Growth of Trypanosomes in Vivo, Host Body Weight Gains, and Food Consumption in Zinc-Deficient Mice

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This study examined the effect of zinc deficiency on food consumption and the growth of mice infected with *Trypanosoma musculi* or immunized with parasite products. In addition, the effects of zinc deficiency on the growth and development of parasites in vivo was studied. Infected mice consumed more food than noninfected mice, and the level of food consumption in the zinc-deficient mice was much less and showed general decline during the observation period. Also, infected mice on both full-complement and zinc-deficient diets gained more body weight than control mice. Throughout the observational period, trypanosomes from zinc-deficient mice showed considerably higher variability in size as determined by coefficient of variation. In both dietary groups, the average length of trypanosomes was not significantly different. *(J Natl Med Assoc.* 1997;89:48-56.*)*

Key words: zinc deficiency ◆ *Trypanosoma musculi* infection ◆ growth of trypanosomes ◆ host food consumption and weight gains

Deficiency in zinc, relative to dietary inadequacy, constitutes a serious and persistent global problem.[1,2] Some manifestations of zinc deficiency in animals include hair loss, anorexia, impaired sexual development, gastrointestinal malfunction and parakeratosis, thymus atrophy, poor wound healing, decreased skin sensitization capacity, and lymphopenia.[3,4] Further, genetic abnormalities such as acrodermatitis enteropathica, sickle cell anemia, and edema disease are associated with a flaw in the function of the intestinal zinc absorption mechanism.[1,5]

There has been some controversy concerning the actual absorption site, but rat studies have shown that zinc uptake occurs partly by a regulated carrier-mediated diffusion mechanism that responds homeostatically to dietary zinc supply. In addition, evidence from transport kinetics has confirmed the presence of both passive and saturable processes.[6] Therefore, even though the actual site of zinc absorption is unclear, it has been estimated that approximately 20% to 30% of ingested dietary zinc is absorbed.[7,8] Further, the distribution of the micronutrients into various organs and tissues is highly variable and can be affected by stress, growth, hormonal activity, and inherited zinc metabolism disorders.[6,9] In plasma, one portion of portal zinc is associated with albumin, alpha-2-macroglobulin, and transferrin; one portion is in ionic form; and another portion exists as an ultrafilterable segment bound to amino acids.[9,10] However, once absorption has occurred, substantial amounts of the metal are taken up by the liver and subsequently redistributed to the bones, muscles, and appropriate cellular sites.[11]

Pekarek et al[12] observed increased susceptibility to experimental tularemia in zinc-deficient rats. With zinc-deficient guinea pigs vaccinated with

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Mycobacterium bovis, there was growth retardation, deminished hematocrit levels, reduced total serum protein and albumin levels, and decreased ability to control mycobacterial population growth.[13] Further such immune dysfunction patterns as atrophied thymus reduced delayed hypersensitivity response to *Listeria monocytogenes*, impaired lymphocyte response to phytohemagglutinin, and increased trapping of *Escherichia coli* by liver, lungs, and kidneys during gram-negative sepsis have been exhibited by zinc-deficient rats.[14,15]

With the scarcity of validated functional criteria to evaluate the relationship between infection and zinc, this study examines the effect of zinc deficiency on food consumption and growth and development of the parasites in mice inoculated with *Trypanosoma musculi* or immunized with parasitic products.

MATERIALS AND METHODS

Mice

Four experimental protocols were completed using a total of 432 Swiss Webster female albino mice of weaning age weighing approximately 12 g. Upon arrival, mice were quarantined for 7 days prior to their designation into their respective dietary groups. All mice groups were housed in separate suspended wire bottom cages. They were fed the appropriate diets from metal cups designed to minimize food spillage. All mice were allowed to eat and drink ad libitum. The water given to the mice did not contain any detectable amount of zinc when analyzed by flame atomic absorption spectrophotometry. The daily food intake of every mouse was determined by subtracting the amount of food remaining in the tared feeding cup from the amount given the previous day. Glass bottles, sipper tubes, and silicone stoppers were used to avoid any metal contamination of water. Water bottles and feeding cups were cleaned daily, and cages were steamed frequently to minimize algal and bacterial contamination.

Diet

Complete (controls) and zinc-deficient (experimental) diets were prepared and purchased commercially from Nutritional Biochemicals, Cleveland, Ohio.

Parasite

Trypanosoma musculi was used as the experimental organism. This organism has been maintained in the laboratory by syringe passage in mice for over 32 years.[16] One thousand trypanosomes in 0.25-mL sterile physiological saline were used to inoculate the mice intraperitoneally.

Parasitic Derivatives

Metabolic products and homogenate of *T musculi* were prepared according to the methods of Lee and Aboko-Cole.[17]

Inoculations

Twenty-eight days after initiation of a dietary regimen, each group (full complement and zinc deficient) was further subdivided as follows:

- *group 1:* uninoculated controls,
- *group 2:* mice were inoculated intraperitoneally with 0.25 mL of physiological saline,
- *group 3:* mice were inoculated with 0.25 mL of physiological saline containing 1×10^3 living trypanosomes,
- *group 4:* mice were inoculated intraperitoneally with 0.25 mL of parasitic metabolic products, and
- *group 5:* mice were inoculated intraperitoneally with 0.25 mL of the homogenate.

Mice were inoculated with the living trypanosomes only once (28 days after initiation of the experiment). The physiological saline and *T musculi* derivatives were injected at 3-day intervals between day 28 and day 80. Mice were sacrificed on every 5th and 7th day until day 80 for various analyses. Sera were collected and stored at −20°C until used.

Parasitemia

Beginning the day after mice were inoculated with the living trypanosomes, wet films of tail blood were prepared daily to determine the time of subsequent appearance of trypomastigotes in the peripheral circulation. Subsequently, numbers of the trypomastigotes were counted using red blood cell pipet, hemacytometer, Toisson's fluid, and a constant dilution factor of 200.[18]

Determination of Zinc Levels in Plasma and Liver

Zinc levels in plasma and liver were analyzed using Perkin-Elmer Model 603 Atomic Absorption Spectrophotometer (Perkin-Elmer Corp, Norwalk, Connecticut). Plasma was diluted fivefold with double-deionized water and the diluted samples analyzed directly for zinc. Plasma concentrations of zinc were measured against zinc standard solutions (Harleco Manufacturers, Gibbstown, New Jersey) diluted with 5% (vol/vol) glycerol in double-deion-

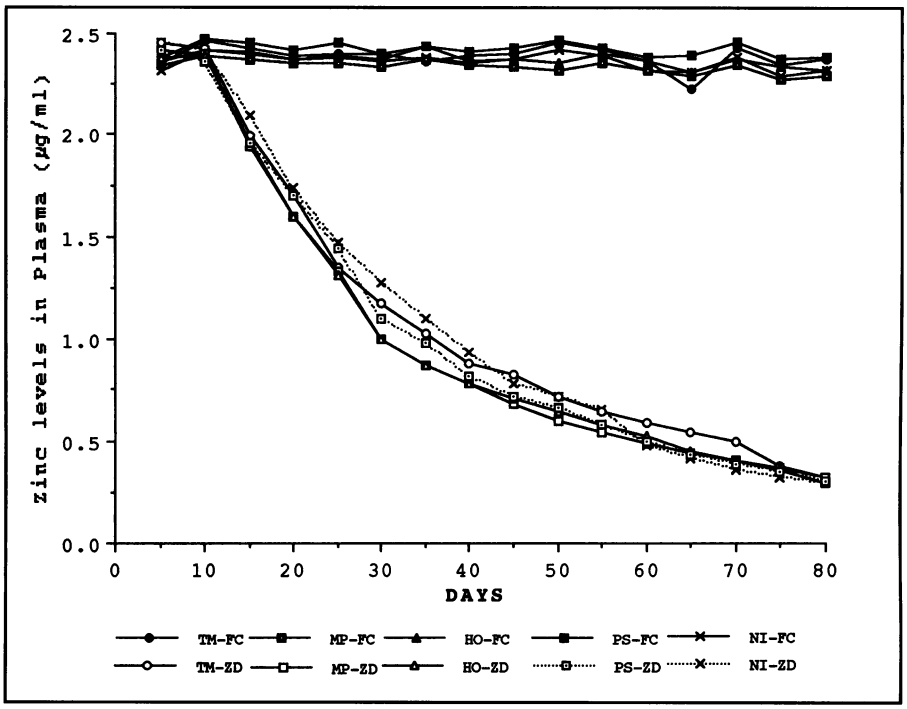

Figure 1A.
Zinc levels in plasma (µg/mL) of mice fed full-complement (FC) or zinc-deficient (ZD) diets and inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), physiological saline (PS), or noninfected (NI).

ized water. The zinc content of the digested liver samples was measured according to the procedure described for plasma, except that the standards were prepared with double-deionized water only.[19]

Measurements of Host Growth and Food Consumption

Mice and food were weighed on a laboratory balance having a sensitivity of 0.01 g. Food consumption were measured daily, and mice body weights were taken every 5 days. Body weight changes were considered as cumulative average percentages relative to initial weights. All computations of food consumption were made as 5-day averages.

Growth and Development of *T musculi*

The reproductive rates and size varibility were determined as described by Lee and Lincicome[20] and Lee et al.[18] The trypanosomes were drawn and measured from random samples. The total length of each trypanosome was recorded in microns, and the coefficients of variation were calculated.

Statistical Evaluation

The statistical treatment of the data involved two-way analysis of variance (replication-Model I) and Duncan's multiple range test at the 5% significant level.[21,22]

RESULTS

Zinc Levels

The average plasma zinc levels in noninfected mice was 2.50±0.05 µg/mL. The levels for mice inoculated with physiological saline, *T musculi*, metabolic products, and homogenate were 2.42±0.03 µg/mL, 2.39±0.06 µg/mL, 2.33±0.01 µg/mL, and 2.36±0.01 µg/mL, respectively. There was a steady decrease in the plasma zinc levels in zinc-deficient mice between day 15 and day 80 (Figure 1A). In general, from day 15 to day 80, cumulative decline for each of noninfected and infected metabolic products-, homogenate-, and physiological saline-inoculated mice averaged about 85%. The average liver zinc concentration was 34.6 mg/g. Zinc-deficient mice showed gradual decreases in the zinc concentration in liver (Figure 1B). Cumulative reductions averaged about 38% for noninfected metabolic products- or physiological saline-inoculated mice, 22% for infected mice, and 31% for homogenate-inoculated mice.

Parasitemia

Throughout the infection, mice fed with zinc-deficient diets exhibited greater numbers of parasites than those fed with full-complement diets. In the full-complement group, trypanosomes appeared in the blood after 4 days of inoculation. The zinc-deficient

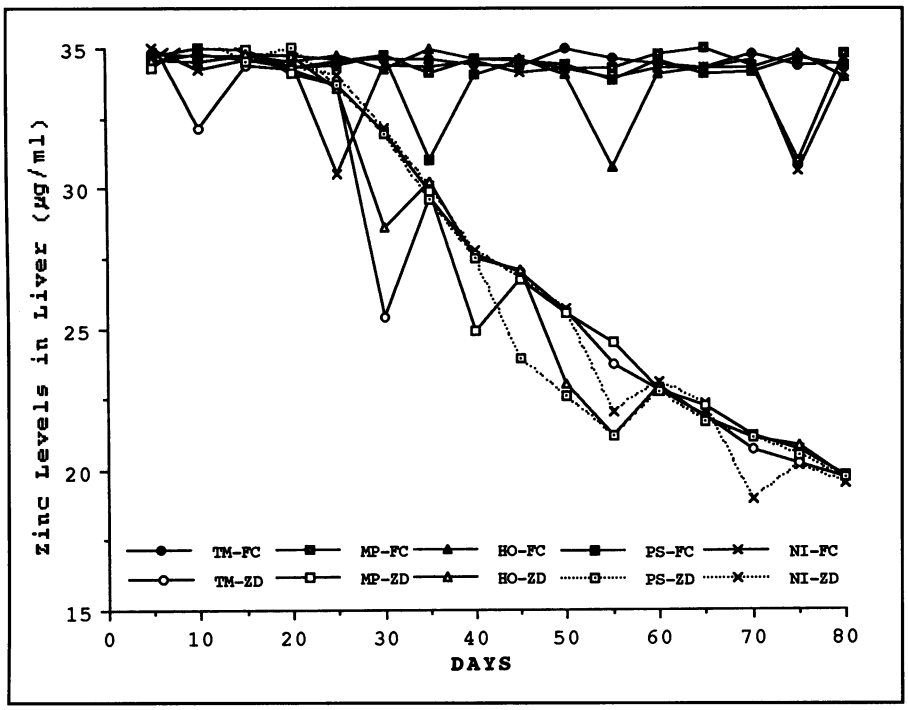

Figure 1B.
Zinc levels in liver (µg/g) of mice fed full-complement (FC) or zinc-deficient (ZD) diets and inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), physiological saline (PS), or noninfected (NI).

animals became positive on day 3. On average, the parasitemias of zinc-deficient hosts were about four times greater than those mice given full-complement diets. Peak parasitemias occurred on day 12 in mice fed full-complement diets and day 15 in zinc-deficient mice. Parasites were no longer visible in the blood of full-complement mice after day 26, but persisted in zinc-deficient mice until day 38 (Figure 2).

Growth of Trypanosomes

The average length of trypomastigotes from the two dietary groups did not differ significantly ($P=.05$) from one another (Figure 3). The range of variability in length of parasites in mice on full-complement diets was 29% to 11% from day 5 through day 14 and 7.7% to 3.6% from day 15 until the parasites disappeared from the blood. The zinc-deficient group showed higher variability in length and a prolonged parasitemia. The range of variability in length of trypomastigotes from day 5 to 26 was 33.6% to 11%. Thereafter, the coefficients of variation ranged from 8.3% to 3.6% (Figure 3).

Host Food Consumption

After 5 days of infection, the mice on full-complement diets started consuming significantly more food than noninfected mice (Figure 4A). The infected mice consumed 0.7 to 2.0 g more food than noninfected mice. Mice inoculated with homogenate showed a similar increase in food consumption. At day 50, infected and homogenate-inoculated mice ate 46.9% and 48.5% more food, respectively, than controls. There were no significant differences in the quantity of food consumed by mice inoculated with metabolic products or physiological saline.

Compared with mice fed full-complement diets, the level of food consumption in the zinc-deficient mice was much less and showed a general decline during the observation period (Figure 4B). Infected and homogenate-incoulated mice consumed 0.5 to 1.0 g more food than control mice. In terms of advantage over controls, food intake reached a maximum of 70% (day 55) for the infected mice and 81% (day 75) for homogenate-inoculated mice. No significant differences were seen in the amount of food consumed by mice fed zinc-deficient diets and inoculated with metabolic products or physiological saline.

Host Body Weight Changes

Mice fed full-complement diets showed a steady rise in body weight gains in all of the treatment groups (Figure 5A). The body weight gains of infected mice and homogenate-inoculated mice became statistically significant ($P=.05$) from day 45 and continued until the last day of observation. With respect to control values, the overall differences in percent-

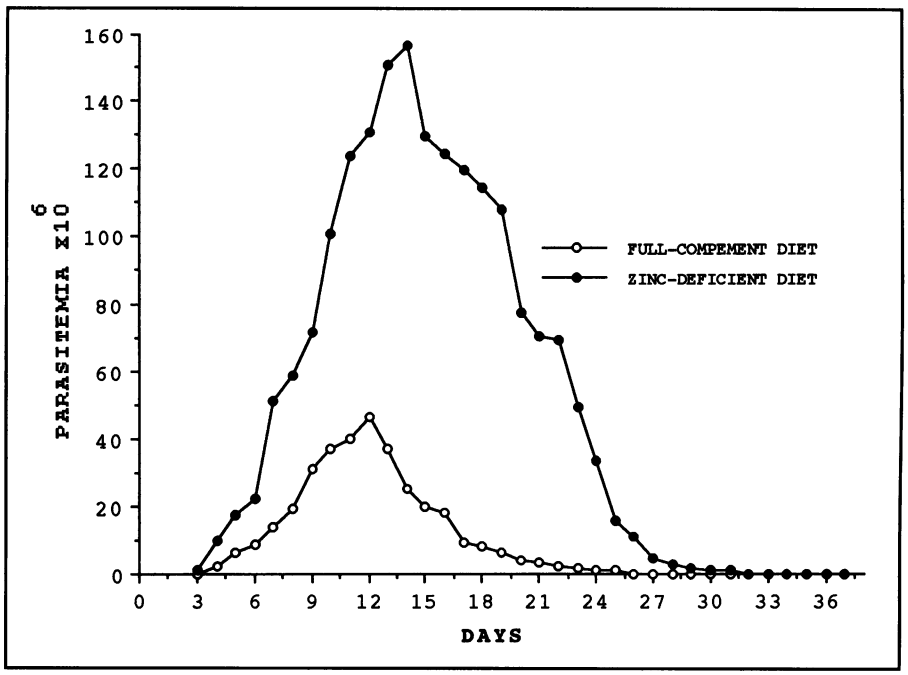

Figure 2.
Daily average parasitemia of mice infected with *Trypanosoma musculi* that were fed full-complement or zinc-deficient diets.

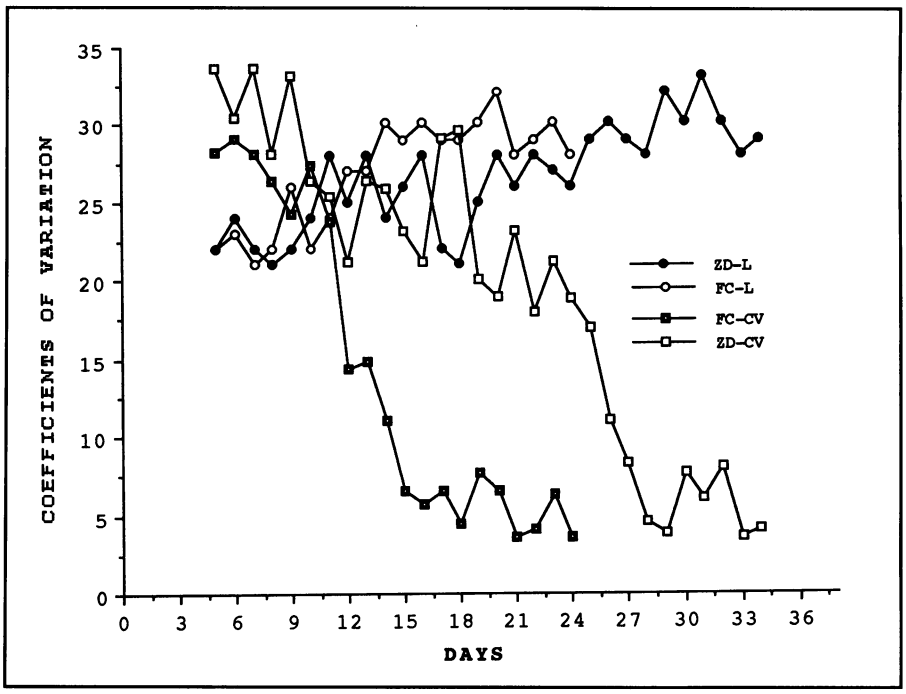

Figure 3.
Coefficients of variation (CV) for total lengths (L) of trypomastigotes during the course of *Trypanosoma musculi* infection in mice fed full-complement (FC) or zinc-deficient (ZD) diets.

age gains ranged from 7.3% to 38.6% for infected mice and 6.2% to 40.8% for homogenate-inoculated mice. There were no significant differences in percent body weight gains in mice inoculated with metabolic products or physiological saline. Compared with mice on full-complement diets, the weight gains in the zinc-deficient mice were on average 3 to 4 times less (Figure 5B). There was no significant difference in all of the treatment group until 40 days after infection. At this time, infected and homogenate-inoculated mice gained significant (P=.05) weight. Compared with control values, the maximum weight gains (30.5% for infected and 31.5% for homogenate inoculated) occurred at day 55. Although the zinc-deficient

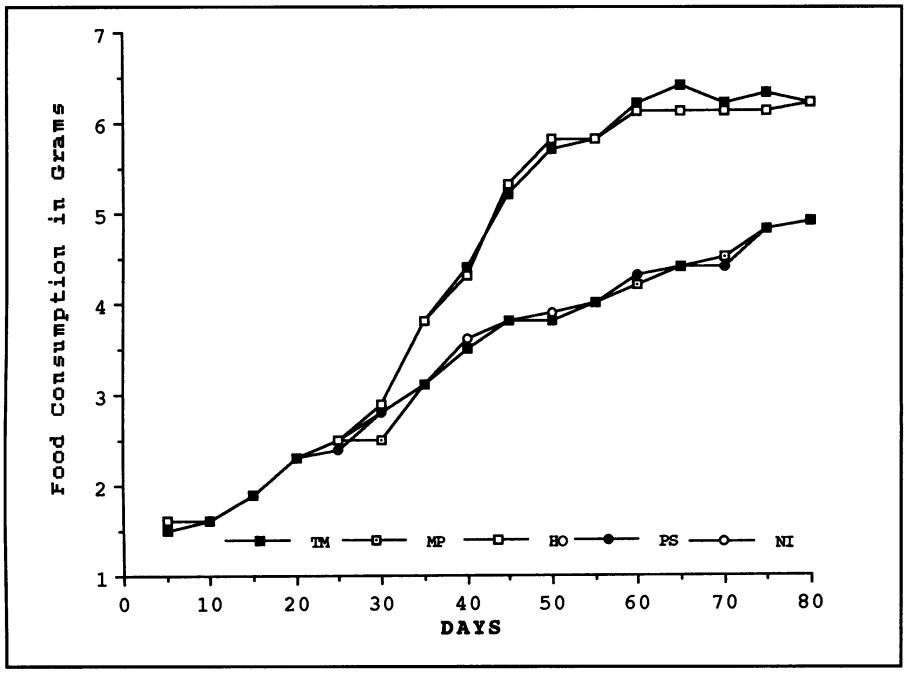

Figure 4A.
Food consumption of noninfected mice (NI) and mice inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), or physiological saline (PS) and fed a full-complement diet.

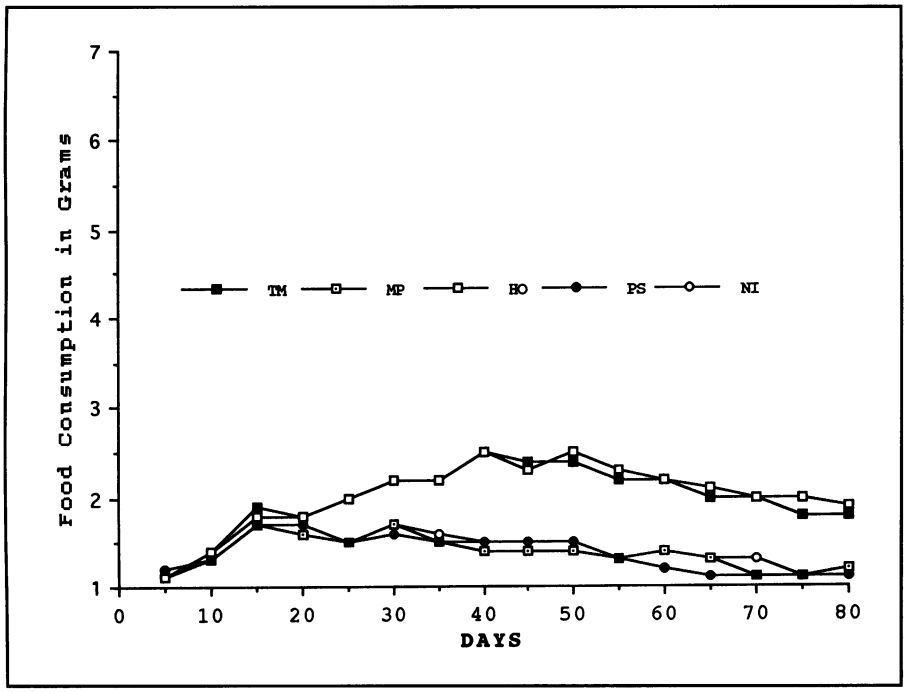

Figure 4B.
Food consumption of noninfected mice (NI) and mice inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), or physiological saline (PS) and fed a zinc-deficient diet.

mice inoculated with metabolic products or physiological saline showed no significant increases, the differences in body weight gains over controls ranged from 0.5% to 4.1%. After day 55, zinc-deficient mice in all of the treatment groups experienced a gradual decrease in weight gains.

DISCUSSION

After a period of 28 days on the experimental diet, the typical signs of zinc deficiency, which included reduced food intake, loss of hair, and lesions on the feet and tail, were manifested.[23,24] As seen in this study, one of the earliest signs of zinc

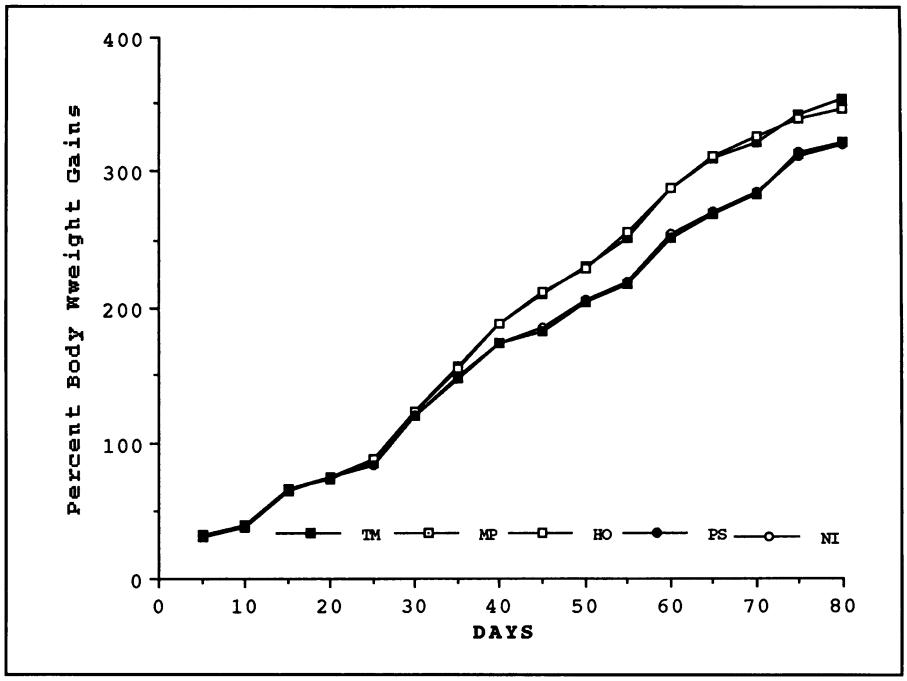

Figure 5A.
Percent body weight gains of noninfected mice (NI) and mice inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), or physiological saline (PS) and fed a full-complement diet.

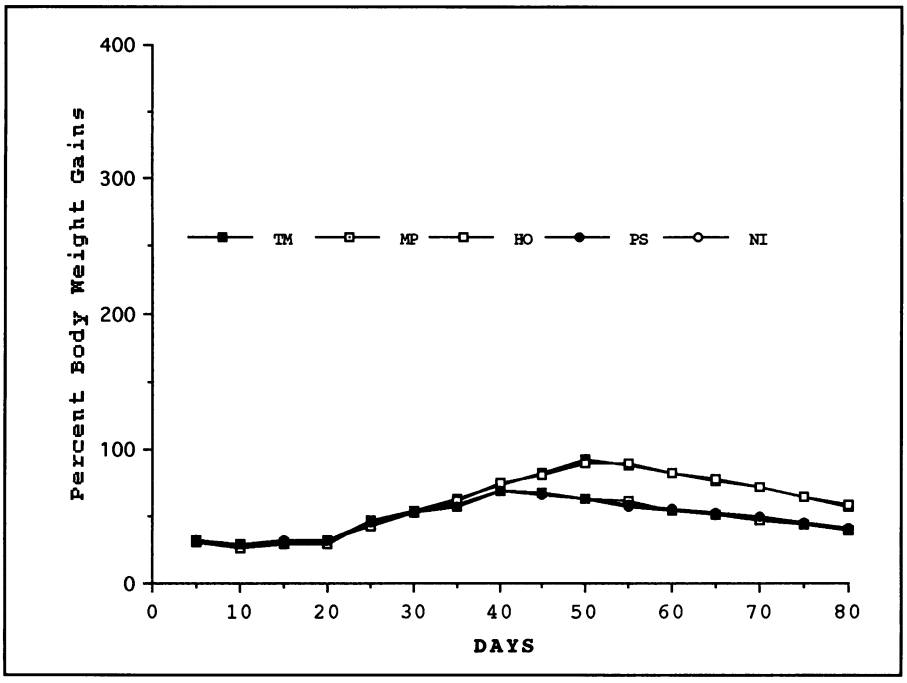

Figure 5B.
Percent body weight gains of noninfected mice (NI) and mice inoculated with *Trypanosoma musculi* (TM), metabolic products (MP), homogenate (HO), or physiological saline (PS) and fed a zinc-deficient diet.

deficiency is the loss of appetite, followed by reduced food intake and failure to grow. This can be accompanied by severe anorexia and gastrointestinal malfunction.[5,25] The data show food consumption and body weight gains of zinc-deficient mice lagged behind that of the full-complement groups and was significantly less for the duration of the study. Here, it is implied as in other studies that with the reduced food intake, an inadequate amount of zinc is available. This inadequacy could be partly responsible for the malfunctioning in the formation and regulation of essential zinc-dependent enzymes. The malfunctioning could contribute to inefficient metabolic processes and reduced growth rate for the

zinc-deficient animals.[7,26] It is clear from the results that weight changes in the zinc-deficient mice were modified when deficiency and inoculation with living cells or homogenate coexisted concurrently in the host. On inoculation with *T musculi* living cells and homogenate, food intake and body weight gains increased substantially. The living cells and homogenate-inoculated mice consumed more food as well as showed significant increases in weight gains over the uninfected controls. This was true for all mice, although for the zinc-deficient mice, the food intake and body weight gains were much smaller than mice on full-complement diet.

Similar results have been reported with *T musculi* living cells and derivative inoculation during mineral or vitamin insufficiency.[18,27] The assumption, therefore, is that mice deficient in zinc had a more favorable metabolic balance on inoculation with living cells or homogenate. It was further postulated that after inoculation with living cells and homogenate, growth stimulating/promoting factors were released in the host, and these factors in some way were responsible for the observed results.[17,28] Another plausible explanation is that after inoculation, undue stress influences the action of the pituitary and thyroid glands resulting in hyperactivity and stimulated growth.[29-31]

With regard to in vivo growth of trypanosomes, the number of parasites in the blood was about four times higher in zinc-deficient mice, and infection was quite prolonged. The indication is that the absence of zinc seemed to elaborate the disease process. Similar effects of nutrient deficiency on other kinds of infection have been reported.[7,12,13,15,32]

It has been postulated that the effect of zinc deficiency on host cellular responses and antibody synthesis accounts in part for the observed heightened parasitemia.[5,23,33] The patterns of trypanosome population growth as indicated by continued high variability in trypomastigote cell size in zinc-deficient mice suggest a delay in the formation of the reproductive-inhibiting antibody, ablastin. High coefficients of variability in the cell length of the parasites also continued much longer in the zinc-deficient mice than in the full-complement and pair-fed mice. In addition, since there was persistence of parasites in the blood and peak population of trypanosomes were found many days later in the zinc-deficient hosts in comparison with full-complement and pair-fed litter mates, there is evidence of interference in action and synthesis of the trypanolytic antibodies.[34-36] Whitelaw et al[37] and MacAskill et al[38] showed that acute fatal infections of *Trypanosoma brucei* and *Trypanosoma congolense* were the results of inability of the host to achieve effective levels of circulating antibodies against rapidly replicating trypanosome clones. It was hypothesized that the degree of parasitemia correlated to the levels of immunoglobulins and the production of antibodies controlled the severity of the disease processes.[39-42]

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