inoculation with Babesia rodhaini during 33-day postinoculation period. Actual Mg levels in p.p.m., left to right: 30.0, 60.1, 91.0, 157.5 and 343.1.

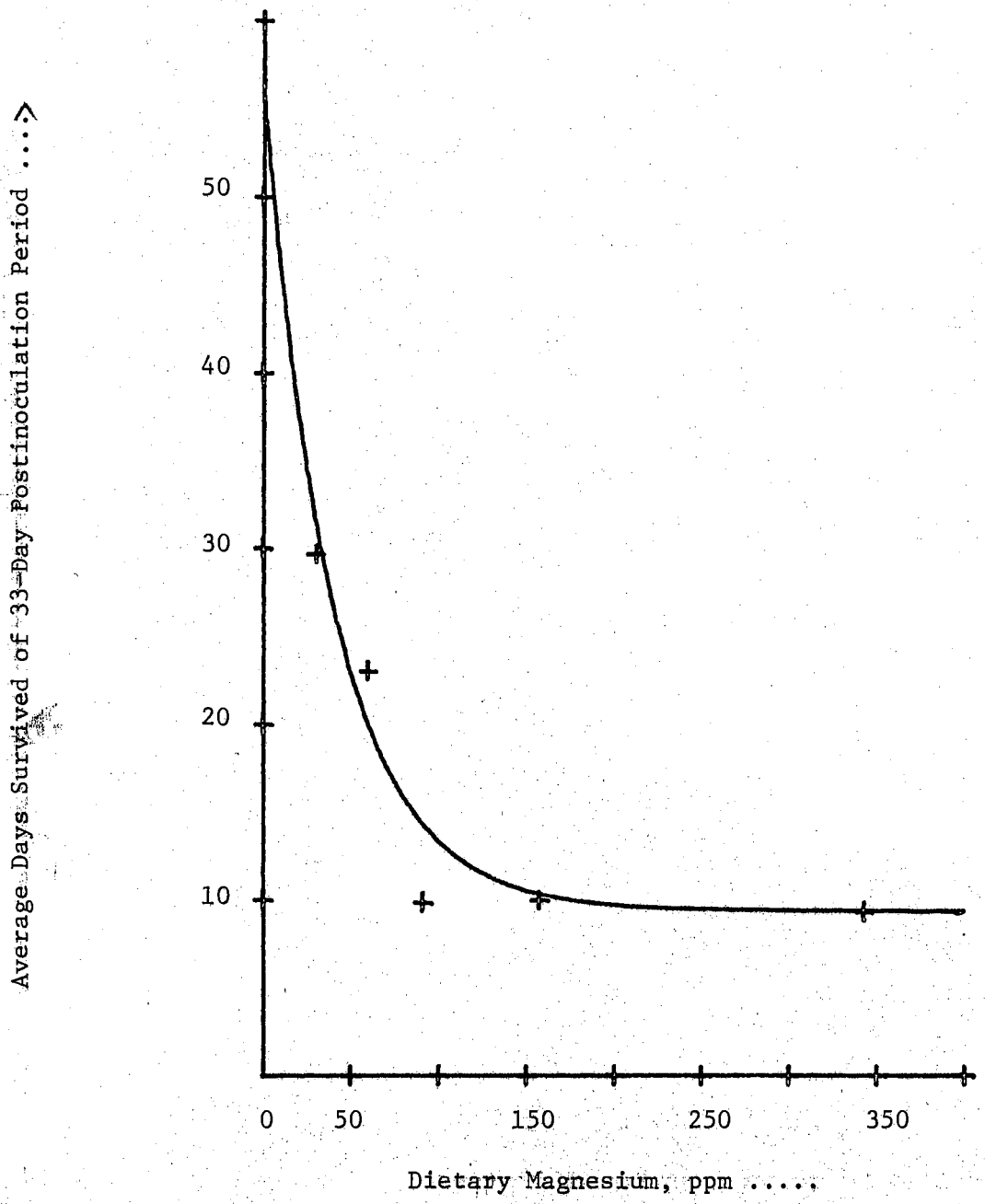

Figure 24. Exponential curve fitted to data for days survived during 33-day observation period after inoculation with Babesia rodhaini. (For curve equation data, see Appendix D, Table XXXIX). Machine plotted graph.[5]

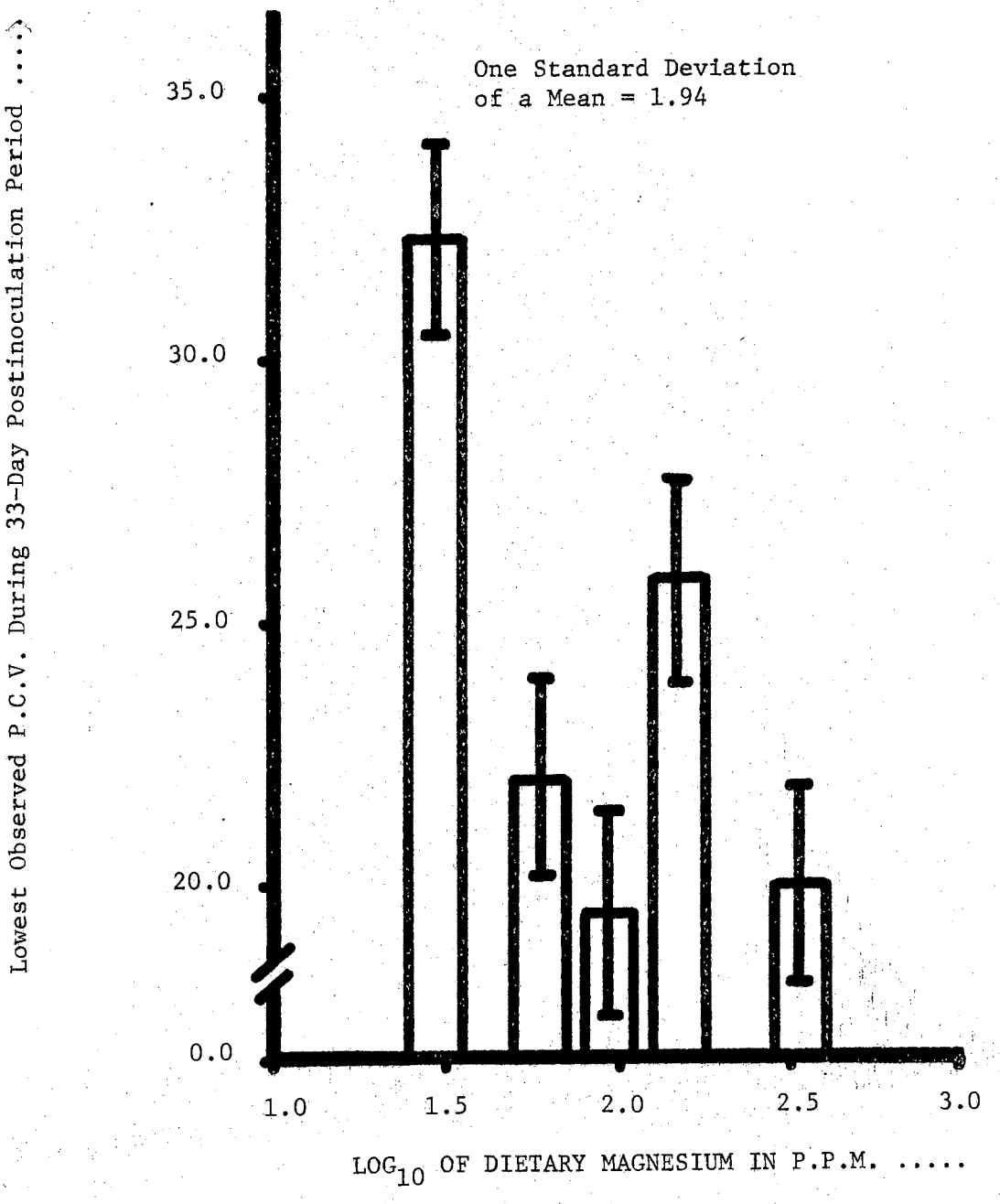

Figure 25. Effect of dietary magnesium (Mg) on lowest packed cell volume (P.C.V.) observed during 33-day period after inoculation with Babesia rodhaini. Actual Mg levels, p.p.m., left to right: 30.0, 60.1, 91.0, 157.5 and 343.1.

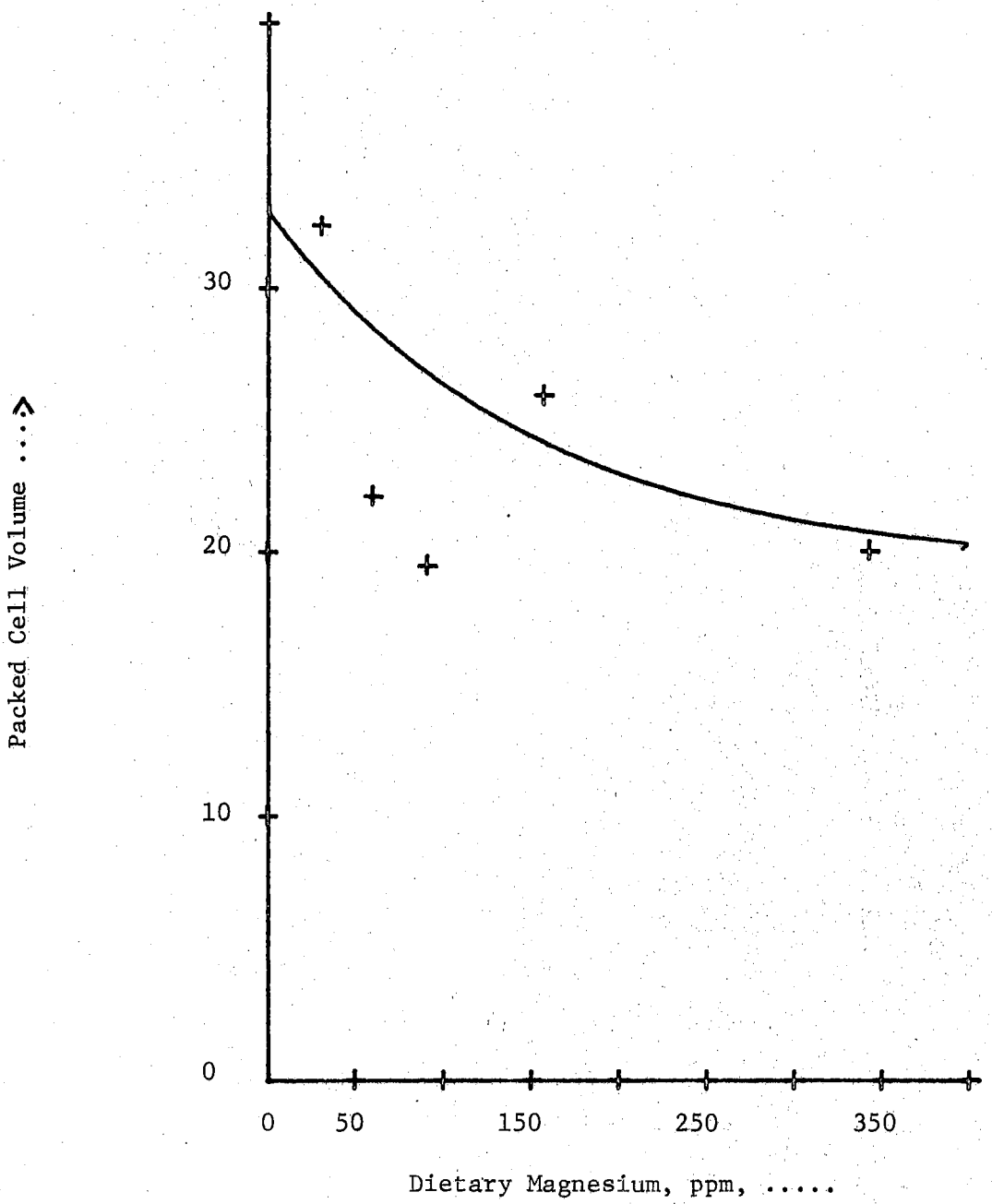

Figure 26. Exponential curve fitted to data for lowest observed packed cell volume during 33-day period after inoculation with Babesia rodhaini. (For curve equation data, see Appendix D, Table XXXIX). Machine plotted graph.[6]

The degree of parasitemia was somewhat proportional to the level of magnesium with higher Mg levels corresponding to higher levels of circulating parasites (Figure 27). This data is fitted with an exponential curve in Figure 28.

Although a significant linear respnose ($P<.05$) was detected for the influence of dietary magnesium on the time interval between inoculation and anemic crisis, its interpretation is clouded by the high value observed at the 60.1 ppm Mg level (Figure 29).

Table VIII indicates the acuteness of the anemic response, once initiated. Significantly lowered packed cell volumes ($P<.01$) were detected only 24 hours prior to the day of peak anemia. Significant cubic effects ($P<.05$) were detected for both days, as well as a linear effect ($P<.05$) for the day of peak anemia.

Subinoculation of rat blood into mice was used to detect those rats surviving the 33-day post-inoculation observation period which were still infected with Babesia rodhaini (See Table IX). Data for 32 survivors were complete enough for purposes of analysis. Of these 32 rats, only 4 failed to infect mice with babesia by subinoculation.

The graphs in Figure 30 summarize the more significant observations for this experiment. It was obvious that a decreased overall anemic response was related to the levels of Mg; the lower levels not only provided protection from the overall anemic response but also decreased death loss during the anemic crisis. Figures 31 through 45 provide similar information for each of the 15 Mg-Zn treatment combinations.

The growth response of the rats during the early part of the experiment is seen in Figure 46. A graded response to dietary magnesium is seen in the third observation period. The first 2 periods indicate

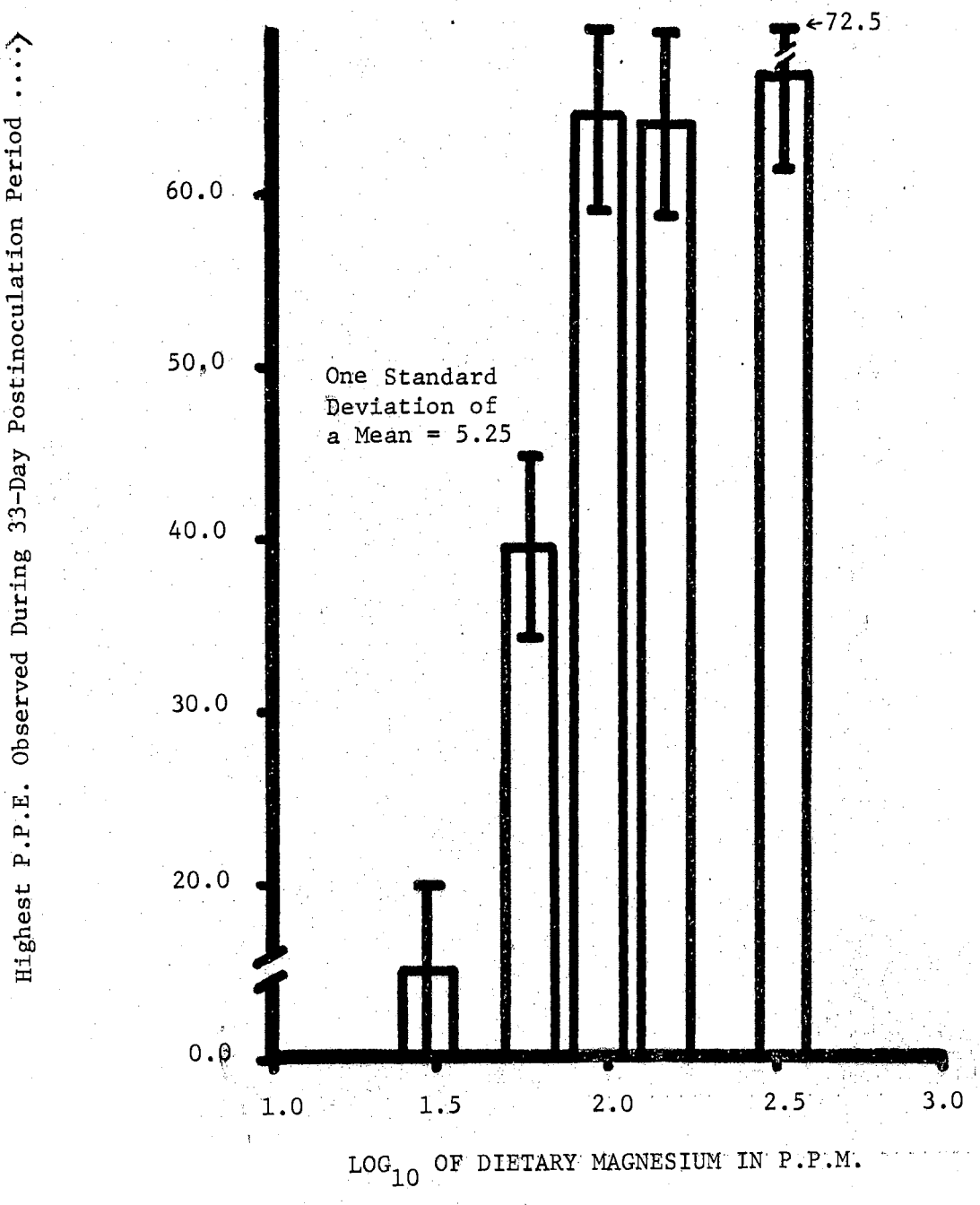

Figure 27. Effect of dietary magnesium (Mg) levels upon highest percentage of circulating erythrocytes observed to be infected with Babesia rodhaini (percent parasitized erythrocytes = P.P.E.). Actual Mg levels, p.p.m., left to right: 30.0, 60.1, 97.9, 157.5 and 343.1.

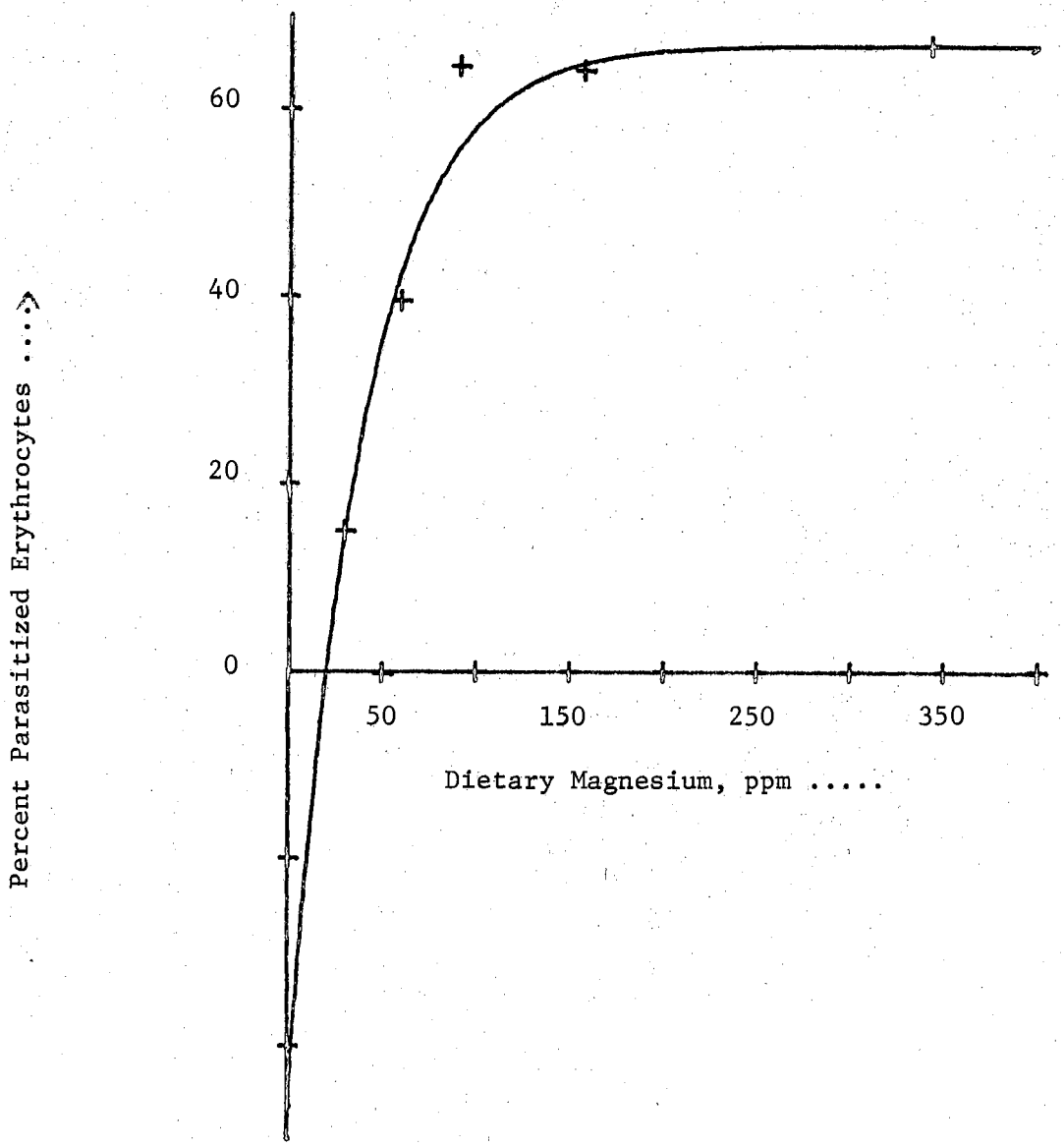

Figure 28. Exponential curve fitted to the data for the highest percent parasitized erythrocytes observed during 33-day period after inoculation with Babesia rodhaini. (For curve equation data, see Appendix D, Table XXXIX). Machine plotted graph.[7]

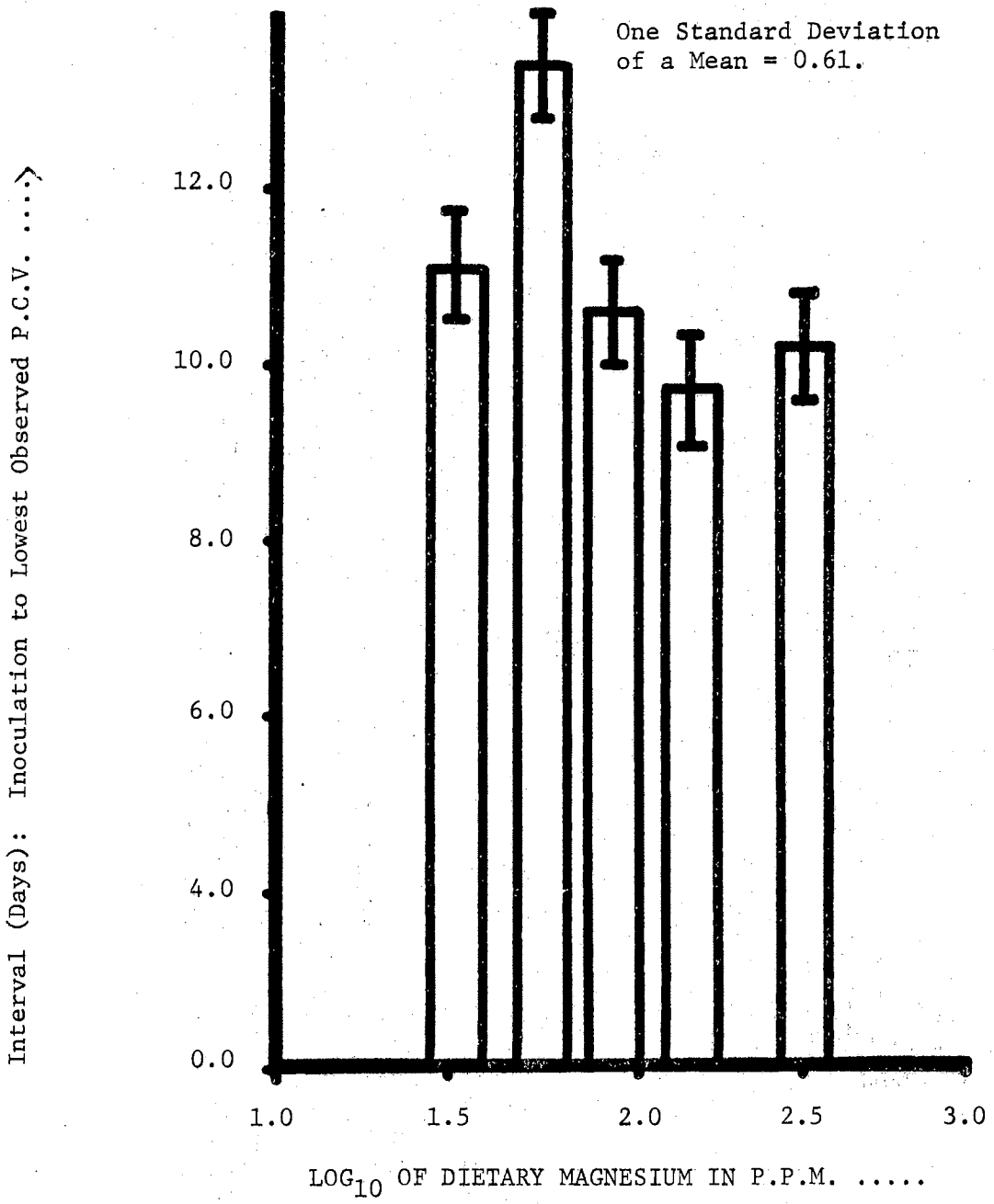

Figure 29. Effect of dietary magnesium (Mg) levels upon the interval between day of inoculation with Babesia rodhaini and lowest observed packed cell volume (P.C.V.) during 33-day postinoculation period. Actual Mg levels, p.p.m., left to right: 30.0, 60.1, 91.0, 157.5 and 343.1.

TABLE VIII

EXPERIMENT III, ANALYSIS OF VARIANCE: SUMMARY OF MEAN SQUARES

Source	df	PCV 3 Days Prior to Low PCV	PCV 2 Days Prior to Low PCV	PCV 1 Day Prior to Low PCV	Lowest Observed PCV
Magnesium (Mg)	4				
Linear	1	3.37	39.78	7.59	659.77*
Quadratic	1	18.27	3.73	14.64	193.06
Cubic	1	4.20	27.66	583.26**	1,857.07**
Quartic	1	4.02	34.75	65.61	3.17
Zinc (Zn)	2				
Linear	1	1.49	0.01	59.31	112.31
Quadratic	1	4.54	6.80	220.62	180.71
Mg x Zn	8	39.83	30.98	46.75	83.63
Blocks	1	22.71	12.81	33.18	277.55
Error	14	23.11	28.77	47.94	90.17
C.V. %		11.6	13.6	19.7	39.8
$\underline{N}$ =		120	120	120	120
$\bar{X}\bar{x}$, P.C.V.		41.5	39.4	35.1	24.0
$\bar{x}$ P.C.V. within Mg,			Treatment Means		
30.0 ppm		41.7	40.7	38.8	32.4
60.1 ppm		41.3	38.2	31.9	22.4
91.0 ppm		41.6	40.0	33.4	19.5
157.5 ppm		40.6	39.9	36.7	25.9
343.1 ppm		42.1	38.1	34.6	20.1

P.C.V. = Packed Cell Volume. C.V. = Coefficient of Variation. Significance: *, P<.05; ** P<.01.

TABLE IX

MICE DYING WITH BABESIA RODHAINI AFTER SUBINOCULATION WITH BLOOD FROM RATS AT END OF EXPERIMENT III

Dietary Mg ppm	Dietary Zn ppm	Mice Died/Mice Inoculated+ 1++	2	3	4	5	6	7
33.0	12.7	4/4	4/4	4/4	1/4	0/4		
33.0	13.5	4/4	4/4	2/4	1/4	1/4	1/4	0/4
33.0	353.0	4/4	4/4	*	*	*		
65.0	12.7	4/4	3/4	2/4				
65.0	13.5	4/4	4/4	2/4	1/4	1/4	0/4	
65.0	353.0	4/4	4/4	3/4	2/4	2/4		
93.0	12.7	-						
93.0	12.7	-						
93.0	353.0	4/4						
165.0	12.7	0/4						
165.0	13.5	2/4						
165.0	353.0	-						
353.0	12.7	-						
353.0	13.5	-						
353.0	353.0	2/4						

+ Four mice were inoculated with 0.2 cc heparinized whole blood from each surviving rat; intraperitoneally injected with a 22 ga., 1 inch needle. Observed for 14 days.

++ Data in each column represents one surviving rat at respective mineral levels.

* Mice in these cages became cannibalistic; observations terminated.

Magnesium (Mg); Zinc (Zn); parts per million (ppm).

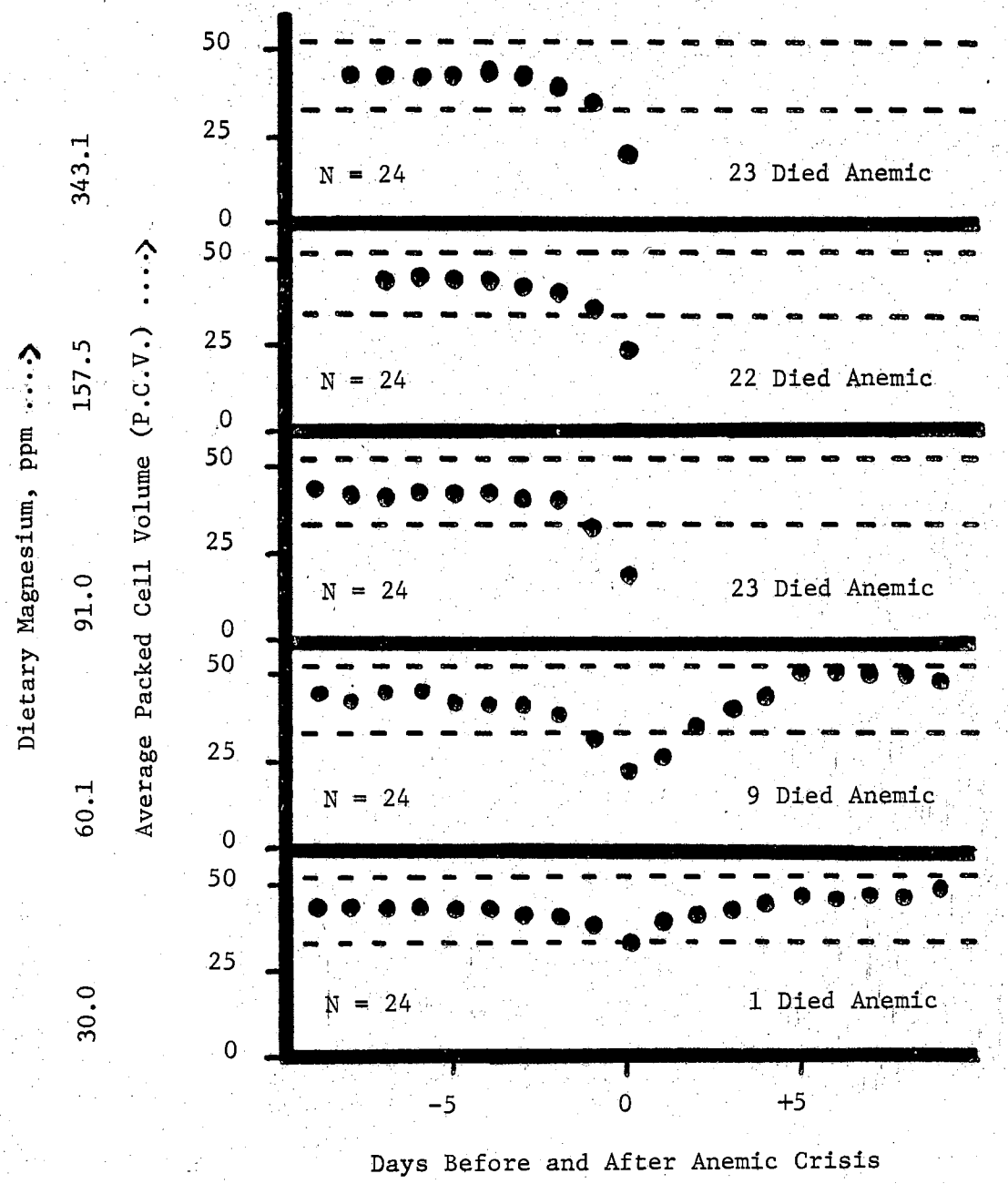

Figure 30. Effect of dietary magnesium (Mg) levels on the course of the anemia during 30-day observation period after inoculation with Babesia rodhaini. The dotted lines enclose the pretreatment grand mean (43.7) plus or minus 1 standard deviation (4.54). N = 24 rats per treatment mean.

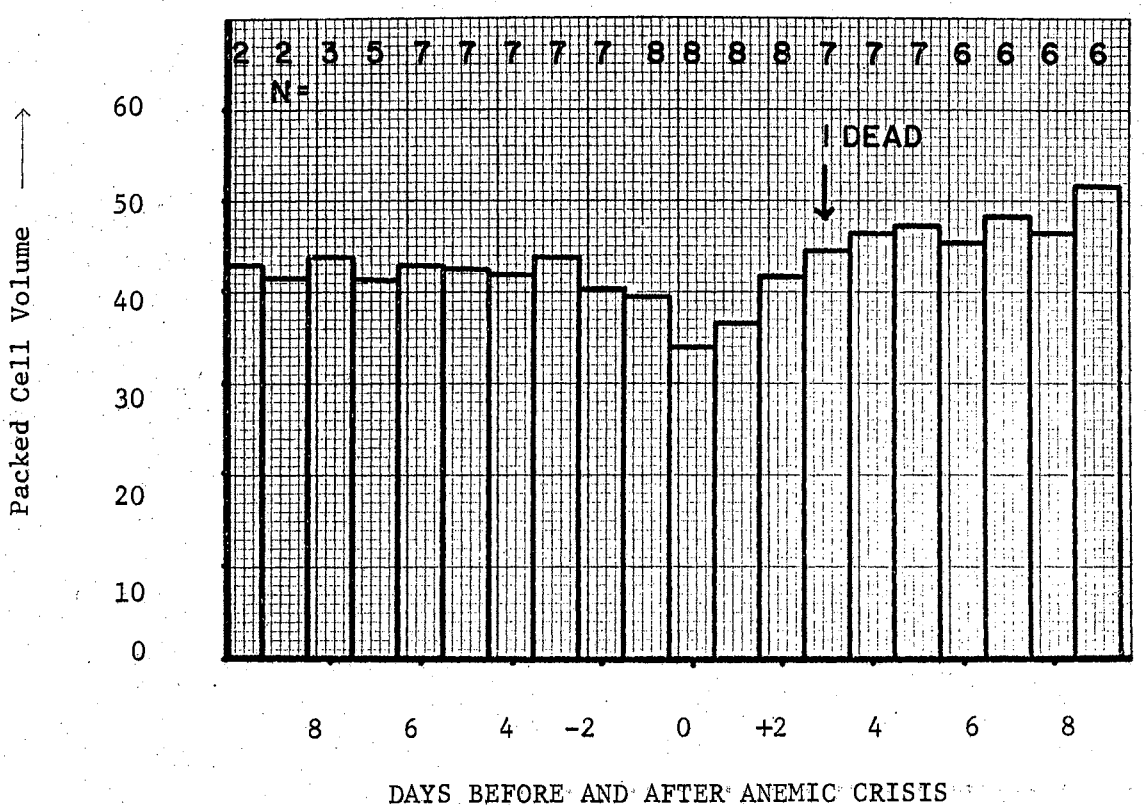

Figure 31. Course of anemia in rats fed 30.0 ppm magnesium and 12.7 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

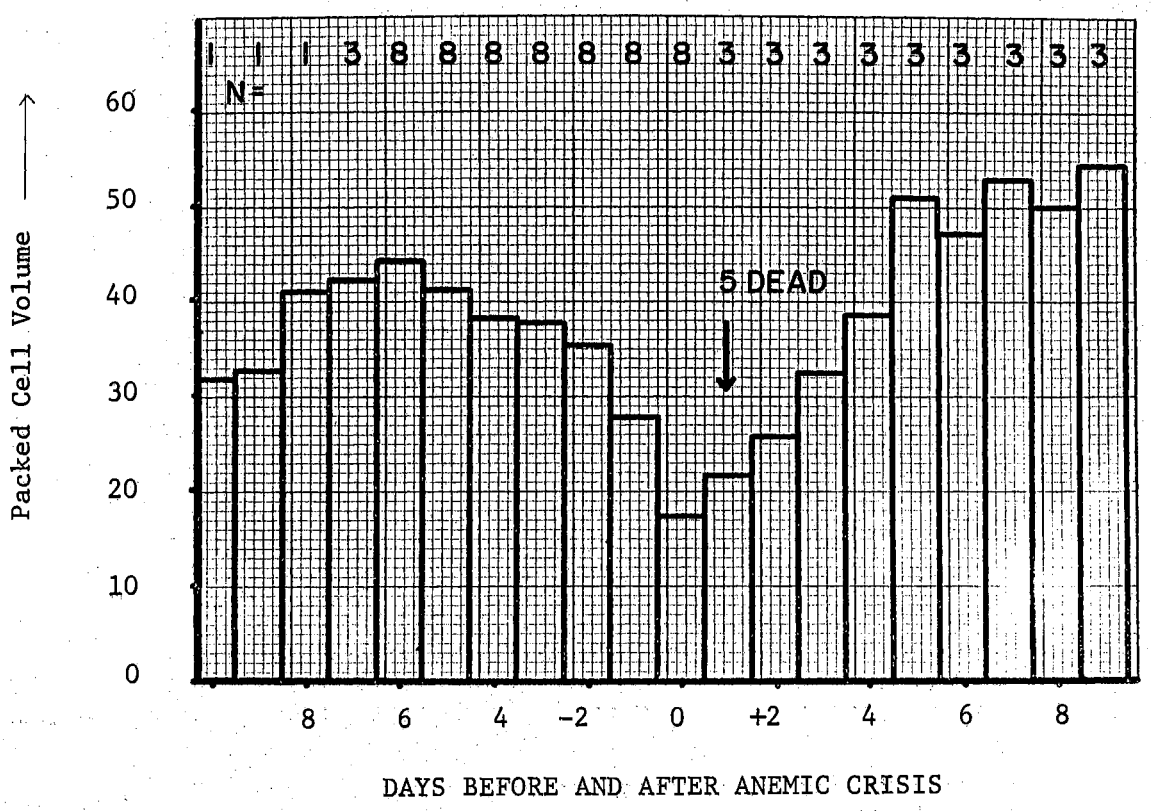

Figure 32. Course of anemia in rats fed 60.1 ppm magnesium and 12.7 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

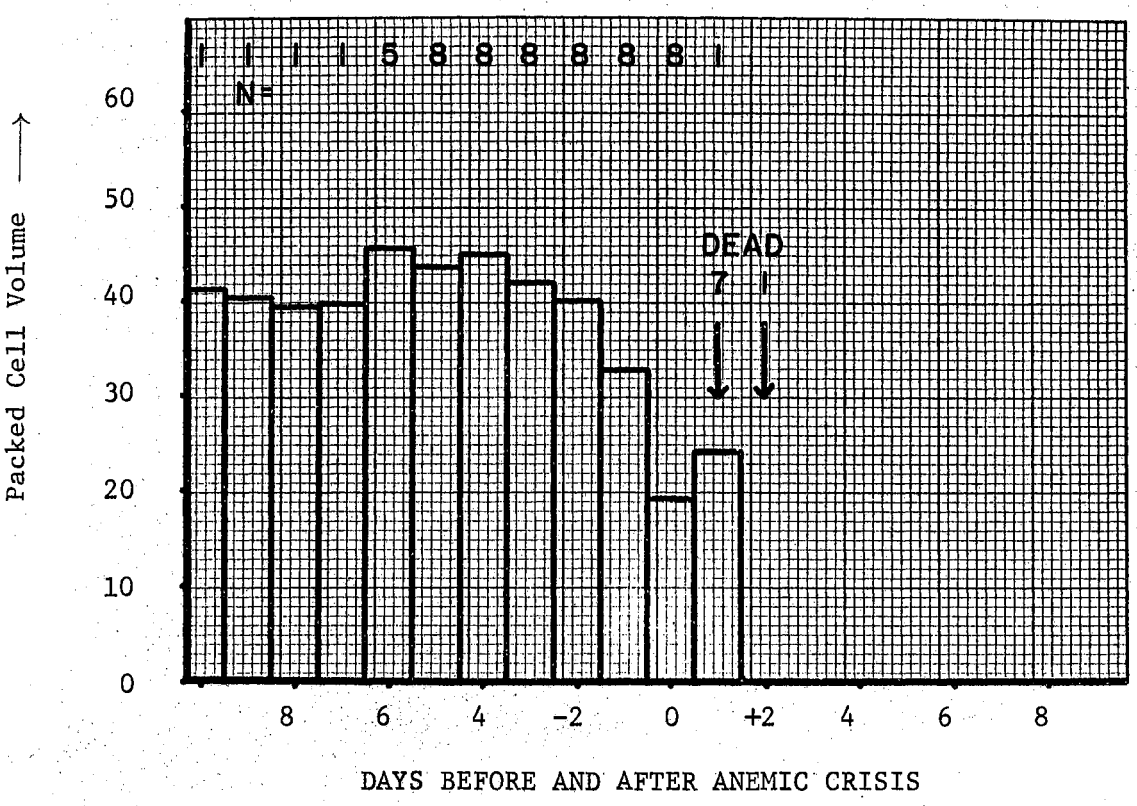

Figure 33. Course of anemia in rats fed 91.0 ppm magnesium and 12.7 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

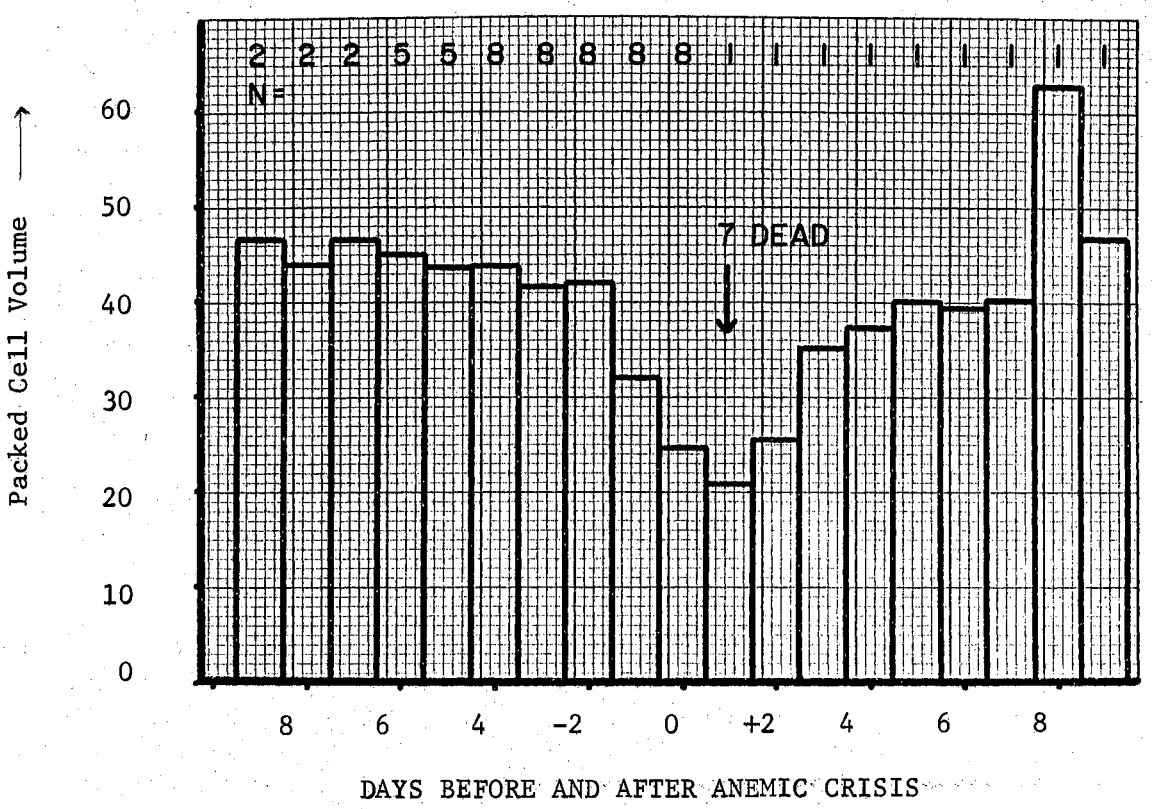

Figure 34. Course of anemia in rats fed 157.5 ppm magnesium and 12.7 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

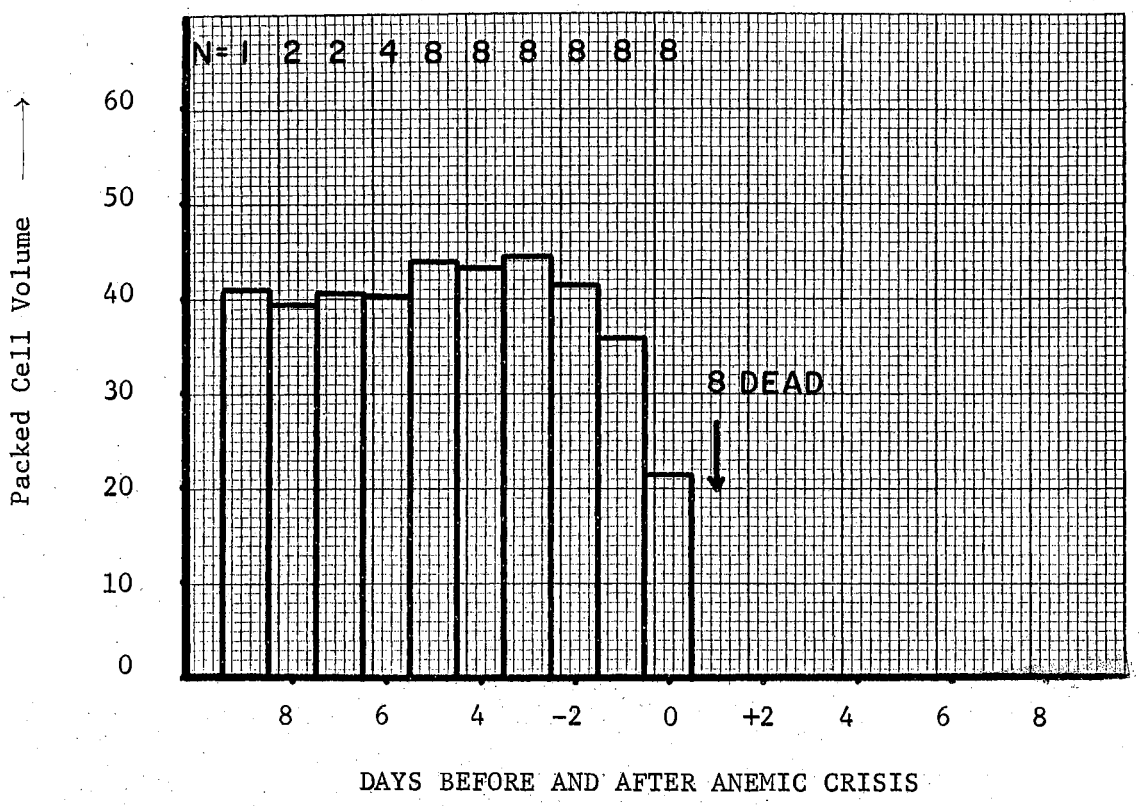

Figure 35. Course of anemia in rats fed 343.0 ppm magnesium and 12.7 ppm zinc during 30 day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

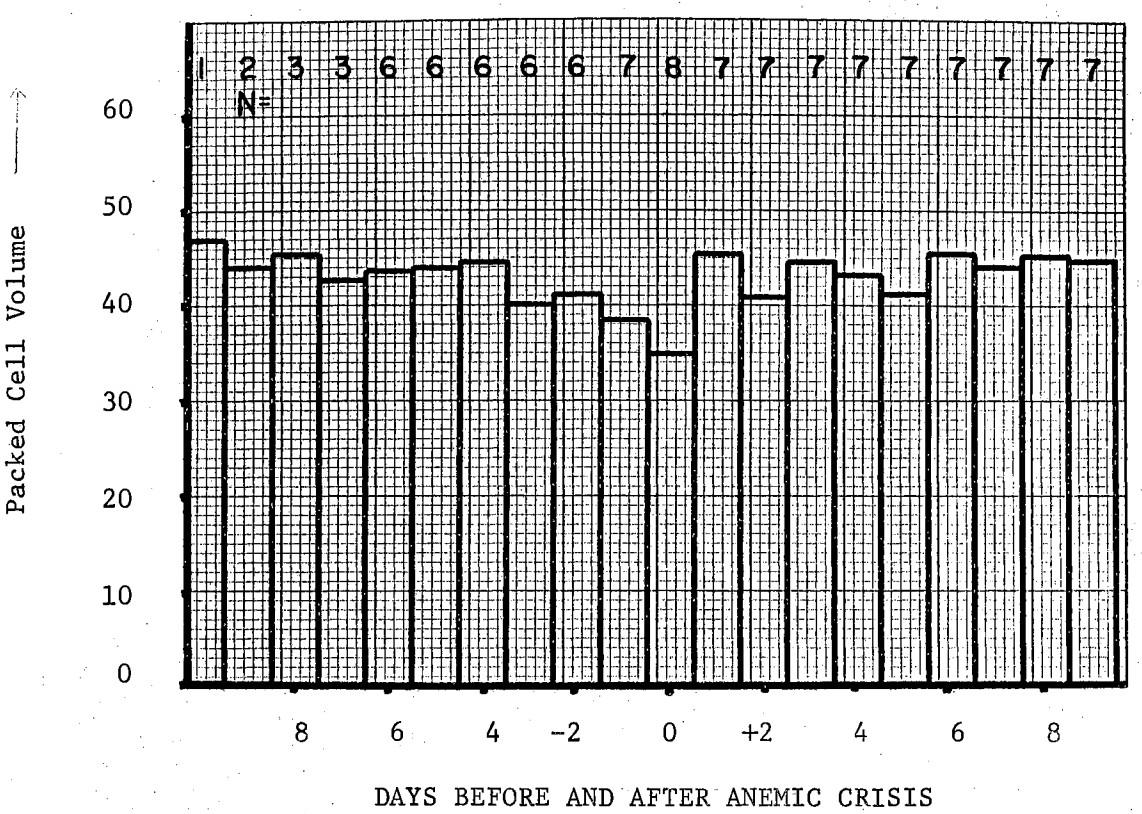

Figure 36. Course of anemia in rats fed 30.0 ppm magnesium and 13.5 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

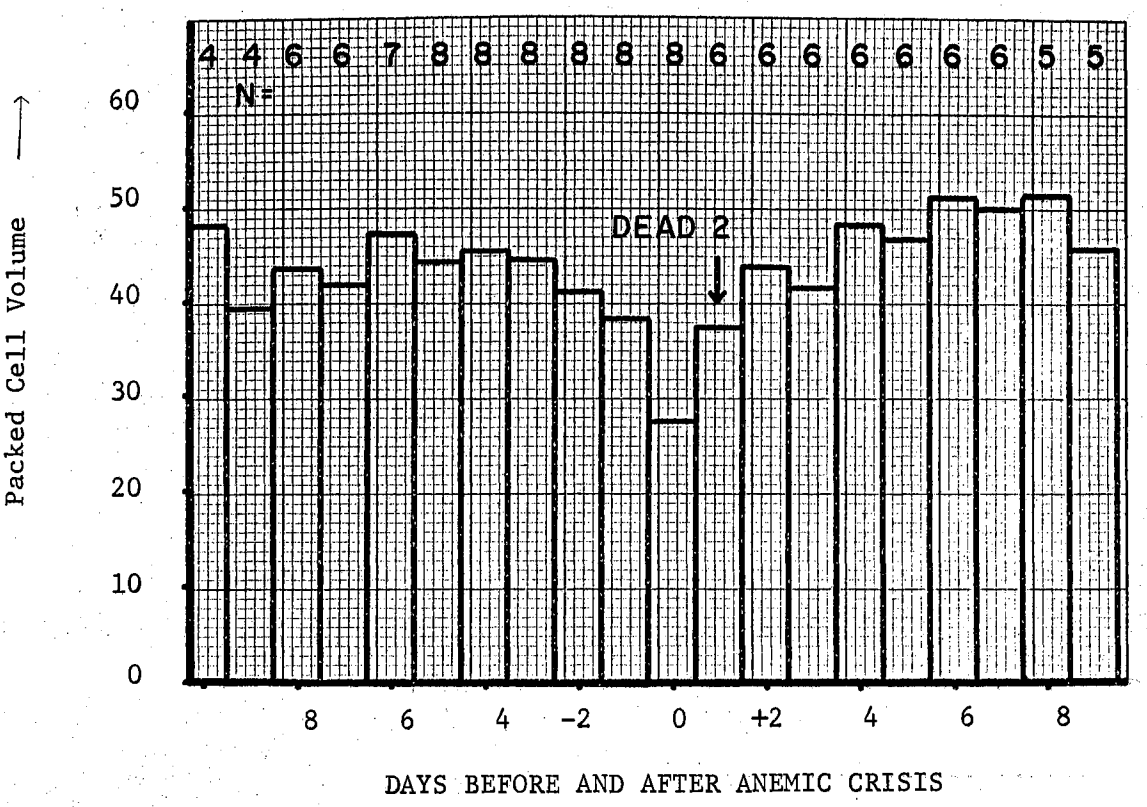

Figure 37. Course of anemia in rats fed 60.1 ppm magnesium and 13.5 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

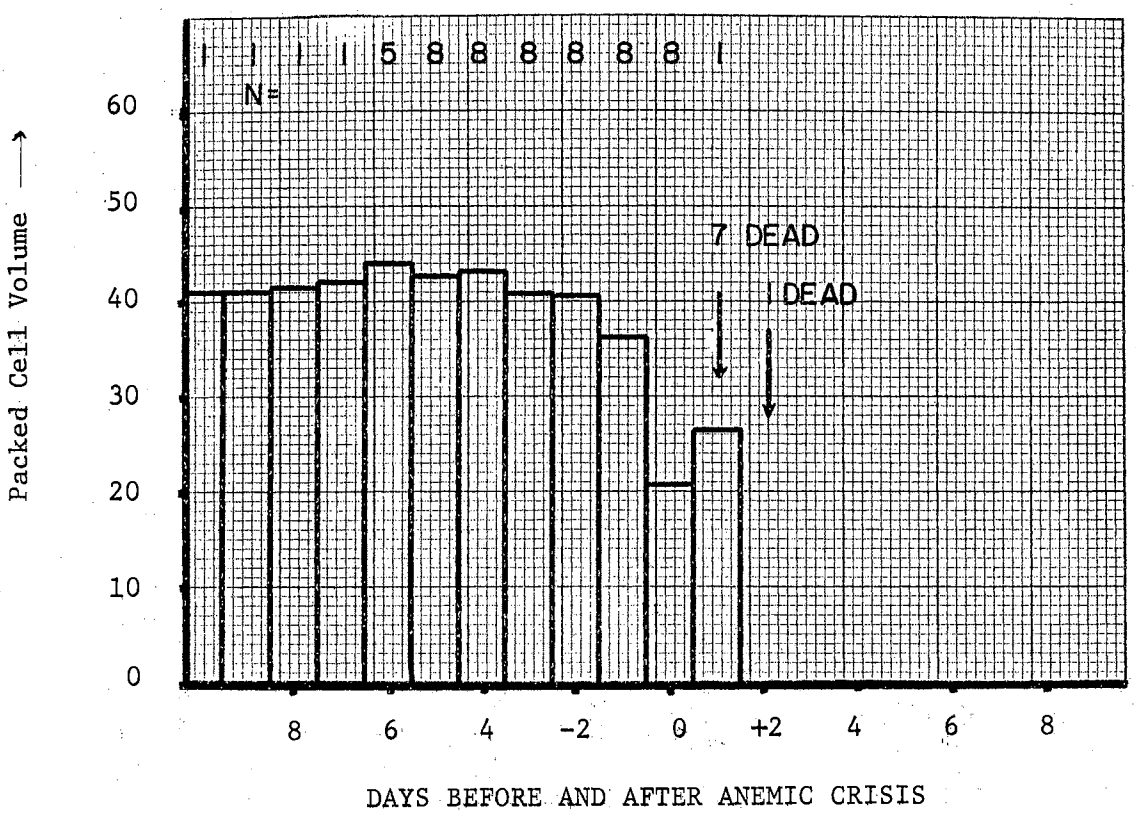

Figure 38. Course of Anemia in rats fed 91.0 ppm magnesium and 13.5 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

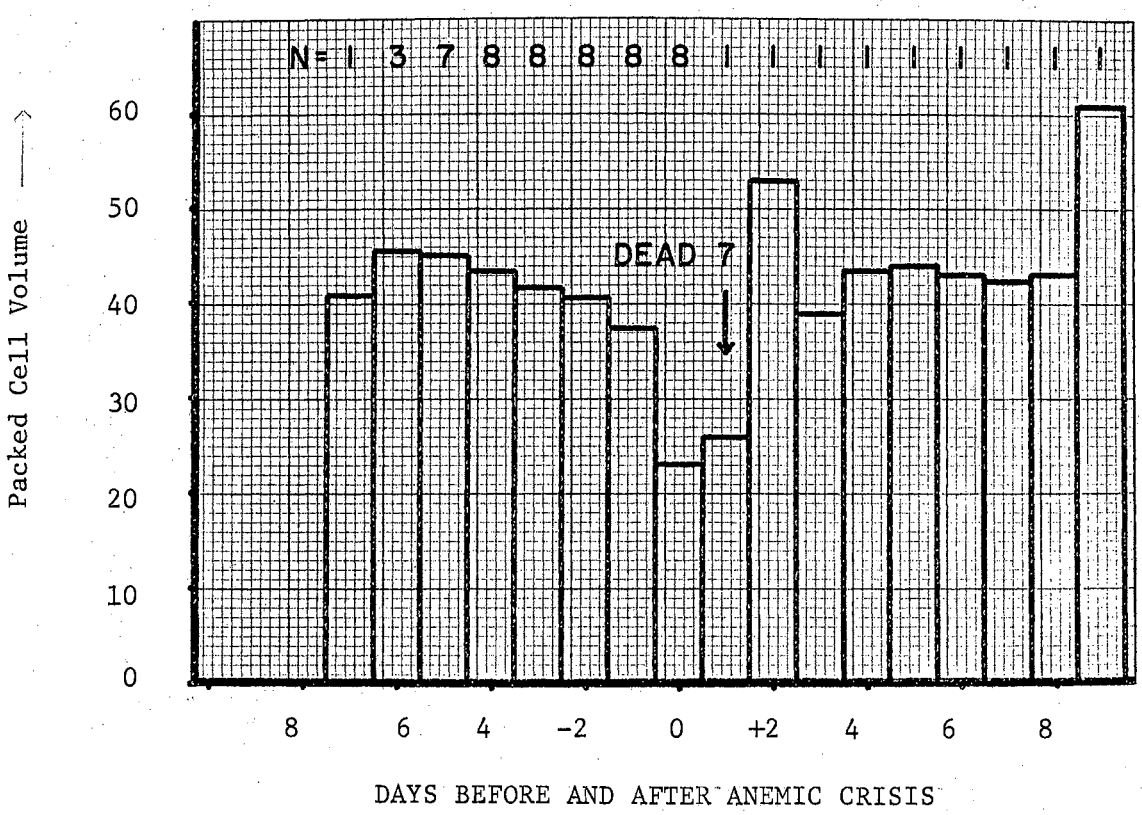

Figure 39. Course of anemia in rats fed 157.5 ppm magnesium and 13.5 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

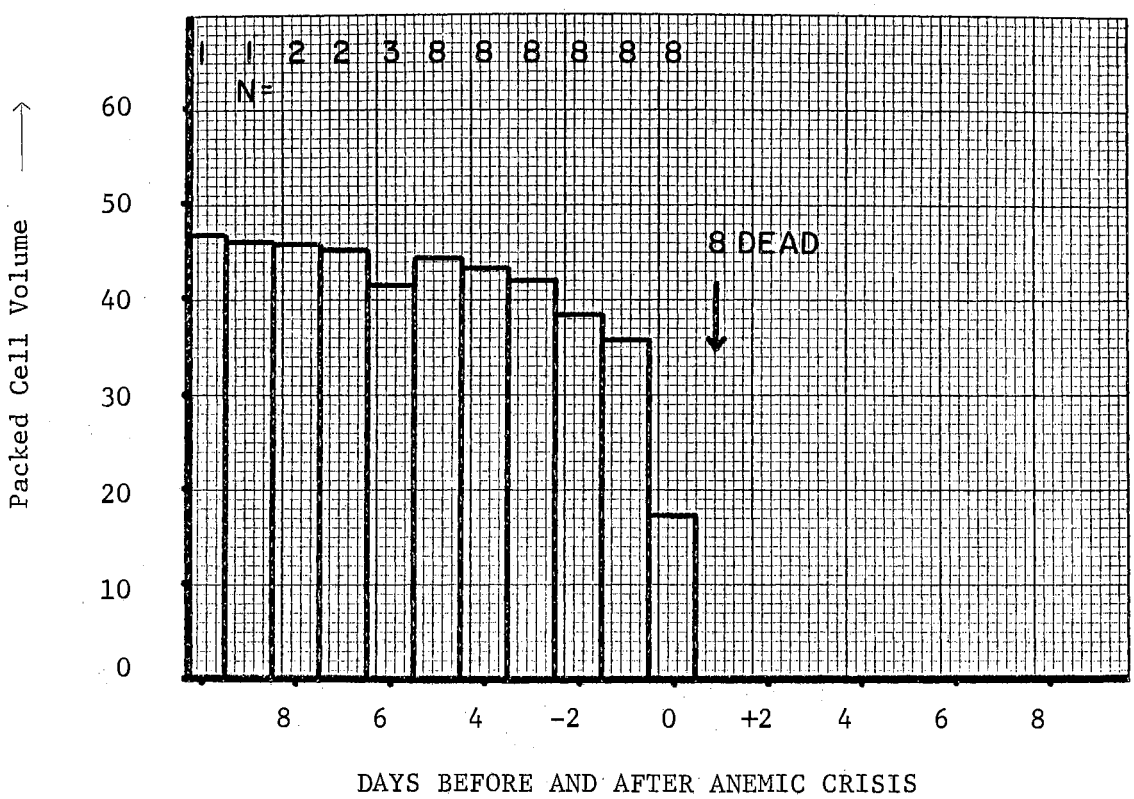

Figure 40. Course of anemia in rats fed 343.1 ppm magnesium and 13.5 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

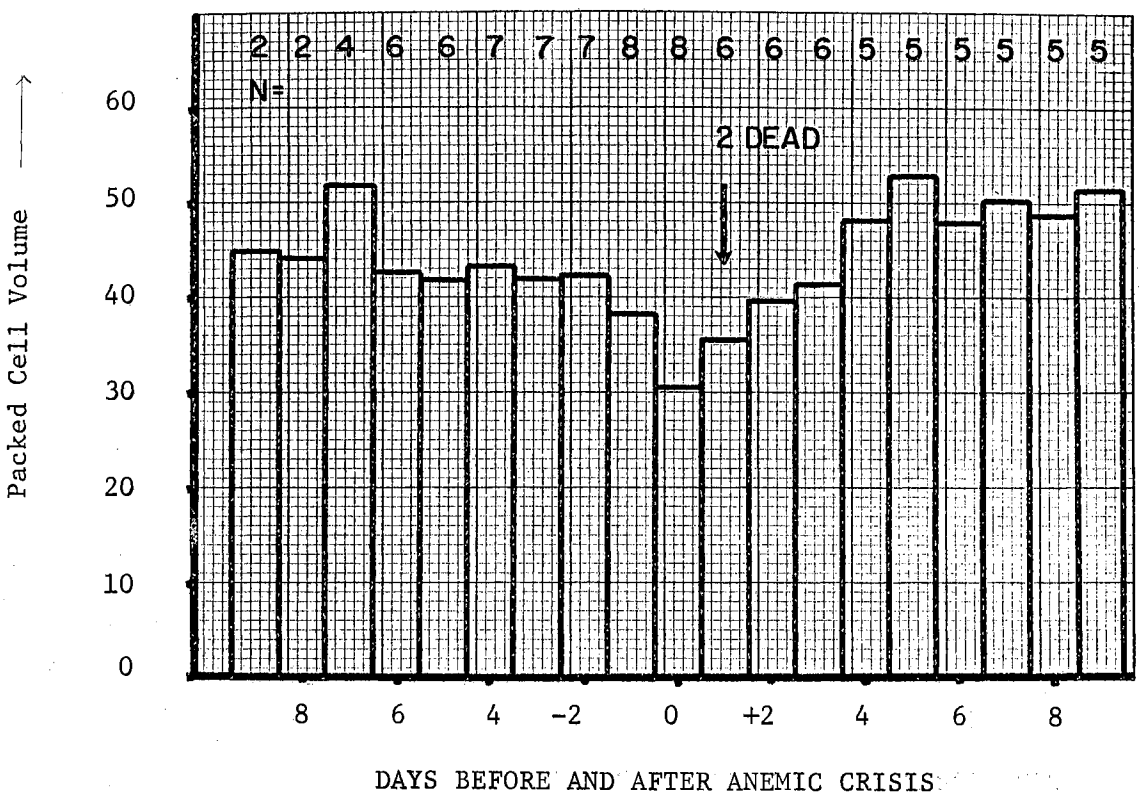

Figure 41. Course of anemia in rats fed 30.0 ppm magnesium and 23.2 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

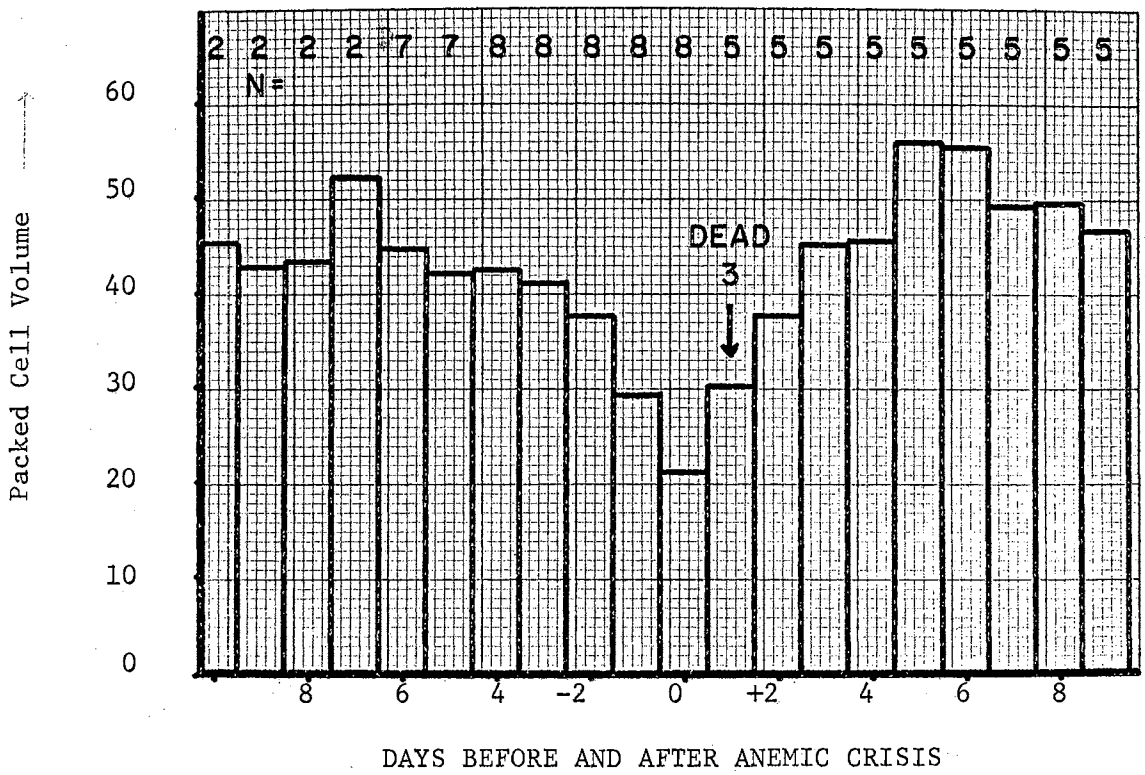

Figure 42. Course of anemia in rats fed 60.1 ppm magnesium and 23.2 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

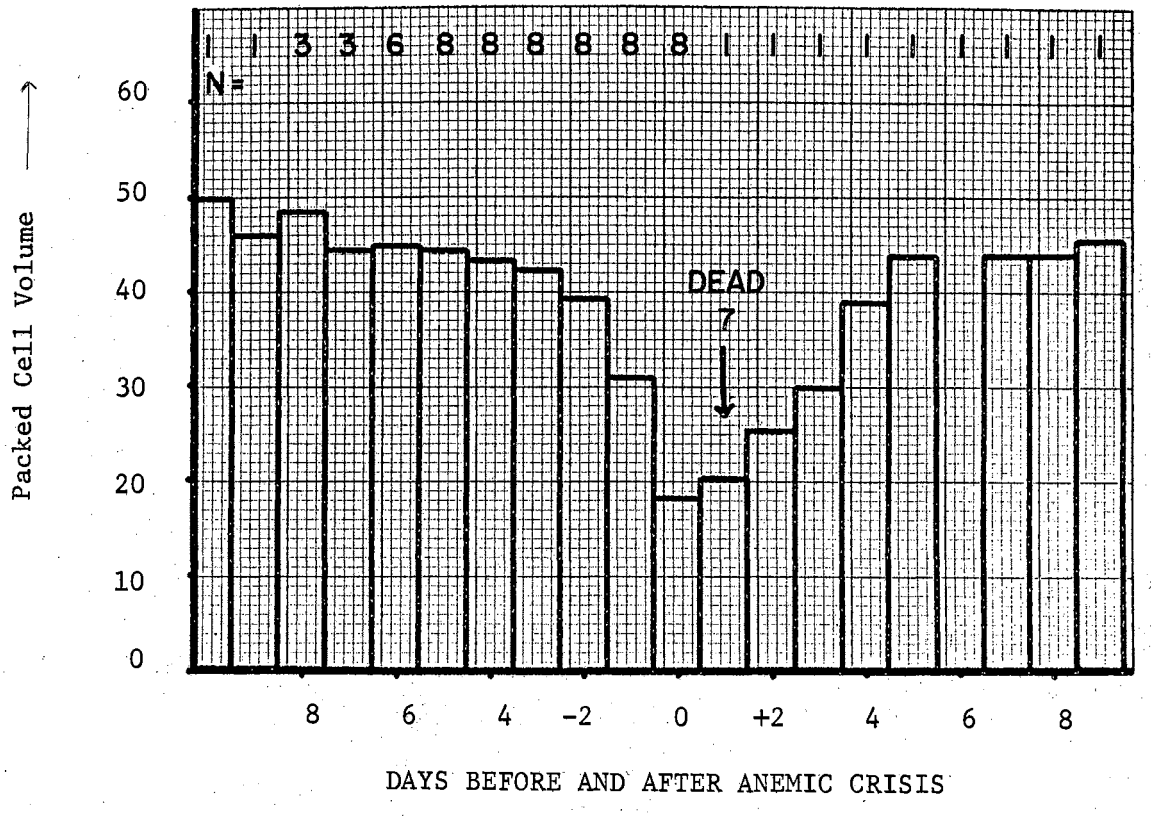

Figure 43. Course of anemia in rats fed 91.0 ppm magnesium and 23.2 ppm zinc during 30 day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

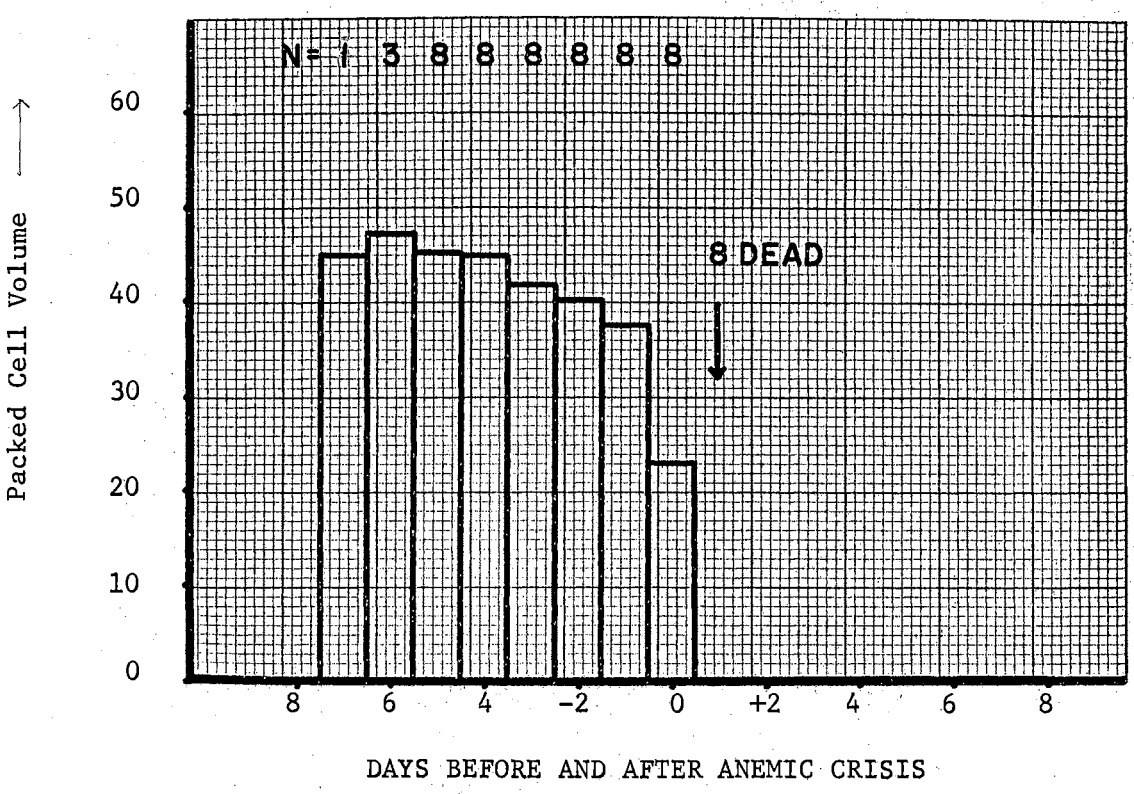

Figure 44. Course of anemia in rats fed 157.5 ppm magnesium and 23.2 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

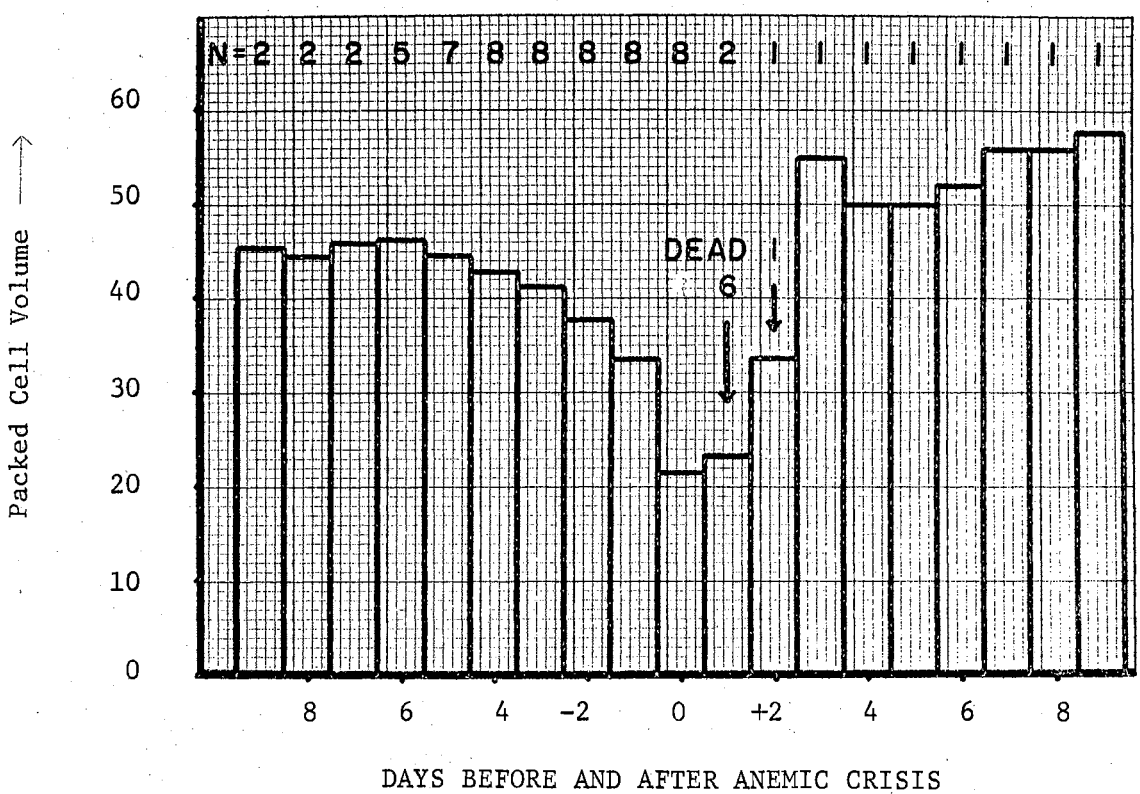

Figure 45. Course of anemia in rats fed 343.1 ppm magnesium and 23.2 ppm zinc during 30-day observation period after inoculation with Babesia rodhaini. N = Observations per mean; 8 rats per treatment.

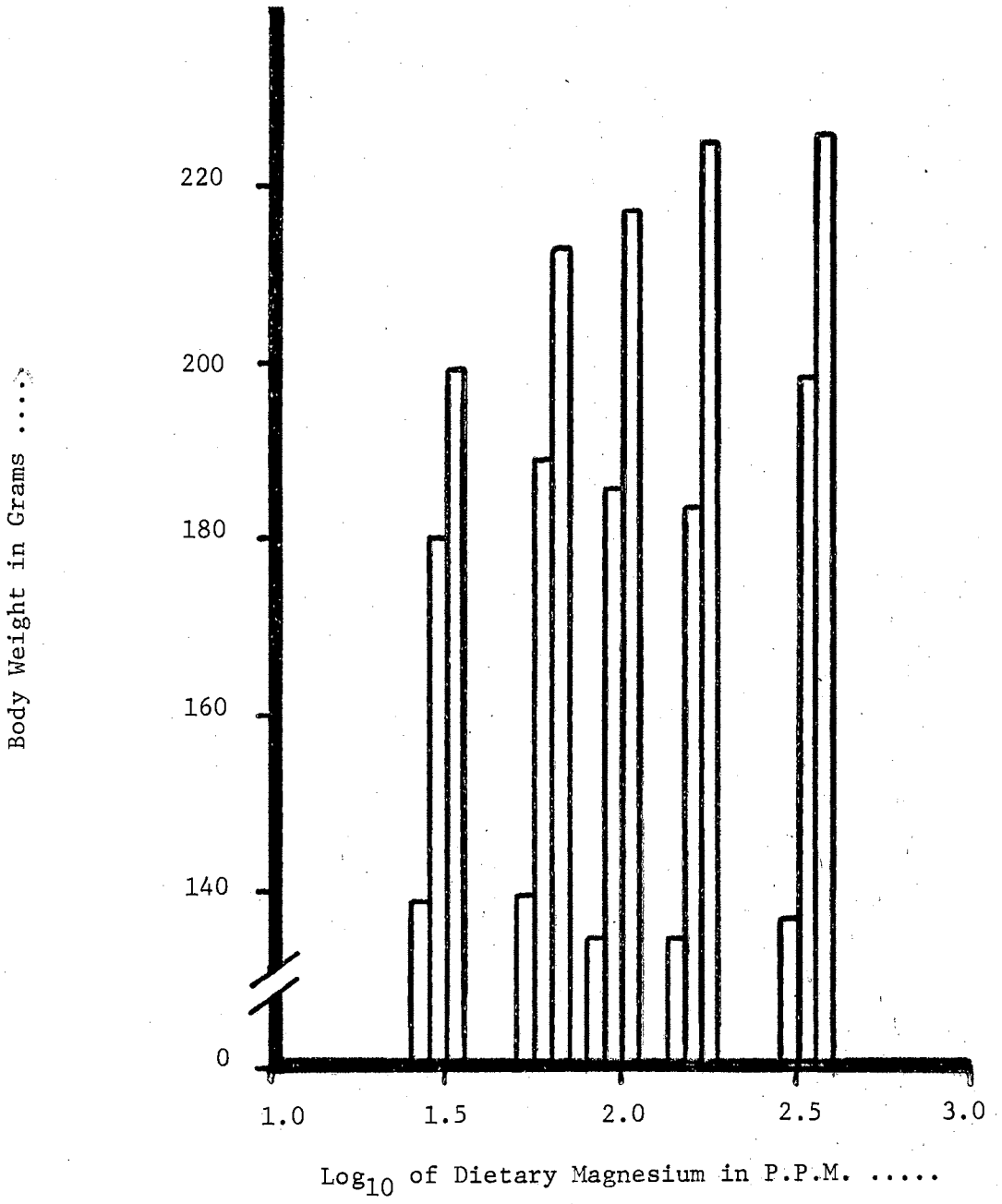

Figure 46. Effect of various dietary magnesium levels on weight gain. Each tripartite column represents 3 growth periods. left to right: weight after 7 days on complete basal diet, uninfected; 7 days on experimental ration, uninfected; weight after 14 days on experimental ration, infected with Babesia rodhaini 5 days. N = 24 rats per treatment.

little difference in weights during the earlier periods. Growth was adequate and similar to that observed in Experiment II.

Simple chemical analysis for levels of Mg in the diet are inadequate to prove differences in Mg intake without looking at the feed intake during the same period. Table X provides a statistical analysis of average daily feed consumption for 3 time periods from the time the experiment diets were started until immediately prior to death of the first animals (see Figure 47). This period included the incubation period for the babesial organism. No significant feed intake differences ($P>.05$) were detected for Mg, Zn or Mg x Zn, or Zn x time. Highly significant responses ($P<.01$) were seen across time within each level of magnesium. Random wastage was assumed for each cage and is consistent with animal room observations.

TABLE X

STATISTICAL ANALYSIS OF FEED CONSUMPTION DURING FIRST 18 DAYS ON EXPERIMENTAL RATIONS

Analysis of Variance: Mean Square (M.S.) Values		
Source:	df	M.S.
Magnesium (Mg)	4	10.21 n.s.
Zinc (Zn)	2	0.65 n.s.
Mg x Zn	8	5.51 n.s.
Error "a"	14	7.81
Time	2	432.82**
Time within Mg Level:		
30.0 p.p.m.	2	36.00**
60.1 p.p.m.	2	73.00**
91.0 p.p.m.	2	118.61**
157.5 p.p.m.	2	248.00**
363.1 p.p.m.	2	84.10**
Time x Zn	4	5.49 n.s.
Error "b"	30	5.33

5 levels magnesium, 3 levels zinc, 2 feed bags per experimental ration. Period #1, first 7 days; #2, second 7 days; #3, last 4 days of 18 day period. Rats inoculated with Babesia on first day of period #2. Rats Became sick during period #3. Total N = 120 rats.

Significance: *P<.05; **P<.01; n.s., P>.10.

Coefficient of Variation = $\sqrt{E.M.S./\bar{X}_{\bar{x}}}$ = $\sqrt{7.81/15.8}$ = 16.8% for error "a".

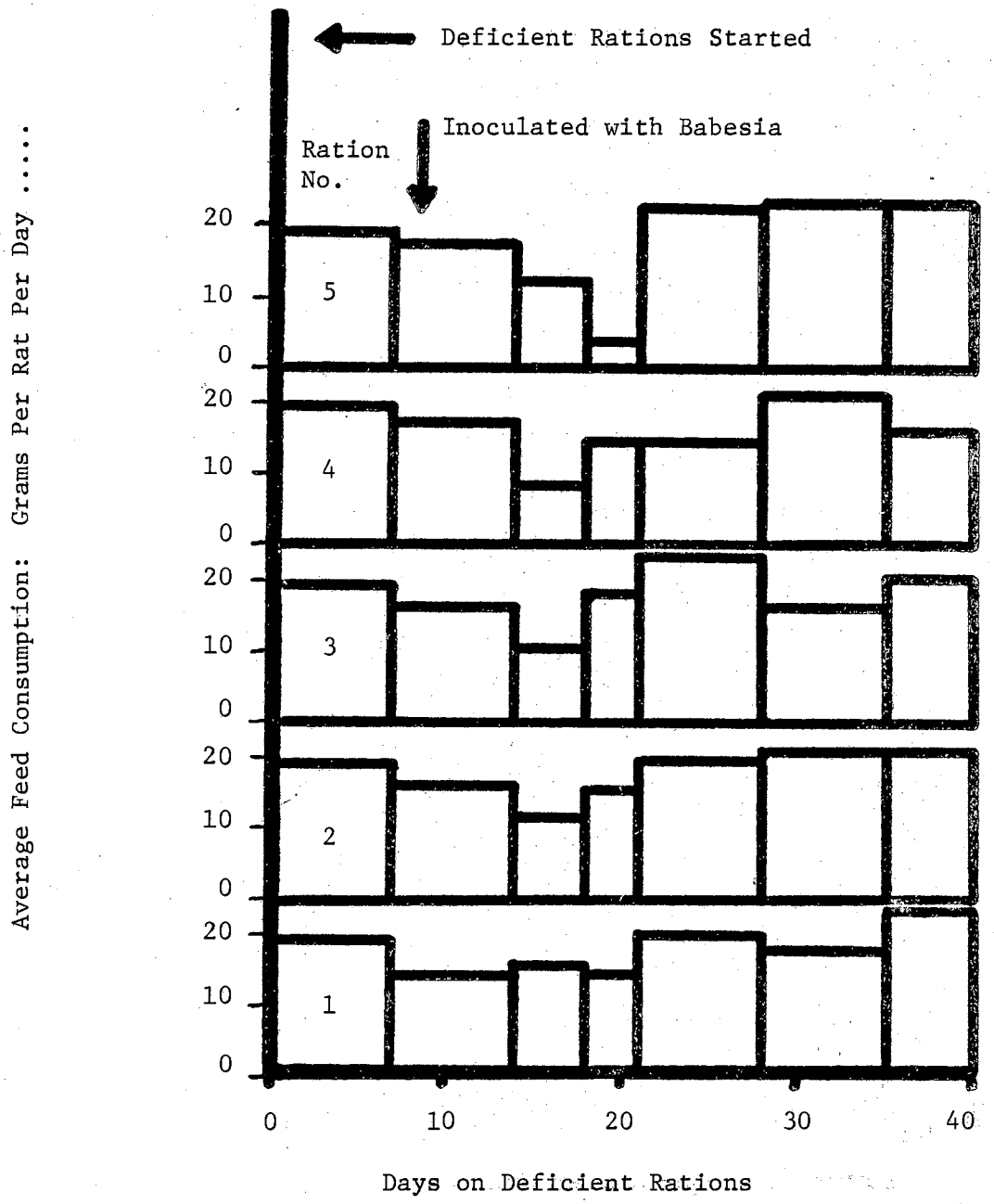

Figure 47. Feed Consumption of rats on various mineral deficient rations, before and after inoculation with Babesia rodhaini. Dietary magnesium, ppm: Ration #1, 30.0; #2, 60.1; #3, 91.0; #4, 157.5; #5, 343.1. Error mean square for treatments 7.18 and for time, 5.33, for first 3 periods (18 days). N = 8 rats per treatment until death loss starts during Period 4 (Day 20).

FOOTNOTES

[1]The square root of the error mean square was divided by the number of observaations per treatment mean to give the standard deviation of a treatment mean.

[2]Laboratory Rat Chow, Ralston-Purina Company, St. Louis, Missouri.

[3]Machine plotted; by XY Plotter of Hewlett-Packard 9100A Computer, Hewlett-Packard Instruments Company, Loveland, Colorado.

[4]Kodacolor print, processed by Kodak Laboratories, Rochester, New York, from a Kodacolor negative taken with 1/1000 second, 5600 degree Kelvin electronic light source; unfiltered, and not retouched.

[5]Ibid.

[6]Machine plotted; by XY Plotter of Hewlett-Packard 9100A Computer, Hewlett-Packard Instruments Company, Loveland, Colorado.

[7]Ibid.

[8]Ibid.

CHAPTER IV

DISCUSSION

The basic diet formulation (See Appendix A) had a 25 percent casein base and was calculated to meet or exceed published National Research Council (N.R.C.) recommendations (55) for the growing rat. It was a slight modification of a basic diet used by Nelson and Tillman (54) in their nutritional work.

All rats were fed on an ad libitum basis. Scrimshaw et al., (61), have put forth a strong case for pair-feeding. They stated:

> "When nutritional alterations of resistance to infection are studied in experiments with both pair-fed and ad libitum controls, the results show that the commonly observed reduced intake of the deficient diet may account for all, none or a specific and measurable part of the total effect on resistance."

Normally, the laboratory rat consumes its feed in many small meals during the entire day (62). Leverille and Chakrabarly (63) have shown significant differences in glucose utilization between ad libitum fed rats and rats offered feed during a 2 hour period every 24 hours. Patel and Mistry (64) have warned that the use of pair-fed rats as controls may lead to erroneous conclusions since they were able to show difference between pair-fed and ad libitum fed animals in such diverse areas as fatty acid metabolism and glycogen synthesis of muscle tissue. Where it was measured in Experiment III, it can be seen from Table X and Figure 45 that a difference in feed consumption during prepatency between dietary magnesium levels was not detected as a significant factor ($P >$

.05). From this data it can be assumed that the observed protective effects were due almost, if not entirely, to differences in dietary magnesium intake.

N.R.C. lists the magnesium (Mg) and zinc (Zn) requirements for the growing rat as 400 and 12 ppm, respectively (59). Diets in Experiments II and III had maximum Mg levels of 363.5 and 343.1 ppm. N.R.C. requirements for Mg were based on the work of McAleese and Forbes (65). They actually listed 115 ppm Mg as the requirement for maximum growth and an average of 365 ppm Mg as the requirement for maximum blood levels. Their rat growth rate was a 4.8-5.1 Gm/day with a daily feed consumption of 17 to 23 grams per animal. The maximum blood Mg levels were 2.33 to 2.44 mg/100 ml for the Mg levels under study. Rats kept 3 weeks on a diet containing 2,000 ppm Mg had an average blood serum magnesium level of only 2.31 Mg/100 ml. Twelve percent casein was used as a protein source.

In Experiment II the uninfected-intact rats on the 343 ppm Mg level had a plasma Mg of 1.40 Mg/100 ml. It has been shown that higher casein levels (36 percent vs. 12 percent) increase the requirement for Mg (32). This may account for the observed plasma and blood cell Mg levels being lower than those described by McAleese and Forbes (33). Experiments with rats on the diet used by Bunce and King (65) indicated that a range of 20 percent to 40 percent dietary casein provided for similar weight gains, feed efficiency, carcass nitrogen deposition and carcass amino acid content.

Experiments II and III equaled or exceeded growth rate recorded by workers in the field (33,65). Feed consumption for healthy animals in Experiment III (See Appendix B) is with the limits reported by McAleese

and Forbes (33). The symptoms observed in the three experiments coincide well with the above reports, with the exception that the reported reappearance of the hyperemia of the skin (33) was not observed in these animals. Once the reddening faded, it did not reoccur during the observation periods of 22 to 33 days. The reason for this is not known, but may be due to different diet formulations and/or rat strains. Kruse (26) observed hyperemic reappearance to occur only in older (age undefined) rats.

The vitamin source in Experiment I was apparently below expected potency levels. The relatively poor growth of animals on that experiment supports this conclusion. The conclusion is also supported by the fact that the average interval between inoculation with Babesia rodhaini and peak anemia for Experiment I was twice as long as the intervals observed in Experiments II and III. Certain vitamin deficiencies have been shown to have antagonistic effects on blood protozoans (16,17). Since the probability exists that the results of Experiment I were confounded by a concurrent multiple vitamin deficiency, it seems wise to limit further discussion to Experiments II and III where the results should have greater repeatability.

The anemia caused by Babesia rodhaini in the rats on the higher mineral levels in Experiments II and III was not markedly different from that reported by several workers for intact and splenectomized rats (22,23,24). It has been shown with Babesia rodhaini that survivability to experimental infection is inversely related to the number of parasites in the inoculum (22). The 50 million infected erythrocytes per dose used in Experiments II and III is 0.5 to 5 times larger than that used by the above-mentioned workers (22,23,24). This could

explain the decreased survivability observed in the groups on higher mineral levels of Experiments II and III. Different husbandry practices and rat strains also may have been contributing factors. Antigenic variation of Babesia rodhaini cannot be ruled out (66,67).

Deficient splenectomized rats were more severely affected by Babesia rodhaini than were the deficient intact animals. This is consistent with other reports on the interaction between splenectomy and babesiosis in rats (67), splenectomy and babesiosis in cattle (68), and splenectomy and anaplasmosis in calves (6().

Zuckerman (13) cited many studies showing the influence of host nutrition on hemoprotozoan parasites. She reported that if host dietary deficiencies involve factors essential for parasitic development, changes in the parasitic population will occur. The depression of a malarial parasitemia in mice by means of a dietary deficiency (such as para-amino-benzoic acid) during the developmental period of immunity permitted the mice to survive this lethal disease and acquire latency (13). Only one of 15 infected rats in Experiment II developed patent babesiosis (See Table VI) when repleted with Mg. The other 14 rats acquired latency and exhibited continued suppression of parasitemia. This is at variance with the observations on anaplasmosis and magnesium deficiency (19), (see Appendix C).

The failure to observe severe babesial recrudescence with the magnesium (Mg) repletion of protected deficient rats may be due to the fact that the animal has been able to develop a state of true premunition (infection immunity). That the calves with low Mg used by Bedell and Miller developed patent anaplasmosis after magnesium repletion indicates a temporary inhibition of that organism as suggested by Barnett

(71) for bovine babesiosis. Thus, the protective effects of magnesium deficiency may operate through different mechanisms for the two organisms in different animal species, or may indicate a more severe degree of anaplasma suppression to the extent that no useful acquired immunity was developed from the initial infection. The latter explanation has been put forth (70) to explain relapses of babesiosis in cattle previously treated with anti-babesial compounds.

That no zinc (Zn) effect was observed may be due to the fact that the Zn levels, although above listed N.R.C. requirements (55), were somewhat below the levels reported necessary to decrease magnesium retention (9).

The data in all 3 experiments is consistent in that infected magnesium deficient rats: 1) survive longer, 2) did not become as anemic as controls, and 3) did not develop high circulating parasitemias. Plasma and intraerythrocytic magnesium levels were influenced by diet (see Appendix B).

It is not likely that the protective effect is mediated through alteration of the immunologic system. Immunologic blockade or enhancement of allergenic tolerance (53) by magnesium deficiency might result in a higher degree of parasitemia in the protected animals, especially in the protected splenectomized rats. This was not observed. Selective early removal of infected erythrocytes would more likely be hindered than enhanced by low magnesium levels, since opsonization (37) and the properdin-lysis system (40) utilize magnesium.

Rickard's work (71) indicates that Babesia rodhaini uses glucose for synthetic purposes and that anaerobic glycolysis via the Embden-Meyerhof pathway is its main energy source. Phosphofructokinase is one

of many enzymes using magnesium (43,44). This enzyme appears to control the Embden-Meyerhof pathway in many tissues (45), including erythrocytes (72). When magnesium levels are lower than ATP levels phosphofructokinase is greatly inhibited. Free ATP is from 6 to 35 times more inhibitory than Mg-ATP complex (45). Mansour (47) presented data indicating strong inhibition of heart phosphosfructokinase by ATP can occur in physiologic concentrations and under intracellular conditions.

Since erythrocytes have several available pathways for carbohydrate metabolism, and do not expend energy to synthesize protein (73) their viability and function might not be significantly altered by low intraerythrocytic magnesium. Suppression of the reproduction of Babesia rodhaini could be due to the inhibition of its known primary energy pathway at the phosphofructokinase control point due to lowered intraerythrocytic magnesium levels.

It has been reported for a human population which was severely undernourished, that with improved food supply, dormant hemoprotozooan infections became clinically apparent (11). If the protective effect provided by Mg deficiency to the rat applies to the bovine animal, then unsupplemented domestic animals nourished on feeds from magnesium deficient soils might have some degree of protection from both anaplasmosis and babesiosis. Nutritional improvement by mineral supplementation could be confounded by increased problems with indigenous infectious anemias.

CHAPTER V

SUMMARY AND CONCLUSIONS

Magnesium deficiency is antagonistic to the development of clinical babesiosis in the rat. It has been shown that growing laboratory rats consuming otherwise adequate diets containing between 30 and 97 ppm magnesium for as few as eight days are significantly protected from the anemia of Babesia rodhaini. This protection is indicated by a marked increase in survivability, a reduced blood loss as measured by packed cell volume changes, and by a significant decrease in the number of circulating parasitized erythrocytes. This protection is not ameliorated by splenectomy, although it was somewhat decreased.

Parasitic recrudescence was observed in only 1 of 15 protected rats known to be carriers of babesia when they were repleted with magnesium for a period twice the time normally required for anemic crisis in control animals. Many animals entered latency without patent disease being observed.

Although little is known about the in vivo metabolism of the organism, there is indirect evidence to indicate that the lack of magnesium might interfere with its energy metabolism and thus, directly depress the multiplication of Babesia rodhaini while the host is acquiring immunity.

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APPENDIX A

TABLE XI

DIET FOR EXPERIMENT I

Ingredient[1]	Grams
Corn Starch	304.090
Dextrose	250.000
Cellulose	52.000
Casein	247.000
Corn Oil	100.000
KH_2PO_4	21.970
$CaCO_3$	15.000
$4MgCO_3 \cdot Mg(OH)_2 \cdot H_2O$	1.650
NaCl	5.780
Trace Mineral Mix	1.100
Vitamin Mix:[2]	
Choline Cl	0.749
Vitamin A & D	0.110
Vitamin E	(0.441 mg/Kg)
B Vitamin Mix	0.500

Trace Mineral Mix

	Grams
$MnSO_4 \cdot H_2O$	0.15350
$FeSO_4$	0.13580
$ZnSO_4 \cdot 7H_2O$	0.10520
$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$	0.01736
KI	0.00039
Na_2SeO_3	0.00019
Dextrose	0.69756

TABLE XII

DIET FOR EXPERIMENTS II AND III

Ingredients	Grams
Corn Starch[3]	300.00
Dextrose[4]	245.10
Cellulose[5]	32.00
Casein[6]	247.00
Corn Oil[7]	100.00
KH_2PO_4	21.97
$CaCO_3$	15.00
NaCl	5.78
Magnesium Supplement[8]	5.00
Zinc Supplement[9]	5.00
Trace Mineral Mix	1.10
Vitamin Mix[10]	22.05
Grams	1,000.00

TRACE MINERAL MIX

$MnSO_4 \cdot H_2O$	0.15350
$FeSO_4$	0.13580
$CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$	0.10173
KI	0.00039
Na_2SeO_3	0.00019
Dextrose	0.79276
Grams	1.10000

TABLE XIII

CONTENTS OF VITAMIN DIET FORTIFICATION MIXTURE
IN EXPERIMENTS II AND III

Nutrients*	Amount
	Gm/45.5 Kg Ration
Vitamin A Concentrate (200,000 units/Gm)	4.50
Vitamin D Concentrate (400,000 units/Gm)	0.25
Alpha tocopherol	5.00
Ascorbic Acid	45.00
Inositol	5.00
Choline Chloride	75.00
Menadione	2.25
ParaAminobenzoic Acid	5.00
Niacin	4.50
Riboflavin	1.00
Pyridoxine Hydrochloride	1.00
Thiamine Hydrochloride	1.00
Calcium Pantothenate	3.00
Biotin	20.00 mg/45.4 Kg Ration
Folic Acid	90.00 mg/45.4 Kg Ration
Vitamin B_{12}	1.35 mg/45.4 Kg Ration

* Nutritional Biochemicals, Inc., Cleveland Ohio. The nutrients are premixed in dextrose such that 1 Kg of the premix is used in each 45.4 Kg of ration.

TABLE XIV

MINERAL SUPPLEMENTS, MAGNESIUM AND ZINC

Magnesium (Mg) Level:		Grams/5 Grams
1	$4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	0.000
	Dextrose	5.000
2	$4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	0.165
	Dextrose	4.835
3	$4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	0.330
	Dextrose	4.670
4	$4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	0.660
	Dextrose	4.340
5	$4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$	1.650
	Dextrose	3.350

The $4\ MgCO_3 \cdot Mg(OH)_2 \cdot nH_2O$ contained 40.2% MgO equivalent.

Zinc (Zn) Level:		Grams/5 Grams
1	$ZnSO_4 \cdot 7H_2O$	0.00000
	Dextrose	5.00000
2	$ZnSO_4 \cdot 7H_2O$	0.01157
	Dextrose	4.98843
3	$ZnSO_4 \cdot 7H_2O$	0.02314
	Dextrose	4.97686
4	$ZnSO_4 \cdot 7H_2O$	0.04629
	Dextrose	4.95371
5	$ZnSO_4 \cdot 7H_2O$	0.11572
	Dextrose	4.88428

Note: In Experiment I, Mg levels 1 and 5 were used. In II, Mg and Zn levels, 1 through 5 were used. In III, Mg levels 1 through 5 and Zn levels 1, 2 and 5 were used. In all cases 5 grams of appropriate supplements was included in each 1000 Gm of experimental ration.

FOOTNOTES

[1]This was a basic diet used by T. E. Nelson and A. D. Tillman in Nutrition Laboratory, Animal Science Department, Oklahoma State University, Stillwater, Oklahoma.

[2]Ibid.

[3]Buffalo Corn Starch, Corn Products Company, Englewood Cliffs, New Jersey, 07632.

[4]Cellulose-Dextrose, Ibid.

[5]Cellulose, International Filler Corporation, North Tonawanda, New York.

[6]Vitamin-Free Casein, Nutritional Biochemicals Corporation, Cleveland, Ohio.

[7]Mazola Corn Oil, Corn Products Company, Englewood Cliffs, New Jersey, 07632.

[8]Magnesium Carbonate Powder, J. T. Baker Chemical Company, Phillipsburg, New Jersey.

[9]Zinc Sulfate, Reagent Grade, Ibid.

[10]Vitamin Diet Fortification Mixture, Nutritional Biochemicals Corporation, Cleveland, Ohio.

APPENDIX B

TABLE XV

TREATMENT MEANS FOR EXPERIMENT I: EFFECTS OF DIETARY MAGNESIUM ON COURSE OF BABESIA RODHAINI INFECTION

Treatment	Treatment I Uninfected Dietary Mg, ppm: 319	Treatment II Infected Dietary Mg, ppm: 13	Treatment III Infected	Error Mean Square
Parameter:	Days Survived of 30-Day Postinoculation Period.			
	30	27.7	20.7	20.94
Parameter:	Highest Postinoculation Observed Parasitemia.			
	00.0	13.8	52.6	330.6
Parameter:	Lowest Postinoculation Observed Erythrocyte Count.			
	5.20	3.19	2.57	2.200
Parameter:	Lowest Postinoculation Observed Packed Cell Volume (P.C.V.).			
	37.6	29.2	17.8	113.4
Parameter:	Day Interval From Inoculation to Low P.C.V.			
	20.2	19.7	14.3	48.67

Inoculation = Inoculation intraperitoneally with 100×10^6 infected erythrocytes. Uninfected group sham inoculated with diluent. Six rats per treatment mean.

TABLE XVI

BODY WEIGHT IN GRAMS: BASAL DIET,
PREINFECTIVE PERIOD

Dietary* Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	140.8	133.5	146.7	128.3	139.1	142.0
Treatment 2: Intact, Not Infected with Babesia rodhaini.	137.7	137.0	138.0	136.5	128.8	137.8
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	150.2	133.0	135.2	133.8	130.6	130.1
$\bar{x}$ for Mineral Level	142.9	134.5	139.9	132.9	132.9	$\bar{X}_{\bar{x}}$=136.6

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 178.7.
* All rats fed basal diet during this period, mineral levels listed for treatment assignment purposes only.

TABLE XVII

BODY WEIGHT IN GRAMS: AFTER 7 DAYS ON VARIOUS DIETARY MINERAL LEVELS, PREINFECTIVE PERIOD

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	200.7	194.3	197.2	193.0	200.2	197.1
Treatment 2: Intact, Not Infected with Babesia rodhaini.	174.3	175.3	178.2	193.8	182.0	189.0
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	183.8	179.2	207.8	190.7	188.0	180.7
$\bar{x}$ for Mineral Levels	186.3	182.9	194.3	192.5	190.1	$\bar{X}_{\bar{x}}$ 189.2

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 309.8.

TABLE XVIII

BODY WEIGHT IN GRAMS: ON VARIOUS DIETARY MINERAL LEVELS FOR 14 DAYS, INFECTED 6 DAYS

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	203.0	213.7	220.2	223.2	232.8	218.6
Treatment 2: Intact, Not Infected with Babesia rodhaini.	196.0	195.0	235.0	218.0	216.0	212.0
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	190.0	194.7	204.5	224.3	200.8	203.0
$\bar{x}$ for Mineral Levels	196.5	201.1	219.9	221.8	216.6	$\bar{X}_x$ 211.2

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 448.0.

TABLE XIX

AVERAGE DAYS SURVIVED DURING 22-DAY POSTINOCULATION PERIOD

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	22.0	22.0	22.0	12.8	10.8	17.9
Treatment 2: Intact, Not Infected with Babesia rodhaini.	22.0	22.0	22.0	22.0	19.8*	21.6*
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	22.0	22.0	11.7	8.7	8.5	14.6
$\bar{x}$ for Mineral Levels	22.0	22.0	18.0	14.5	13.1	$\bar{X}_{\bar{x}}$ 18.0

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 9.37.
* One of 40 animals became infected by accidental transfer.

TABLE XX

TIME IN DAYS FROM DAY OF INOCULATION WITH *BABESIA RODHAINI* TO DAY OF LOWEST OBSERVED PACKED CELL VOLUME DURING 22-DAY POSTINOCULATION PERIOD

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with *Babesia rodhaini*.	11.8	9.7	10.3	8.0	8.7	9.7
Treatment 2: Intact, Not Infected with *Babesia rodhaini*.	15.2	13.0	13.8	10.0	6.5	11.7
Treatment 3: Splenectomized, Infected with *Babesia rodhaini*.	15.3	18.2	9.3	7.8	8.2	11.8
$\bar{x}$ for Mineral Levels	14.1	13.6	11.1	8.6	7.8	$\bar{X}_{\bar{x}}$ 11.1

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 16.22.

TABLE XXI

HIGHEST PERCENT PARASITIZED ERYTHROCYTES OBSERVED DURING 22-DAY PERIOD AFTER INOCULATION WITH *BABESIA RODHAINI*

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with *Babesia rodhaini*.						
	16.5	18.5	32.7	55.4	61.1	36.8
Treatment 2: Intact, Not Infected with *Babesia rodhaini*.						
	0.0	0.0	0.0	0.0	10.8	2.2
Treatment 3: Splenectomized, Infected with *Babesia rodhaini*.						
	39.7	37.1	68.1	80.7	65.0	58.1
$\bar{x}$ for Mineral Levels	18.7	18.5	33.6	45.4	45.6	$\bar{\bar{x}}$ 32.4

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 239.50.

TABLE XXII

LOWEST ERYTHROCYTE COUNT OBSERVED DURING 22-DAY POSTINOCULATION PERIOD (CELLS x $10^6/mm^3$ WHOLE BLOOD)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.						
	2.90	2.40	1.90	2.18	2.33	2.34
Treatment 2: Intact, Not Infected with Babesia rodhaini.						
	3.43	3.82	3.62	3.96	3.24	3.62
Treatment 3: Splenectomized, Infected with Babesia rodhaini.						
	2.18	1.57	2.00	2.61	2.87	2.25
$\bar{x}$ for Mineral Levels	2.84	2.60	2.51	2.92	2.83	$\bar{X}_{\bar{x}}$ 2.74

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 1.207.

TABLE XXIII

MEAN CORPUSCULAR VOLUME OCCURRING ON DAY LOWEST ERYTHROCYTE COUNT OBSERVED, (CUBIC MICRONS)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	176.5	165.1	165.0	147.9	112.1	153.3
Treatment 2: Intact, Not Infected with Babesia rodhaini.	129.3	116.4	132.2	125.4	131.9	127.0
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	173.0	198.1	139.0	126.9	118.4	151.1
$\bar{x}$ for Mineral Levels	159.6	159.8	145.4	133.4	120.8	$\bar{X}_{\bar{x}}$ 143.8

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 2248.9

TABLE XXIV

AVERAGE PRETREATMENT PACKED CELL VOLUME OBSERVED DURING BASAL DIET PERIOD, (VOLUMES PERCENT)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	39.3	37.6	40.7	39.0	39.9	39.3
Treatment 2: Intact, Not Infected with Babesia rodhaini.	38.5	36.9	39.1	38.9	41.6	39.6
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	38.1	39.6	37.6	39.2	38.4	39.9
$\bar{x}$ for Mineral Levels	38.6	38.1	39.1	39.0	39.9	$\bar{X}_{\bar{x}}$ 39.0

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 7.046.

TABLE XXV

PACKED CELL VOLUME (PCV) THREE DAYS PRIOR TO LOWEST OBSERVED PCV, (VOLUMES PERCENT)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with *Babesia rodhaini*.						
	49.9	45.1	44.6	43.3	45.8	45.6
Treatment 2: Intact, Not Infected with *Babesia rodhaini*.						
	51.3	53.0	49.9	45.8	47.4	44.4
Treatment 3: Splenectomized, Infected with *Babesia rodhaini*.						
	45.2	33.3	44.0	45.8	43.8	42.4
$\bar{x}$ for Mineral Levels	48.8	43.8	46.2	44.9	45.7	$\bar{X}_{\bar{x}}$ 45.9

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 39.07.

TABLE XXVI

PACKED CELL VOLUME (PCV) TWO DAYS PRIOR TO LOWEST OBSERVED PCV, (VOLUMES PERCENT)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.	45.4	44.0	42.3	40.4	44.8	43.4
Treatment 2: Intact, Not Infected with Babesia rodhaini.	43.5	46.9	49.7	47.5	44.6	46.4
Treatment 3: Splenectomized, Infected with Babesia rodhaini.	39.3	31.9	44.8	46.5	42.3	41.0
$\bar{x}$ for Mineral Levels	42.7	40.9	45.6	44.8	43.9	$\bar{X}_{\bar{x}}$ 43.6

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 82.52.

TABLE XXVII

PACKED CELL VOLUME (PCV) ONE DAY PRIOR TO LOWEST OBSERVED PCV, (VOLUMES PERCENT)

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Treatments
Treatment 1: Intact, Infected with Babesia rodhaini	37.5	41.0	34.7	32.3	39.2	36.9
Treatment 2: Intact, Not Infected with Babesia rodhaini	41.0	43.3	48.9	47.9	42.8	44.8
Treatment 3: Splenectomized, Infected with Babesia rodhaini	42.1	24.8	34.1	45.3	41.7	37.6
$\bar{x}$ for Mineral Levels	40.2	36.4	39.2	41.9	41.2	$\bar{X}_{\bar{x}}$ 39.8

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 83.86.

TABLE XXVIII

LOWEST PACKED CELL VOLUME OBSERVED DURING 22 DAY POSTINOCULATION PERIOD

Dietary Mineral Level	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	$\bar{x}$ for
Zn, ppm	13.0	14.2	15.3	20.0	26.0	Treatments
Treatment 1: Intact, Infected with Babesia rodhaini.						
	31.7	29.6	22.2	23.2	22.6	25.9
Treatment 2: Intact, Not Infected with Babesia rodhaini.						
	38.0	39.3	41.7	43.0	37.9	40.0
Treatment 3: Splenectomized, Infected with Babesia rodhaini.						
	31.0	20.5	20.9	23.7	21.8	23.6
$\bar{x}$ for Mineral Levels	33.6	29.8	28.3	30.0	27.4	$X_{\bar{x}}$ 29.8

Column and row means include rounding error.
Six rats per treatment-mineral mean; 90 rats total.
Error Mean Square = 62.60.

TABLE XXIX

PLASMA MAGNESIUM LEVELS (MILLIGRAMS OF MAGNESIUM/100 ml. OF PLASMA), UNINFECTED - INTACT RATS, BLED OUT AFTER 22 DAYS ON EXPERIMENTAL DIETS. (ANIMALS FROM TREATMENT #2)

Dietary Mineral Levels	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Blocks
Block 1	0.402	0.495	0.750	1.656	1.478	0.956
Block 2	0.257	0.527	0.699	1.164	1.318	0.793
N =	6	6	6	6	6	30
$\bar{x}$ for Mineral Levels	0.330	0.511	0.725	1.410	1.398	$\bar{\bar{x}}$ 0.875

Column and row means include rounding error.
"Error Mean Square" = 0.1344 (for only this data set).

TABLE XXX

ERYTHROCYTE MAGNESIUM LEVELS: UNINFECTED INTACT RATS, BLED OUT AFTER 22 DAYS ON EXPERIMENTAL DIETS. (ANIMALS FROM TREATMENT #2)
(MILLIGRAMS OF MAGNESIUM/100 ml. OF ERYTHROCYTES)

Dietary Mineral Levels	1	2	3	4	5	
Mg, ppm	35.5	65.1	97.7	172.6	363.5	
Zn, ppm	13.0	14.2	15.3	20.0	26.0	$\bar{x}$ for Blocks
Block 1	3.34	3.43	4.56	5.53	5.83	4.54
Block 2	3.73	3.81	4.80	5.29	7.02	4.93
N =	6	6	6	6	6	30
$\bar{x}$ for Mineral Levels	3.53	3.62	4.68	5.41	6.42	$\bar{X}\bar{x}$ 4.73

Column and row means include rounding error.
"Error Mean Square" = 2.396 (only for this data set).

TABLE XXXI

AVERAGE DAILY FEED CONSUMPTION DURING FIRST 18 DAYS ON EXPERIMENTAL RATIONS. PERIOD #1, FIRST 7 DAYS; #2, SECOND 7 DAYS; #3, LAST 4 DAYS OF 18-DAY PERIOD. EIGHT RATS PER TREATMENT MEAN; VALUES GIVEN IN GRAMS PER RAT PER DAY

Dietary Mineral, ppm Magnesium	TIME PERIOD 1	2*	3**	$\bar{x}$
30.0	19.3	14.9	16.4	16.9
60.1	19.1	15.1	12.3	15.5
91.0	19.0	16.6	10.3	15.3
157.5	19.8	17.9	7.9	15.3
363.1	19.3	17.7	12.2	16.4
$\bar{x}$	19.3	16.4	11.8	$\bar{x}\bar{x}$ 15.8
Zinc, ppm,				
12.7	20.2	16.3	11.5	16.0
13.5	19.3	16.6	12.4	15.8
23.2	19.4	16.4	11.6	15.8
$\bar{x}$	19.3	16.4	11.8	$\bar{x}\bar{x}$ 15.8

Row and column means include rounding error.

Error Mean Square: for minerals, 17.8; for time, 5.33.

* Animals inoculated with Babesia rodhaini on first day of this period.

** Animals became sick during this period.

N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXII

DAYS RATS SURVIVED AFTER INOCULATION WITH BABESIA RODHAINI
DURING 33-DAY POSTINOCULATION PERIOD
Mg x Zn, 5 x 3 FACTORIAL DESIGN

Dietary Mg, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	33.0	18.3	8.6	11.3	8.6	16.0
13.5	33.0	27.1	8.6	11.0	8.3	17.6
23.2	23.2	23.7	12.4	7.8	11.3	15.7
$\bar{x}$ for Mg 24	29.7	23.0	9.9	10.0	9.4	$\bar{\bar{X}}$ 16.40

Column and row means include rounding error.
Error Mean Square = 174.6.
N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXIII

PRETREATMENT PACKED CELL VOLUME, TWO OBSERVATIONS PER RAT: FEED COMPLETE BASAL DIET Mg x Zn, 5 x 3 FACTORIAL DESIGN

Mg*, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn*, ppm						
12.7	41.3	42.3	44.0	44.0	43.3	43.0
13.5	41.6	44.5	44.4	45.5	45.2	44.2
23.2	43.8	46.2	44.0	40.8	44.8	43.9
$\bar{x}$ for Mg	42.2	44.3	44.1	43.4	44.4	$\bar{X}\bar{x}$ 43.7

* Note: Mineral levels indicate animal assignments only, all animals on basal diet, animals not infected.

Column and row means include rounding error.

Error Mean Square = 20.75.

N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXIV

PACKED CELL VOLUME (PCV) THREE DAYS PRIOR TO LOWEST OBSERVED PCV (VOLUMES PERCENT), Mg x Zn, 5 x 3 FACTORIAL DESIGN

Mg, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	43.9	37.9	42.1	41.4	42.9	41.6
13.5	39.7	44.7	40.8	38.6	42.1	41.2
23.5	41.6	41.2	42.3	41.9	41.4	41.7
$\bar{x}$ for Mg	41.7	41.3	41.6	40.6	42.1	$\bar{X}\bar{x}$41.5

Column and row means include rounding error.
Error Mean Square = 23.11.
N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXV

PACKED CELL VOLUME (PCV) TWO DAYS PRIOR TO LOWEST OBSERVED PCV (VOLUMES PERCENT), Mg x Zn, 5 x 3 FACTORIAL DESIGN

Dietary Mg, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	39.7	35.4	40.1	41.9	38.5	39.1
13.5	40.4	41.4	40.6	37.6	39.4	39.7
23.2	42.0	37.8	39.4	40.3	37.6	39.4
$\bar{x}$ for Mg	40.7	38.2	40.0	39.9	38.1	$\bar{X}\bar{x}$39.4

Column and row means include rounding error.
Error Mean Square = 28.77.
N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXVI

PACKED CELL VOLUME (PCV) ONE DAY PRIOR TO LOWEST OBSERVED PCV (VOLUMES PERCENT), Mg x Zn, 5 x 3 FACTORIAL DESIGN

Dietary Mg, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	39.7	27.9	33.0	35.3	34.4	34.0
13.5	38.8	38.2	36.1	37.3	33.6	37.0
23.2	37.9	29.5	31.1	37.7	33.6	34.0
$\bar{x}$ for Mg	38.8	31.9	33.4	36.7	34.6	$\bar{X}\bar{x}$35.1

Column and row means include rounding error.
Error Mean Square = 47.93.
N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXVII

LOWEST PACKED CELL VOLUME OBSERVED AFTER INOCULATION WITH *BABESIA RODHAINI* DURING 33-DAY POSTINOCULATION PERIOD
Mg x Zn, 5 x 3 FACTORIAL DESIGN

Dietary Mg, ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	34.0	17.4	19.3	24.7	21.4	23.4
13.5	34.9	27.7	20.9	30.1	17.4	26.2
23.2	28.3	28.3	18.4	23.2	21.5	22.5
$\bar{x}$ for Mg	32.4	22.1	19.5	25.9	20.1	$\bar{\bar{x}}$ 24.0

Column and row means include rounding error.
Error Mean Square = 90.25.
N = 8 rats per Mg-Zn mean; 120 rats total.

TABLE XXXVIII

HIGHEST PERCENT PARASITEMIA OBSERVED AFTER INOCULATION WITH BABESIA RODHAINI DURING 33-DAY OBSERVATION PERIOD MAGNESIUM x ZINC 5 x 3 FACTORIAL DESIGN

Dietary Mg ppm	30.0	60.1	91.0	157.5	343.1	$\bar{x}$ for Zn
Zn, ppm						
12.7	9.2	55.0	67.2	59.0	66.6	51.4
13.5	4.5	27.2	62.0	67.2	70.5	46.3
23.2	31.9	37.2	65.1	66.9	64.6	53.2
$\bar{x}$ for Mg	15.2	39.8	64.8	64.4	67.2	$\bar{x}\bar{x}$ 50.3

Column and row means include rounding error.
Error Mean Square = 662.8.
N = 8 rats per Mg-Zn mean; 120 rats total.

APPENDIX C

EFFECT OF REDUCED SERUM Mg++ ON INCUBATION TIMES IN SPLENECTOMIZED CALVES

NORMAL SERUM Mg^{++} INCUBATION TIME (DAYS)	REDUCED SERUM Mg^{++} INCUBATION TIME (DAYS)*
27	59+
28	63+
23	64+
29	64+
30	46+

**Calves died in tetany. Blood passed to next low Mg^{++} calf and to normal Mg^{++} calf*

Figure 48. Effect of reduced dietary magnesium (Mg) on incubation period of Anaplasma marginale in splenectomized calves. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Used with permission of the senior author).

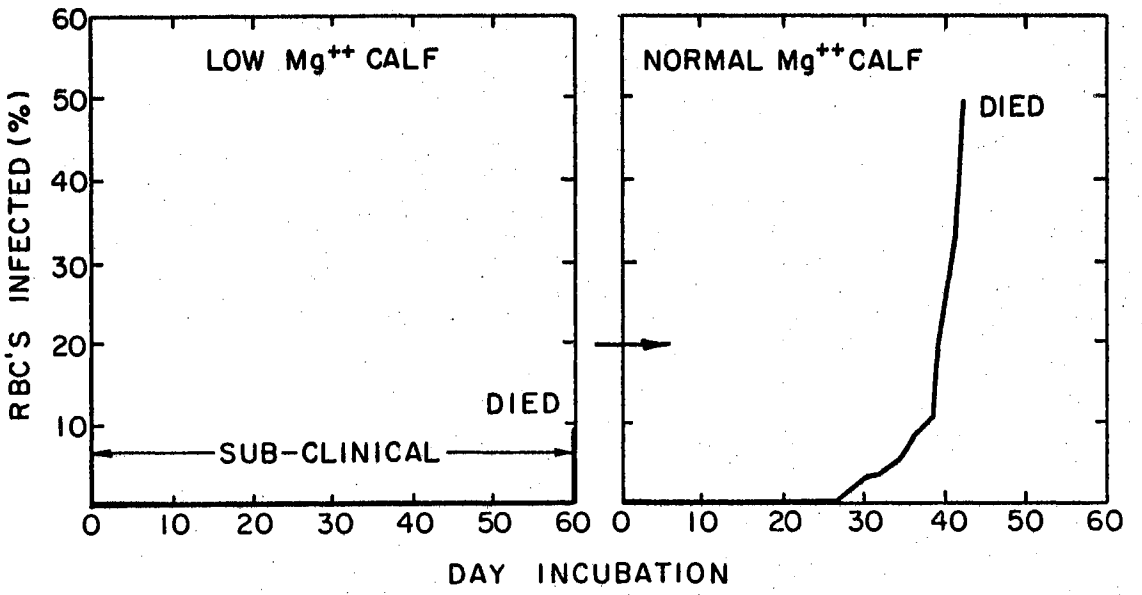

Figure 49. Effect of dietary magnesium (Mg) on the course of Anaplasmosis in two calves. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Research Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Used with the permission of the senior author).

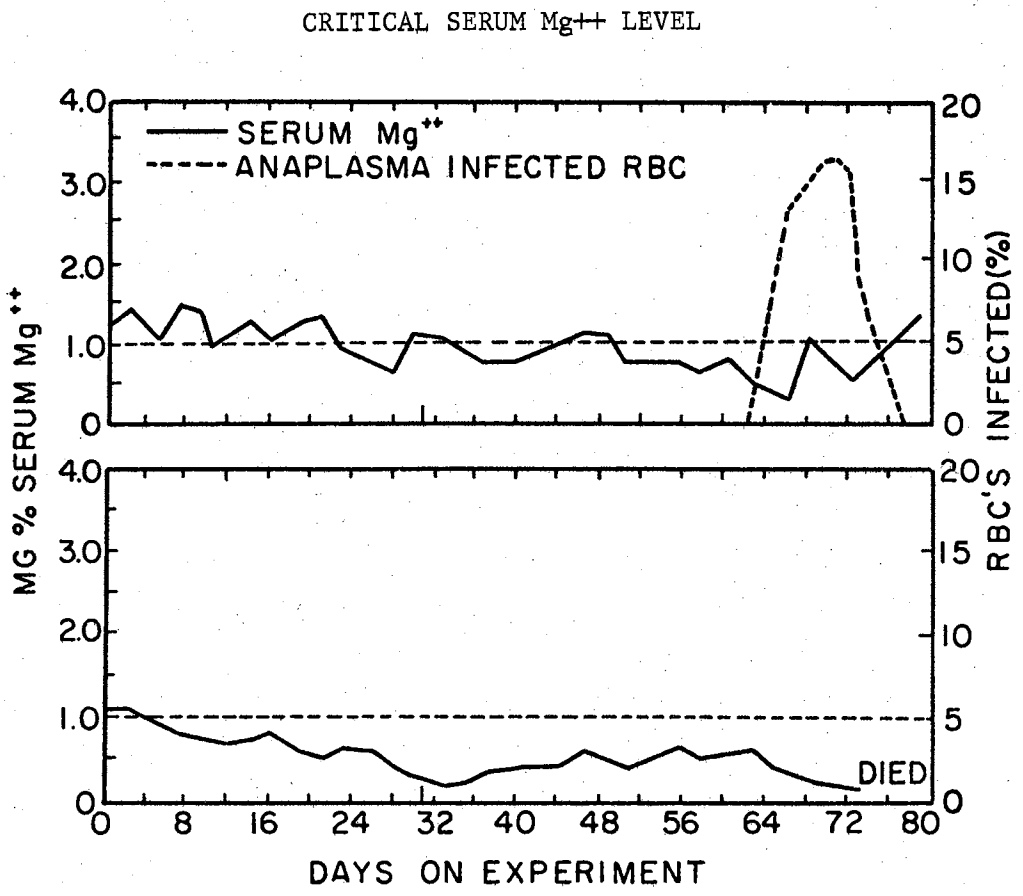

Figure 50. Response of two calves with different serum magnesium (Mg) levels to inoculation with Anaplasma marginale. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Research Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Used with permission of the senior author).

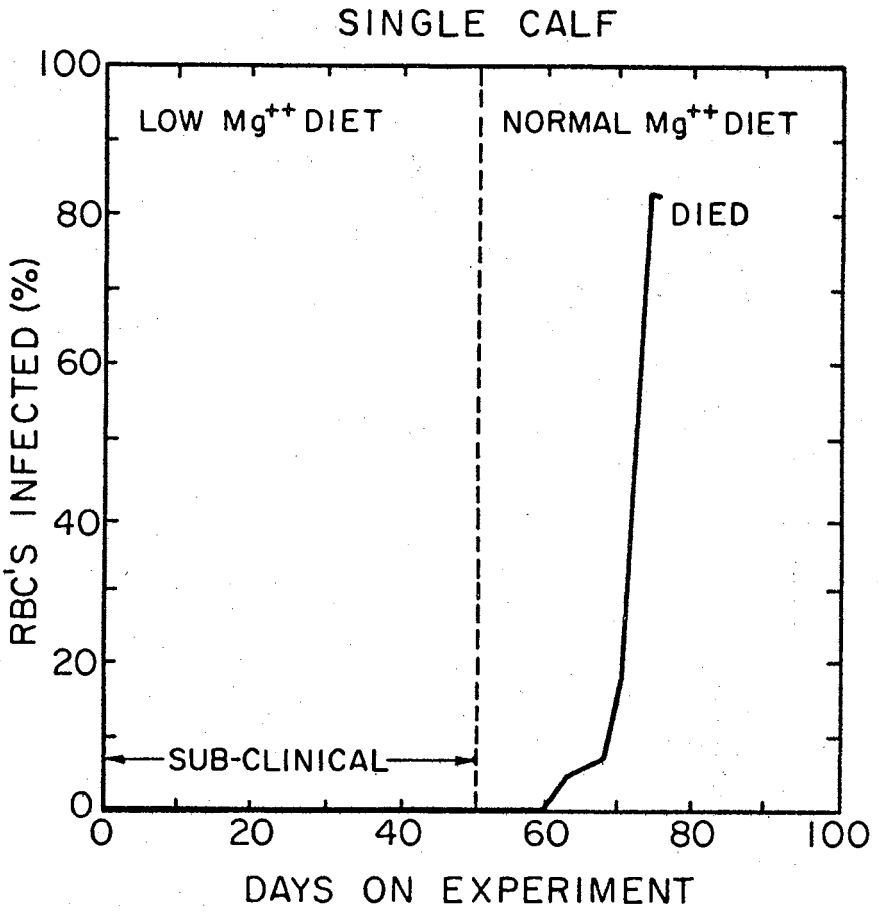

Figure 51. Effect of placing a magnesium (Mg) deficient calf previously inoculated with Anaplasma marginale on a commercial calf ration. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Research Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Used with permission of the senior author).

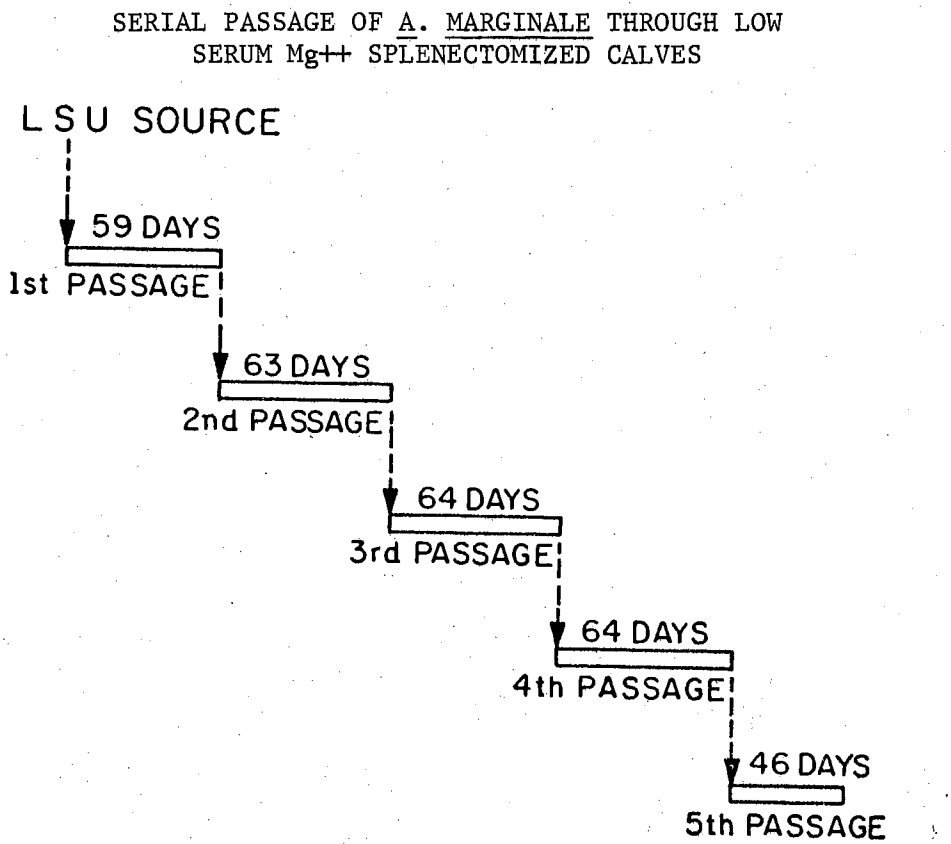

Figure 52. Effect of serial passage of Anaplasma marginale through magnesium (Mg) deficient splenectomized calves. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Research Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Used with permission of the senior author).

INFECTIVITY OF A. MARGINALE AFTER FIVE SERIAL PASSAGES THROUGH REDUCED SERUM Mg++ CALVES

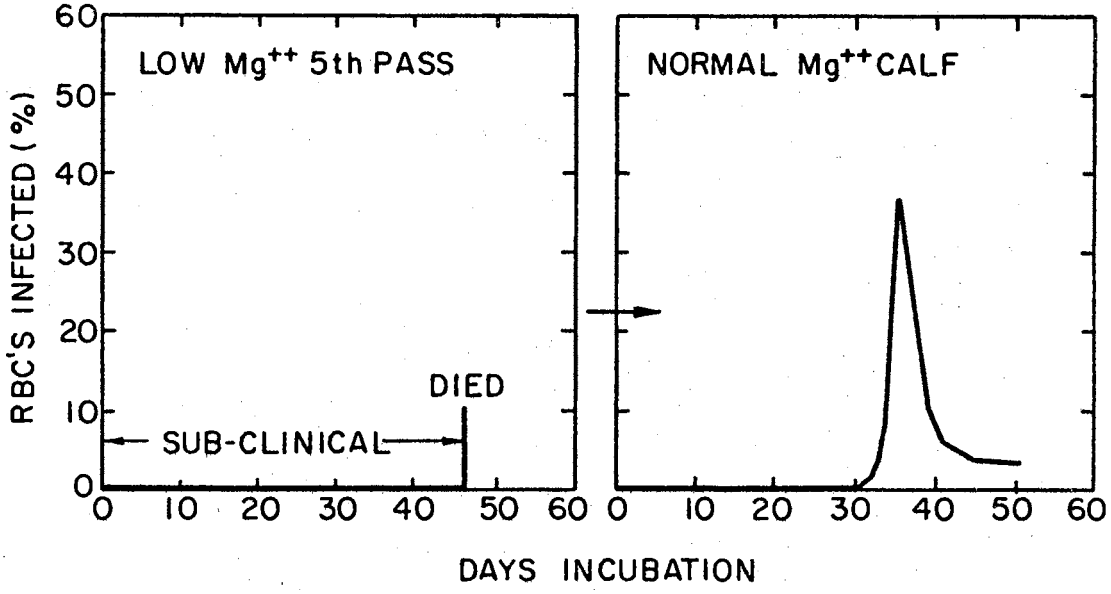

Figure 53. Infectivity of Anaplasma marginale after five serial passages through splenectomized calves fed a magnesium (Mg) deficient diet and exhibiting reduced serum magnesium values. Redrawn from a slide presented by David M. Bedell and James G. Miller at the Southern Region Animal Disease Research Workers Conference, Clemson University, Clemson, South Carolina, 1964. (Presented with the permission of the senior author).

APPENDIX D

TABLE XXXIX

CURVE EQUATION DATA FOR VARIOUS FIGURES.

c = Y at X = infinity.
a = Y intercept at X = 0.
b = Slope of the curve.
e = Error term.
r = Correlation
C.V. = Coefficient of Variation (C.V. · 100 = %).

For Figure 7: Growth Data, Experiment II

Ration	#1	#2	#3	#4	#5
c =	+357.000	+595.000	+525.000	+610.000	+880.000
a =	-264.867	-506.087	-435.903	-528.245	-791.638
b =	-0.02439	-0.01122	-0.01895	-0.01492	-0.00903
e =	+44.9308	+23.3797	+17.7080	+12.2155	+17.8388
r =	-0.99264	-0.99719	-0.99843	-0.99913	-0.99868
CV=	+0.03140	+0.02180	+0.01630	+0.01370	+0.01620

For Figures in Experiment III

	Figure 24 (Survival)	Figure 26 (Low P.C.V.)	Figure 28 Parasitemia
c =	+9.37499	+19.4000	+67.2000
a =	+47.2810	+13.5624	+113.523
b =	-.024819	-.006661	-.025627
e =	+6.70189	+20.2221	+16.9268
r =	-0.85455	-0.65637	-0.92769
CV =	+0.15000	+0.17200	+0.08350

TABLE XL

LOGIC USED TO DEVELOP THE EXPONENTIAL CURVE FITTING PROGRAM FOR THE I.B.M. 1620

Basic Equation: $Y = c + ae^{bx}$

Exponential Regression: $\log_e(ae^{bx}) = \log_e a + bx$

of form: $z = k + b \cdot x$

Weighted Linear Regression:

$$b = \frac{\Sigma(W_i)\Sigma(W_iX_iZ) - \Sigma(W_iX_i)\Sigma(W_iZ_i)}{\Sigma(W_i)\Sigma(W_iX_i^2) - (\Sigma W_iX_i)^2}$$

$$k = \frac{\Sigma(W_iZ_i) = b\cdot\Sigma(W_iX_i)}{\Sigma(W_i)}$$

$$a = e^k$$

Note: To compensate for the log of Y being used in the regression, weighting of $W_i = (Y_i - c)$ is used.

Correlation = r

$$r = \frac{\Sigma(W_i)\Sigma(W_iX_iZ_i) - \Sigma(W_iX_i)\Sigma(W_iZ_i)}{\sqrt{[\Sigma(W_i)\Sigma(W_iX_i^2) - \Sigma(W_iX_i)^2]\cdot[\Sigma(W_i)\Sigma(W_iZ_i^2) - (\Sigma W_iZ_i)^2]}}$$

Error Term = e

$$e = \frac{1}{N}\Sigma(c+ae^{bx}-Y_i)^2$$

Coefficient of Variation = C.V.

$$C.V. = \frac{\sqrt{e}}{\frac{1}{N}\Sigma Y_i}$$

TABLE XLI

EXPONENTIAL CURVE FIT PROGRAM

```
C          EXPFIT - EXPONENTIAL CURVE FIT - LEVEL 70.0608.
C          LEAST SQARE ERROR FIT OF EXPONENTIAL CURVE TO DATA USING
C          LOGARITHMIC TRANSFORMATION AND LINEAR REGRESSION FOR THE
C          IBM 1620 COMPUTER

C          JOHN WITZ AND FRED WITZ, PROGRAMMERS


      DIMENSION X(150),Y(150),M(150),ME(150)
      COMMON X,Y,M,ME
    1 FORMAT(11HN,D=(I3,F2)    )
    2 FORMAT(I3,F2.0)
    3 FORMAT(2F10.0)
    4 FORMAT(13HC=(F10) CMIN=,E15.7)
    5 FORMAT(13HC=(F10) CMAX=,E15.7)
    6 FORMAT(F10.0)
    7 FORMAT(9HM(I)=(I2)    )
    8 FORMAT(10HME(I)=(I2)    )
    9 FORMAT(40I2)
   10 FORMAT(4H  A=,E15.7,4H  B=,E15.7,4H  R=,E15.7,4H  E=,E15.7)
   11 TYPE 1
      ACCEPT 2,N,D
      YMAX=-1.E40
      YMIN=1.E40

      DO29 I=1,N
      READ3,X(I),YI
      Y(I)=YI
      IF(YI-YMAX)22,22,21
   21 YMAX=YI
      GO TO 24
   22 IF(YI-YMIN)23,24,24
   23 YMIN=YI
   24 M(I)=1
      ME(I)=1
   29 CONTINUE

   30 IF(D)31,11,33
   31 TYPE4,YMAX
      GO TO 35
   33 TYPE5,YMIN
   35 ACCEPT6,C
      IF(SENSE SWITCH 1)11,42
   42 IF(SENSE SWITCH 2)43,46
   43 TYPE7
      ACCEPT9,(M(I),I=1,N)
   46 IF(SENSE SWITCH 3)47,50
   47 TYPE8
      ACCEPT9,(ME(I),I=1,N)
   50 SX=0.
      SXX=0.
      SZ=0.
      SZZ=0.
      SXZ=0.
      SW=0.
      SE=0.
      SME =0.

      DO59 I=1,N
      XI=X(I)
      YD=(Y(I)-C)*D
      IF(YD)30,30,55
   55 Z=LOG(YD)


      W=M(I)
      W=W*YD
      SX=SX+W*XI
      SXX=SXX+W*XI*XI
      SZ=SZ+W*Z
      SZZ=SZZ+W*Z*Z
      SXZ=SXZ+W*XI*Z
      SW=SW+W
   59 CONTINUE

      T1=SW*SXX-SX*SX
      T2=SW*SXZ-SX*SZ
      B=T2/T1
      AL=(SZ-B*SX)/SW
      A=EXP(AL)
      T3=T1*(SW*SZZ-SZ*SZ)
      R=T2/SQRT(T3)

      DO69 I=1,N
      XI=X(I)
      WEI=ME(I)
      YC=C+D*A*EXP(B*XI)
      ER=YC-Y(I)
      SE=SE+WEI*ER*ER
      SME=SME+WEI
   69 CONTINUE

      EMS=SE/SME
      TYPE10,A,B,R,EMS
      GO TO 35
      END
```

VITA

2

Benny Burge Norman

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Doctor of Philosophy

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Professional Experience: Private Practice, 1960; Disease Control Veterinarian, U. S. Department of Agriculture, 1961; Supervisor, Hematology Research Laboratory, Oklahoma State University, 1962-63; Fulbright Lecturer in Physiology, Guatemala, 1964-65; Training Coordinator, Oklahoma State University, 1966; Director, Field Clinics Program, Colombia, South America, for the University of Nebraska, 1967-68.

Professional and Honorary Societies: American Society of Animal Science, American Veterinary Medical Association, American Association of Bovine Practitioners, American Veterinary Society for the Study of Breeding Soundness, and the Society of Sigma Xi.