INTERACTION OF NUTRITION AND INFECTION: EFFECT OF ZINC DEFICIENCY ON IMMUNOGLOBULIN LEVELS IN TRYPANOSOMA MUSCULI INFECTION

Clarence M. Lee, PhD, Patricia A. Humphrey, MS, and Georgiana F. Aboko-Cole, PhD
Washington, DC

Accumulative evidence in the scientific literature relating malnutrition and susceptibility to infection in human beings and animals has contributed significantly to the understanding of the maladies among the malnourished of the world. In the dispensation of health care systems around the world, little or no emphasis has been placed on trace-element nutrition. This paper adds to the available evidence by using a metabolic imbalance technique to investigate the effects zinc deficiency exert on the course of a parasitic infection. A model composed of zinc-deficient mice and Trypanosoma musculi was used to study changes in serum immunoglobulins.

Zinc, an essential element for humans and animals, has been shown to be important for stabilization or function of numerous metalloenzymes involved in protein synthesis and necessary for the proper operation of the immune system.[1,2] Deficiencies in zinc, related to dietary inadequacies, occur worldwide and are not confined to underdeveloped and developing countries.[3,4]

According to the Council on Food and Nutrition,[5] the official recommended daily dietary allowance for zinc is 15 mg/day for children and adults and 25 mg/day for nursing mothers. However, in some countries, meat and fish diets are replaced by diets high in cereal content, resulting in inadequate amounts of zinc available for absorption. It has been shown in recent years that many communities, even the affluent ones, do not achieve the recommended level of zinc in their dietary intake.[4,6]

Prasad[7] noted that some manifestations of zinc deficiency in humans and animals include retarded growth, anorexia, gastrointestinal malfunction, hypogonadism, and skin diseases characterized by parakeratosis. Miller et al[8] showed that zinc-deficient animals exhibited growth retardation and indicated that this resulted partly from a reduced food intake and partly from an impaired utilization of food. Sandstead and Rinaldi[9] found that in the absence of zinc, the in vivo synthesis of deoxyribonucleic acid (DNA) in the rat liver parenchyma cells was greatly impaired. Eckhert and Hurley[10] demonstrated that DNA synthesis in the central nervous system of rat embryos was markedly reduced after adequate zinc was withheld.

Likewise, Lieberman et al[11] showed that when zinc was not present, activities of DNA polymerase and thymidine kinase were greatly reduced. In these studies, it was theorized that the absence of zinc contributed to the malfunctioning in ribonucleic acid, a mediator in the synthesis of the above enzymes and a participant in DNA synthesis. Frost et al[12] found depression in the weights of the thymus and other lymphatic organs and reduction in peripheral lymphocyte counts when zinc deficiency in mice was investigated. The depression

Requests for reprints should be addressed to Dr. Clarence M. Lee, Associate Dean, College of Liberal Arts, PO Box 470, Administration Building, Howard University, Washington, DC 20059.

TABLE 1. AVERAGE INITIAL BODY WEIGHT OF MICE FED COMPLETE, ZINC-DEFICIENT, AND PAIR-FED DIETS AND INOCULATED WITH TRYPANOSOMA MUSCULI

Dietary Group	Weight (g) ± SD Experiment 1	Experiment 2
Complete diet		
Noninfected mice (n=40)	12 ± 0.4	12 ± 0.3
Infected mice (n=40)	12 ± 0.5	12 ± 0.5
Zinc-deficient		
Noninfected mice (n=40)	12 ± 0.3	12 ± 0.1
Infected mice (n=40)	12 ± 0.1	12 ± 0.3
Pair-fed		
Noninfected mice (n=40)	12 ± 0.5	12 ± 0.4
Infected mice (n=40)	12 ± 0.2	12 ± 0.3

Note: Twenty infected and 20 noninfected mice were used for each dietary group in each experiment

was attributed to an impairment in DNA synthesis.

Other investigations into the biochemical aspects of zinc showed that it is required for the optimum growth of many organisms. Webley[13] reported that a lack of zinc caused marked morphological changes in Nocardia opaca. Similarly, Falchuk et al[14] demonstrated that zinc was necessary for each step in the growth cycle of Euglena gracilis. On the other hand, Ratledge and Winder[15] noted that zinc caused a reduction in the growth rate and yield of Escherichia coli. Numerous studies have also shown that zinc plays a significant role in immune responses.[16-23]

Although there are many reports on the effects of zinc in man and laboratory animals, there are few studies dealing with zinc deficiency and infection. In the present study a zinc-deficient mouse trypanosome system was employed to determine changes in serum immunoglobulin levels of mice infected with Trypanosoma musculi and fed adequate zinc diets, calorically restricted zinc diets (pair-fed), and inadequate zinc diets.

MATERIALS AND METHODS

Experimental Hosts

Two hundred and forty Swiss Webster female albino mice obtained from the National Institute of Health, Bethesda, Maryland were used in two separate experiments. Table 1 shows the distribution number, initial body weights, and dietary groupings that formed the structure of each experiment.

Housing and Feeding of Mice

Mice were housed individually in wire-bottom cages and fed the appropriate diets from metal feeding cups. All mice in the control diet group (complete group) and zinc-deficient diet group (experimental group) were allowed to eat ad libitum. Those in the pair-fed group (caloric-control group) were fed the control diet daily in amounts equal to the food consumed by their zinc-deficient paired mates in twenty-four hours. The average daily food intake of the mice of each cage was determined by subtracting the amount of food remaining in the food cup from the amount given the previous day. All animals had free access to water. Water bottles, feeding cups, and cages were cleaned frequently to minimize algal and bacterial contamination. All diets were purchased commercially from ICN Nutritional Biochemicals, Cleveland, Ohio.

Parasitic Cells

Trypanosoma musculi was employed as the experimental organism. This parasite has been maintained in the laboratory by syringe passage in mice over the last 20 years.

Experimental Infection

Twenty-eight days after initiation of a dietary regimen, one half of the mice in each dietary group

TABLE 2. IMMUNOGLOBULIN LEVELS IN UNINFECTED AND TRYPANOSOMA MUSCULI-INFECTED MICE FED A FULL-COMPLEMENT DIET (mg/dL ± SD)

Day	IgA		IgG_1		IgG_{2a}		IgG_{2b}		IgM	
	I	NI	I	NI	I	NI	I	NI	I	NI
3	38 ± 2	40 ± 4	193 ± 5	202 ± 8	46 ± 2	47 ± 4	101 ± 3	105 ± 6	10 ± 2	9 ± 3
10	39 ± 2	43 ± 2	182 ± 4	187 ± 5	50 ± 4	48 ± 3	89 ± 2	86 ± 5	10 ± 3	10 ± 2
17	42 ± 2	43 ± 3	202 ± 4	199 ± 6	46 ± 6	48 ± 4	95 ± 4	96 ± 4	8 ± 2	9 ± 1
24	47 ± 4	45 ± 2	204 ± 3	203 ± 5	71 ± 3	73 ± 8	91 ± 6	93 ± 3	11 ± 4	13 ± 2
31	46 ± 2	46 ± 2	208 ± 7	206 ± 2	65 ± 7	67 ± 5	98 ± 8	111 ± 3	12 ± 0	10 ± 1**
38	46 ± 3	56 ± 2*	430 ± 12	237 ± 5*	23 ± 3	50 ± 2*	108 ± 3	109 ± 4	59 ± 2	10 ± 3*
45	39 ± 2	62 ± 3*	1951 ± 20	251 ± 8*	26 ± 4	56 ± 3*	158 ± 5	95 ± 5*	95 ± 4	9 ± 3*
59	38 ± 1	61 ± 2*	592 ± 7	234 ± 5*	24 ± 5	50 ± 5*	185 ± 3	104 ± 5*	40 ± 2	10 ± 1*

I = Infected animals
NI = Uninfected animals
*Significance at 1% level
**Significance at 5% level

were injected with 10^3 live T musculi cells. The T musculi cells were freshly isolated from the blood of donor mice. The appropriate experimental mice were injected with 0.5 mL of pure suspension of trypanosomes in physiological saline by intraperitoneal inoculation. Pure suspensions of trypanosome cells were prepared by the method of Lincicome and Watkins.[24] The number of trypanosomes per unit volume of suspension was estimated with red blood cell pipet hemacytometer, Toison's fluid, and a constant dilution factor of 1:200.

Immunoglobulin Assay

The serum immunoglobulin levels of IgG_1, IgG_{2a}, IgG_{2b}, IgA, and IgM were measured and compared in mice fed full-complement, zinc-deficient, and pair-fed diets. Beginning on day three and every seventh day thereafter, up to day 59, blood was collected by aseptic heart puncture and serum was extracted after the blood had cooled to room temperature. Serum samples were put in separate labelled vials. These were sealed, frozen in dry ice alcohol, and stored at 20°C. Radioimmunodiffusion assay was performed according to the techniques of Mancini et al[25] and Fahey and McKelvey.[26] Serial dilutions of mouse immunoglobulin standard (Meloy Laboratories, Springfield, Virginia) were used to quantitate each serum immunoglobulin level. Using magnifying comparator with reticle, the diameters of the precipitin rings of standards and unknown serum samples were measured and compared. The Digital pdp11 Computer with Digital Decprinter 1 and the Regression Analysis Program Curve were employed to compute and tabulate the concentrations of the immunoglobulins found in the unknown serum samples.

Statistical Evaluation

The data were studied as averages calculated from two experiments. Statistical treatment of this data followed reported techniques of standard deviations and application of Student's *t* test for significance of differences.[27,28] Statistical significance was assigned to all differences of means having a probability scale of 1 percent or less.

Immunoglobulin Levels

Serum immunoglobulin levels of IgG_1, IgG_{2a}, IgG_{2b}, IgA, and IgM of mice fed a full-complement diet and infected with T musculi are shown in Table 2. The infected animals fed the complete diet had higher IgG_1 levels over the controls. At day 45, there was about an eightfold increase in IgG_1 levels over the noninfected mice fed the same diet. Statistically significant differences between the two groups were observed from day 38 and persisted until day 59. In terms of percentages of control values, the greatest IgG_1 level occurred in the infected animals on day 45. With the IgG_{2a} levels, measurable decreases occurred between days 38 and 59. The infected, full-complement diet

TABLE 3. IMMUNOGLOBULIN LEVELS IN UNINFECTED AND TRYPANOSOMA MUSCULI-INFECTED MICE FED A ZINC-DEFICIENT DIET (mg/dL ± SD)

Day	IgA		IgG_1		IgG_{2a}		IgG_{2b}		IgM	
	I	NI	I	NI	I	NI	I	NI	I	NI
3	42 ± 2	45 ± 4	184 ± 3	188 ± 4	46 ± 1	48 ± 2	62 ± 4	64 ± 5	10 ± 1	11 ± 2
10	43 ± 5	48 ± 2	180 ± 3	180 ± 6	46 ± 2	48 ± 3	63 ± 2	62 ± 2	8 ± 2	10 ± 1
17	43 ± 2	46 ± 2	182 ± 4	183 ± 2	69 ± 7	75 ± 3	64 ± 3	63 ± 1	10 ± 2	12 ± 1
24	41 ± 6	44 ± 3	186 ± 4	181 ± 5	50 ± 2	45 ± 4	64 ± 3	63 ± 1	9 ± 1	11 ± 3
31	42 ± 2	41 ± 3	206 ± 6	182 ± 4*	45 ± 5	46 ± 4	63 ± 3	62 ± 4	12 ± 1	8 ± 0*
38	46 ± 5	48 ± 2	366 ± 7	272 ± 8*	15 ± 2	81 ± 3*	166 ± 1	105 ± 2*	21 ± 2	12 ± 1*
45	45 ± 1	51 ± 1*	1291 ± 18	188 ± 5*	16 ± 2	36 ± 5*	389 ± 16	66 ± 3*	44 ± 5	10 ± 2*
59	43 ± 2	49 ± 1*	510 ± 12	186 ± 6*	16 ± 2	39 ± 4*	258 ± 7	62 ± 2*	19 ± 2	10 ± 1*

I = Infected animals
NI = Uninfected animals
*Significance at 1% level

group exhibited maximum significant decreased levels at day 45. On the average, the IgG_{2b} levels in serum of T musculi infected mice was higher than that experienced by the noninfected controls. Overall differences in IgG_{2b} serum immunoglobulin levels became statistically significant by day 45 when a maximum of approximately 66 percent was achieved.

The average IgA levels in adequately fed animals showed lower values for the trypanosome-infected mice over the noninfected controls fed the same diet. Statistically significant decreases between the two groups occurred between days 38 and 59. For the IgM serum immunoglobulin levels, the values for the trypanosome-infected group were higher than those observed for the noninfected group fed the complete diet. Significant differences were observed between days 31 and 59. The increase was progressive, reaching a tenfold maximum by day 45. At the end of the experiment, the levels of IgM for the infected animals were still four times that of the noninfected animals fed the complete diet.

Table 3 contains serum immunoglobulin levels of mice fed the zinc-deficient diet and infected with T musculi. The zinc-deficient infected mice showed higher IgG_1 levels than the noninfected mice fed the same diet. On a percentage basis in relation to controls, an approximate 600 percent increase was observed on day 45 of the experimental animals. Statistically significant differences occurred between days 31 and 59. Throughout the period of observation, IgG_{2a} levels in infected mice exhibited decreases of 0.78 mg/dL to 66 mg/dL over the control group. However, statistically significant decreases were seen between days 38 and 59. IgG_{2b} values for the trypanosome-infected zinc-deficient mice showed progressive increases which became statistically significant between days 38 and 59. At the height of the increase (day 45), a fivefold higher value was recorded for infected over noninfected animals. The highest percentage increase reached 491 percent. In comparison to noninfected zinc-deficient animals, the IgA values for infected mice were lower except on day 31. Statistically significant decreases occurred between days 45 and 59. Trypanosoma musculi-infected mice exhibited higher IgM values than that of the noninfected group fed the same zinc-deficient diet. As in the complete-diet animals, differences in the IgM immunoglobulin levels between the two groups became statistically significant by day 31 and continued until day 59. Concentration differences of IgM ranged between 1.2 mg/dL and 33.65 mg/dL with the maximum percentage increase of 338 percent for the infected over noninfected zinc-deficient being reached by day 45.

Table 4 contains serum immunoglobulin levels of mice fed a pair-fed diet and inoculated with T musculi. In comparison to noninfected animals, infected animals in this dietary group showed significant higher IgG_1 values from day 31 until the end of the experimental period. The greatest IgG_1 difference occurred at day 45 where a value seven times greater was seen for the infected pair-fed hosts over their noninfected counterparts. Overall IgG_{2a} values were statistically significant

TABLE 4. IMMUNOGLOBULIN LEVELS IN UNINFECTED AND TRYPANOSOMA MUSCULI-INFECTED MICE FED A PAIR-FED DIET (mg/dL ± SD)

Day	IgA		IgG_1		IgG_{2a}		IgG_{2b}		IgM	
	I	NI	I	NI	I	NI	I	NI	I	NI
3	40 ± 4	38 ± 5	193 ± 5	201 ± 9	44 ± 4	47 ± 3	92 ± 5	90 ± 3	10 ± 2	11 ± 4
10	45 ± 2	39 ± 2	182 ± 4	180 ± 3	50 ± 5	49 ± 3	83 ± 2	82 ± 6	9 ± 2	9 ± 1
17	40 ± 3	37 ± 3	188 ± 6	189 ± 5	47 ± 3	48 ± 3	95 ± 3	93 ± 3	10 ± 1	12 ± 3
24	39 ± 2	41 ± 1	182 ± 6	184 ± 5	50 ± 5	50 ± 2	97 ± 2	94 ± 5	9 ± 3	8 ± 3
31	62 ± 6	37 ± 2*	240 ± 5	209 ± 5*	46 ± 1	50 ± 1*	75 ± 4	73 ± 6	9 ± 2	8 ± 4
38	46 ± 2	35 ± 2*	358 ± 4	242 ± 6*	23 ± 2	50 ± 5*	120 ± 3	109 ± 1*	41 ± 7	11 ± 2*
45	38 ± 4	55 ± 2*	1352 ± 10	242 ± 7*	21 ± 2	49 ± 2*	163 ± 8	71 ± 2*	76 ± 5	10 ± 1*
59	38 ± 2	54 ± 3*	534 ± 12	236 ± 6*	23 ± 3	50 ± 2*	128 ± 3	73 ± 2*	37 ± 1	13 ± 1*

I = Infected animals
NI = Uninfected animals
*Significance at 1% level

between days 31 and 59. Decreases of between 1.4 and 58 percent were observed for the pair-fed infected over the uninfected group. In the pair-fed group, there were no differences in the IgG_{2b} serum levels between days 3 and 31 for the infected over the noninfected animals. However, from day 38 to day 59, statistically significant increases in the level of IgG_{2b} were observed for the infected pair-fed group when compared to the uninfected group. After the T musculi inoculation, percentage increases in IgG_{2b} levels ranged from approximately 3 to 129 percent.

With the IgA levels, statistically significant increases occurred between days 31 and 38 while significant decreases were recorded from day 45, until the final day of the observational period for the trypanosome-infected pair-fed mice over the uninfected controls. Compared to noninfected animals, infected pair-fed animals showed statistically significant IgM increases from day 38 until day 59. A peak level increase of about 695 percent was achieved on day 45. The level increases of IgM for the infected pair-fed animals over the noninfected mice ranged from 1.1 mg/dL to 66.80 mg/dL.

In all three dietary groups, higher serum levels of IgG_1, IgG_{2b}, and IgM and lower levels of IgG_{2a} and IgA were seen for the infected over the uninfected animals. IgM showed progressive increments reaching a maximum of approximately 886, 338, and 488 percent for infected complete, zinc-deficient and pair-fed groups respectively over the noninfected counterparts.

It is noted that in the infected zinc-deficient animals, the increase in the IgM was only half as much as that of the complete infected group, and the increase in the IgG_{2b} level was two times greater over the infected complete-diet mice. This suggests that in addition to trypanosomiasis, zinc deficiency does play an important role in enhancement and/or suppression of the host's immunological response.

In slight contrast to the present study, Navalkar et al[29] noted higher levels of IgA, IgG_1, IgG_2 and IgM_1 in mice infected with Mycobacterium leprae and Mycobacterium lepraemurium. IgG_1 and IgA appeared to increase as the infection progressed. IgG_2 and IgM rose and then remained constant for the remainder of the testing period.

Luckins[30] reported increases in IgM and IgG in Zebu cattle infected with Trypanosoma congolense and Trypanosoma vivax. Cunningham and Vickerman[31] and Cross[32] showed that the antigenic variation expressed through the changes in the structure of glycoproteins present on the surface coat of the trypanosome contributed to the immunological response of the host. Welch[33] believed that the increased immunoglobulin levels that occurred during trypanosomal infection was due to the ability of the host to respond to the multiple antigenic variants with specificity.

Along the same line, Mattern et al[34] and Lumsden et al[35] reported elevations in IgM levels in humans suffering from African sleeping sickness. It was felt by the authors that the increased levels of immunoglobulins could result from B-cell mitogen produced by the trypanosomes themselves or by the induction of IgG production of helper T cells acting nonspecifically.[33]

In the present study the increased levels of IgG and IgM correlate with the findings of Viens et al[36] and give additional supporting evidence that ablastin, the thymus-dependent antibody responsible for the elimination of dividing parasites and the establishment of a stable level of infection, could be an IgG; and the trypanocidal antibody, which terminates the infection by removal of the adult parasites, could be an IgM. Thus, regardless of the dietary status of the host, during trypanosomal infection, the activities of ablastin and the trypanocidal antibody become relative to the immunoglobulin levels in the intricate mechanisms of the immune systems.

Literature Cited

1. Kirchgessner M, Schwarz FJ, Roth HP, et al. Interactions between the trace elements zinc, copper and iron after zinc depletion in dairy cows. Arch Tierernaehr 1978; 28:723-733.
2. Fraker PJ, Haas SM, Luecke RW. Effect of zinc deficiency on the immune response of the young adult A/J mouse. J Nutr 1977; 107:1889-1895.
3. Sanstead HH. Zinc nutrition in the United States. Am J Clin Nutr 1973; 26:1251-1260.
4. Underwood EJ. Trace metals in human and animal health. J Nutr 1981; 35:37-48.
5. Council on Food and Nutrition Board: Recommended Dietary Allowances. Washington DC: National Academy of Science/National Research Council, 1974, pp 1-20.
6. Prasad AS, Oberleas D. Zinc: Human nutrition and metabolic effects. Ann Int Med 1970; 73:631-636.
7. Prasad AS. Deficiency of zinc in man and its toxicity. In: Prasad AS, ed. Trace Elements in Human Health and Disease. New York, Academic Press, 1976, pp 1-20.
8. Miller ER, Luecke RW, Ullrey DE, et al. Biochemical, skeletal and allometric changes due to zinc deficiency in the baby pig. J Nutr 1968; 95:278-286.
9. Sandstead HH, Rinaldi RA. Impairment of deoxyribonucleic acid synthesis by dietary zinc deficiency in the rat. J Cell Physiol 1969; 73:81-83.
10. Eckhert CD, Hurley LS. Reduced DNA synthesis in zinc deficiency: regional differences in embryonic rats. J Nutr 1977; 107:855-861.
11. Lieberman L, Abrams R, Hunt N. Levels of enzyme activity and DNA synthesis in mammalian cells cultured from animals. J Biol Chem 1963; 238:3955-3966.
12. Frost P, Chen JC, Rabban I, et al. The effect of zinc deficiency on the immune response in zinc metabolism. In: Brown GJ, Prasad AS, eds. Current Aspects in Health and Disease. New York, Alton R. Liss Company, 1977, pp 143-150.
13. Webley DM. The effect of deficiency of iron zinc and manganese on the growth and morphology of Nocardia opaca. J Gen Microbiol 1960; 23:87-92.
14. Falchuk KH, Fawcett DW, Vallee BL. Role of zinc in cell division of Euglena gracilis. J Cell Sci 1975; 17:57-58.
15. Ratledge C, Winder FG. Effect of iron and zinc on growth patterns of Escherichia coli in an iron-deficient medium. J Bacteriol 1964; 87:823-827.
16. Haas S, Fraker P, Luecke R. The effect of zinc deficiency on the immune response of A/J mice. Fed Proc 1976; 35:659.
17. Fraker PJ, DePasquale-Jardiew P, Zwickl CM, et al. Regeneration of T-cell helper function in zinc-deficient adult mice. Proc Nat Acad Sci USA 1978; 75:5660-5664.
18. Ferrante A, Jenkin CR, Reade PC. Changes in the activity of the reticuloendothelial system of rats during an infection with Trypanosoma lewisi. Austr J Exp Biol Med Sci 1978; 56:47-59.
19. Fernandes G, Nair M, Onoe K, et al. Impairment of cell-mediated immunity functions by dietary zinc deficiency in mice. Proc Nat Acad Sci USA 1972; 76:457-461.
20. Luecke RW, Simond CE, Fraker PJ. The effect of restricted dietary intake on the antibody medicated response of the zinc deficient A/J mouse. J Nutr 1978; 108: 881-887.
21. Zwickl CM, Fraker PJ. Restoration of the antibody mediated response of zinc/caloric deficient neonatal mice. Immunol Commun 1980; 9:611-626.
22. Frost P, Rabbani I, Smith J, et al. Cell mediated cytotoxicity and tumor growth in zinc deficient mice. Proc Soc Exp Biol Med 1981; 167:333-337.
23. Mehrmofakham S, Treagan L. Antibody suppression by zinc and nickel. Biol Trace Element Res 1981; 3:7-11.
24. Lincicome DR, Watkins RC. Method for preparing pure cell suspensions of Trypanosoma lewisi. Am Inst Biol Sci Bull 1963; 13:53-54.
25. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965; 2:235-254.
26. Fahey JL, McKelvey EM. Quantitative determination of serum immunoglobulins in antibody-agar plates. J Immunol 1965; 94:84-90.
27. Snedecor GW. Statistical methods. Ames, Iowa, Iowa State College Press, 1959.
28. Sokal RR, Rohlf FJ. Biometry: The Principles and Practice of Statistics in Biological Research. San Francisco, Freeman, 1969.
29. Navalkar RG, Patel PJ, Dalvi RS, et al. Electrophoretic patterns of serum proteins and immunoglobulin levels in Mycobacterial infections: Studies in mice infected with Mycobacterium leprae and Mycobacterium lepraemurium. J Natl Med Assoc 1976; 68:500-505.
30. Luckins AG. Immunoglobulin response in Zebu cattle infected with Trypanosoma congolense and Trypanosoma vivax. Trans R Soc Trop Med Hyg 1974; 68: 144-149.
31. Cunningham MP, Vickerman K. Antigenic analysis in the Trypanosoma brucei group, using the agglutination reaction. Trans R Soc Trop Med Hyg 1962; 56:48-59.
32. Cross GAM. Identification, purification and properties of variant-specific glycoprotein antigens constituting the surface coat of Trypanosoma brucei. Parasitology 1975; 71:393-417.
33. Welch TM. Parasitic Diseases. In: Fudenberg HH, Stites DP, Caldwell JL, et al, eds. Basic Clinical Immunology. Los Altos, Lange Medical Publications, 1971, pp 44-58.
34. Mattern P, Masseyeff R, Michel R, et al. Etude immunochimique de le B_2-macroglobuline des serums de malades atteints de trypanosomiasi africaine a T. gambiense. Ann De L'institut Pasteur 1961; 101:382-388.
35. Lumsden WHR, Herbert WJ, McNeillage GJC. Nomenclature of antigenic types of trypanosomes. Vet Rec 1967; 81:237-238.
36. Viens P, Targett GAT, Leuchers E, et al. The immunological response of CBA mice to Trypanosoma musculi. Initial control of the infection and the effect of T-cell deprivation. Clin Exp Immunol 1974; 16:279-294.