ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

IVol. 47 N° 2, 1997

Serum level of Zn, Cu and Fe in healthy schoolchildren residing in Mérida, Venezuela

Oscar M Alarcón [1], José Reinosa Fuller [2], Tania M. Silva [1], Coromoto Angarita [1], Elfida Terán [1], Maritza Navas [3], Pedro Solano [1], and Milena Agostinelli [1]

SUMMARY. Levels of Zn, Cu and Fe were measured in blood serum samples of 320 children: 160 boys and 160 girls randomly selected, ages between 7 and 14 years, all considered healthy and residing in the City of Merida, Venezuela. The metals were determined using flow injection analysis-flame atomic absorption spectrometry. There was a tendency for serum Zn (SZn) to increase with age. There was no significant difference in SZn levels between males and females in the different age groups. Serum copper (SCu) decreases significantly ($p<0.05$) with age in male children, whereas it increases in female children. The concentration of serum iron (SFe) tends to be lower than that reported in the literature. However, the age groups studied showed no statistically significant sex and age-related differences. The results are compared with values previously reported for healthy children studied in other communities. The present study has shown that there is a complex interaction between SZn, SCu, SFe and age and sex of the children. On the other hand, our observations also suggest that more detailed studies of these metals should be done, and that the study should include metabolic balances and associations between SZn, SCu, SFe and anthropometric variables (height, weight, body mass index and skinfold thickness).
Keywords: serum zinc, serum copper, serum iron, schoolchildren.

RESUMEN. Niveles séricos de Zn, Cu y Fe en escolares sanos residentes en Mérida, Venezuela.. En la presente investigación se determinaron los niveles séricos de Zn, Cu y Fe en 320 escolares sanos, entre 7 y 14 años, seleccionados al azar. Los metales se determinaron mediante una técnica combinada de inyección en flujo contínuo-espectrometría de absorción atómica. Los resultados muestran que el Zn sérico (SZn) se incrementa con la edad mientras que el sexo no influye significativamente en la cincemia, al comparar niños y niñas en los diferentes grupos de edad. El Cu sérico (SCu) disminuye ($p<0.05$) con la edad en los niños, mientras que se incrementa ($p<0.05$) en las niñas. La concentración del Fe sérico (SFe) es menor que la señalada en la literatura. Sin embargo, en los grupos estudiados no se demostraron diferencias estadísticamente significativas en relación con la edad o con el sexo de los escolares. Los resultados se comparan con los valores previamente publicados para escolares sanos estudiados en otras comunidades. El presente estudio demuestra que existe una compleja interrelación entre SZn, SCu, SFe y la edad y el sexo de los escolares. Nuestras observaciones también sugieren que se deben hacer estudios más detallados, los cuales deben incluir balances metabólicos y asociaciones entre SZn, SCu, SFe y variables antropométricas (talla, peso, índice de masa corporal y pliegue cutáneo).
Palabras claves: zinc sérico, cobre sérico, hierro sérico, escolares.

INTRODUCTION

It is now recognized that zinc, iron and copper are essential trace nutrients involved in normal growth and development. Zinc has been shown to be an integral constituent and cofactor of more than one hundred metalloenzymes that play an important role in DNA, RNA, and protein synthesis. Copper is an important factor for several enzyme systems and is involved in the mobilization and release of stored iron from liver, formation of myelin and bone, and maintenance of elastin in the great blood vessels (1). Iron plays an important function in energy metabolism, in oxygen transport and storage; it participates in a variety of enzyme activities (1) and is needed for bactericidal activity in cell mediated host defense (2). These trace elements have been associated with normal lymphocyte maturation and regulation of the immune function (3).

Much interest has been centered on plasma and serum levels of these elements in health and disease. There are numerous reports related to the element concentration in adult blood sera (4-6). However, reports on the simoultaneous determination of serum Zn (SZn), serum Cu (ZCu) and serum Fe (SFe) concentration in school age children are scarce (7,8). Consequently, the aim of this work is to obtain information about the «reference values» of zinc, iron and copper in blood serum of 320 schoolchildren without overt disease in an age group of 7-14 years, residing in the City of Mérida, Venezuela. The influence of age and sex on the serum value of these trace elements was evaluated.

MATERIAL AND METHODS

Subjects: The sample size was determined by the stratified sampling method. 320 children were studied: 160 boys (50%) and 160 girls (50%) randomly selected, ages between 7 and 14 years, all considered healthy, according to the following criteria: a) adequate height and weight corresponding to chronological age; b) absence of any acute or chronic pathology clinically evident at the momento of examination and c) normal blood chemistry, hematology, faeces and urine analysis. Information regarding diet, exercise, nutritional supplements and familiy medical history were obtained by questionnaire.

The children were stratified balanced by age and gender into one-year groups from 7 to 14 yr with 20 boys and 20 girls in each group.

Blood preparation and analysis: The blood samples (10 mL)

1 Escuela de Nutrición y Dietética. Laboratorio de Bioquímica y Nutrición. Facultad de Medicina, Universidad de Los Andes.
2 Dpto. de Bioquímica, Facultad de Farmacia, Universidad de Los Andes, Mérida.
3 Dpto. de Puericultura y Pediatría. Hospital Universitario de Los Andes, Mérida.

were obtained between 8:00 and 10:00 am and were drawn from an antecubital vein using vacutainer tubes. Blood was transferred into an acid-washed polystyrene tube (with a polystyrene stopper), allowed to clot in 30 min and centrifuged at approximately 1000 g for 10 min in the same tube. The serum was then separated, transferred to an acid-washed sample polystyrene tube and stored at -20 °C until analyzed. Hemolyzed samples were discarded. In each case, special care was also taken regarding the quality of sampling and any possible contamination during the sample preparation. Aliquots of the sera were analyzed within one month.

Instrumentation: The flow injection analysis (FIA)-atomic absorption spectrophotometric technique (AAS) was used for the determination of Zn, Cu (9) and Fe (10). The FIA+AAS system used in this work is the same as that described by Burguera et al. (11).

Reagents: The chemicals were all analytical grade. The water was de-ionised and then distilled twice. Ammonium iron (II) sulphate, copper nitrate and zinc nitrate (BDH) were used to prepare stock solutions. Working solutions were freshly prepared daily. All standars were prepared in 20% (v/v) glycerol containing physiological amounts of sodium, phosphate, chloride, sulphate and human albumin. All glassware, pipettes, syringes and sample tubes were cleaned with 20% (v/v) nitric acid and throughly rinsed with metal free-water before use.

Statistical analysis: The mean and standard deviation were calculated, and the results were expressed as mean±standard deviation. One-way analysis of variance (ANOVA) and Tukey test were used to analyze vertically along the columns of Tables 1-3, at a significance level of 0.05, which permited to establish the significant difference between the various average values. Student «t» test was used for comparisons horizontally across rows of Tables 1-3. Relationships between variables (age, sex, SZn, SCu and SFe) were assessed by using backward stepwise-regression analyses.

TABLE 1
Serum Zn level[1] in 320 healthy schoolchildren residing in Mérida, Venezuela

Age (years)	M+F	M	F	p2
7	67 ± 14 (47-104)	68 ± 18	66 ± 12	NS
8	68 ± 10 (54-90)	69 ± 14	67 ± 14	NS
9	75 ± 15 (60-110)	76 ± 22	73 ± 13	NS
10	77 ± 15 (60-114)	78 ± 27	75 ± 15	NS
11	79 ± 19 (60-109)	80 ± 12*	78 ± 25	NS
12	82 ± 20* (60-108)	84 ± 11*	79 ± 15*	NS
13	83 ± 20* (60-117)	86 ± 18*	80 ± 13*	NS
14	89 ± 13* (60-117)	89 ± 15*	89 ± 16*	NS
7-14	78 ± 14 (47-117)	79 ± 17 (58-117)	76 ± 13 (47-117)	

1 µg/dL (mean ± standar deviation). M+F= male+female. M=male. F=Female.
* statistically significant difference p<0.05 on comparing with 7 yr.
N.S. Non statistically significant on comparing males and females on the same age group.
Concentration ranges are in parentheses.

TABLE 2
Serum Cu level[1] in 320 healthy schoolchildren from Mérida, Venezuela

Age (years)	M+F	M	F	p2
7	123 ± 21 (99-178)	129 ± 15	116 ± 15	<0.02
8	121 ± 22 (91-159)	126 ± 16	117 ± 17	<0.05
9	122 ± 19 (80-155)	123 ± 32	120 ± 25	NS
10	121 ± 13 (75-139)	119 ± 19	122 ± 16	NS
11	120 ± 16 (95-149)	117 ± 16*	122 ± 18	NS
12	119 ± 18 (90-153)	114 ± 19*	124 ± 25	<0.05
13	118 ± 19 90-158	112 ± 16*	124 ± 12	<0.05
14	117 ± 13 (90-140)	109 ± 13*	125 ± 17*	<0.05
7-14	120 ± 13 (75-118)	119 ± 17 (75-159)	121 ± 19 (80-178)	

1 µg/dL (mean ± standar deviation). M=Males. F=Female.
* p<0.05 on comparing with 7 yr.
2p<0.05 on comparing males vs. females on the same age group
N.S. = No significative
Concentration ranges are in parentheses.

TABLE 3
Serum Fe level[1] in 320 healthy schoolchildren from Mérida, Venezuela

Age (years)	M+F	M	F	p2
7	66 ± 19 (38-114)	69 ± 11 (38-120)	62 ± 24 (55-134)	NS
8	71 ± 19 (42-118)	74 ± 33 43-114)	67 ± 22 (42-138)	NS
9	70 ± 23 (50-138)	70 ± 18 (50-138)	70 ± 13 (50-137)	NS
10	71 ± 21 (55-133)	71 ± 24 (50-128)	71 ± 14 (55-133)	NS
11	72 ± 19 (48-134)	73 ± 14 (58-132)	71 ± 19 (48-134)	NS
12	72 ± 18 (67-140)	73 ± 22 (67-140)	71 ± 19 (57-131)	NS
13	72 ± 19 (68-125)	73 ± 15 (64-125)	70 ± 13 (68-125)	NS
14	73 ± 13 (51-142)	75 ± 13 (60-117)	70 ± 13 (51-142)	NS
	71 ± 19 (38-142)	72 ± 20 (38-140)	69 ± 19 (42-142)	

1 µg/dL (mean ± standar deviation). M+F= Male+female. M=male. F=Female.
2 N.S. Non statistically significant on comparing males and females on the same age group.
Concentration ranges are in parentheses.

RESULTS AND DISCUSSION

Table 1 shows the serum zinc concentration and extreme values of Zn for the different age groups, also separate data are given for males and females. The mean concentration of SZn was 78±14 µg/dL (males, 79±17 µg/dL; females, 76±13 µg/dL) with a range of 47-117 µg. These values are in close agreement with those reported by Butrimowitz and Purdy (12), Chuwa et al (13) and Udomkesmalee et al (14) who studied 283 rural schoolchildren aged 7-13 in Norotheast Thailand, but they are lower than those reported by Ontake and Tamura (15), Delves et al (7), Bekarogly et al (16), Buxaderas and Farré-Rovira (17), Kozielec et al (18), Fons et al (19) and Varadvithya et al (200 for well-nourished Thai children aged 7-13 years (88±4 µg/dL). On the other hand, van Wouve and Waser (21) reported control values for SZn of 67 µg/dL in healthy Dutch children.

The gradually increase in zinc level, observed here with age, is in accordance with observations previously reported by Buxaderas and Farré-Rovira (17) and Butrimovitz and Purdy (12), who found an increase in the zinc plasma levels at approximately 6 to 8 years of age, but conflicting with the previous reports of Ohtakee and Tamura (15).

There was no significant difference in serum zinc level between males and females in the different groups; this observation is in agreement with reports of Ontake and Tamura (15), Carpentieri et al (8), and Malvy et al (22). However, healthy Dutch boys over 9 yr of age showed higher SZn compared with girls of the same age ($p<0.08$) (21). In the group of 10-19 yr age, Buxaderas and Ferré-Rovira (18) found definetely higher serum and whole blood zinc level in females than in males, although the difference was only statistically significant in whole blood. Brun et al (23) measured serum zinc in 20 adolescent gymnasts (9 boys, 11 girls, age 12-15 yr), exploring for detection of possible adverse effects of intense training on pubertal maturation and growth and found that girls had lower SZn than boys.

The mean concentration of SCu was 120±13 µg/dL (Table 2). These values show a close agreement with those reported by Carpentieri et al (8), Delves et al (7), and Ontake and Tamura (15) in healthy Japanese children. Tessmer et al (24) reported mean values of SCu in children (6-12 years old) of 133 µg/dL for both Caucassians and Mexican-Americans, and 141 µg/dL for Negroes. Ogihara et al (25) in 19 age-matched controls (7 boys and 12 girls; mean age 12.3 yr) found a mean concentration of SCu of 107 µg/dL. Other reports in the same age group show slightly higher values for the mean concentration of SCu than those of our study (26,27).

The changes in SCu levels during school age (7-14 yr) are indicated in Table 2. Statistically significant differences ($p<0.05$) were found in the values in the 12-14 yr group between males and females. The mean concentration of SCu in younger male age group (7 yr) was higher than those in older age groups (12-14 yr), whereas SCu levels increases in girls with age.

Sass-Kortsak (26) demonstrated a highly significant ($p<0.005$) negative correlation between SCu levels and increase in age with mean values of 140, 129 and 117 µg/dL for 2, 6- and 10 year-old children, respectively. The same author (26) showed that serum copper levels decreases gradually during childhood and reaches adult levels at 13 to 16 yr of age. Hrgovcic et al (27) observed an essentially linear relation between serum copper and age between 2 and 18 yr, decreasing to adult levels at about 20 yr of age. Tessmer et al (24) also found a significant linear decrease in SCu concentration with increasing age during childhood (6-12 yr).

The concentration of SFe found in this study (Table 3) tend to be lower than that reported in the literature for normal adolescents (28) and from 4 days to 15 years old children (29), but are in some agreement with those reported by Koerper and Dalhman (30) for children aged 0.5 to 12 years. Our average value is smaller than the majority of values reported in the literature; these observations could be related to dietetic reasons. The values for SFe may partly reflect the marginal iron reserves that are characteristics of childhood. There is no basis, however, for considering these values abnormal. It is of interest to mention the nutritional iron deficiency frequently detected both in developed as in under developed countries (31). In spite of what is actually known in the area of infant nutrition, iron deficiency and its significant consequence, the hypochromic microcytic anemia continues to be a very serious pediatric problem (32).

The mean and range iron concentration reported in this study tend to increase with age. It is well known that during childhood the SFe values undergo a slow variation up to the value considered normal in the adult population. The age groups studied showed no statistically significant sex- and age-related difference, as previously reported by Carpentieri et al (8), Koerper and Dallamn (30), Card et al (33), and Dahlstron et al (34), González-Silva et al (35). The detection of iron deficiency in children is important due to the influence of this precursor in the physical and intellectual development.

Factors influencing serum metal concentration were explored in more detail by using backward stepwise-multiple regression analysis (Table 4). There was not statistically significant positive simple linear correlation between SZn-SCu ($r=0.037$), SZn-SFe ($r=0.094$) and SCu-SFe ($r=0{,}089$), SFe-age ($r=0.181$), SZn-sex ($r=0.091$) and SFe-sex ($r=0.068$) whereas between SCu-age ($r=0.034$) and SCu-sex ($r=0.071$) there was no a significant negative correlation. When SZn plus age, sex, SCu and SFe were included in a backward stepwise-regression analysis, and a $P<0.05$ used for exclusion, SFe dropped out the equation. For those variables left in the equation age, sex and SCu were strongly related to SZn. The overall F ratio was $F_{(4,316)}=3363.49$ ($p<0.00$); the adjusted R^2 was 0.9768, suggesting that variables in the equation accounted for 98% of the variance in SZn. For every unit change in SCu there was a 0.31 unit change in serum zinc, while all other factors in the equation were taken into account. A similar analysis was made for serum iron. When this variable plus age, sex, SZn and SCu were included in a backward stepwise-regression analysis, age and serum copper were the only variables that remained in the equation. The overall F ratio was $F_{(4,316)}=3527.49$ ($P<0.05$); the adjusted R^2 was 0.9781. In this case, the variables in the equation accounted for 98% of the variance in SFe. For every unit change in SCu there was a 0.45 unit change in SFe and for every unit change in age there was a 1.13 unit change in SFe. This analyses suggests that there is a statistical effect of age and SCu on SFe.

When SCu, sex, age, SZn and SFe were included in a backward stepwise-regression analysis, sex and age dropped out of the equation; SZn and SFe were the only variables that remained in the equation. The overall F ratio was $F_{(4,316)}=4494.32$ ($P<0.05$) and the adjusted R^2 was 0.981. Thus, the variables in the equation accounted for 98% of the variance in SCu. For every unit change in SCu there was a 0.56 unit and 0.99 unit change in SZn and SFe, respectively.

These findings are in close agreement with the previous works of Delves et al (7) and Laitinen et al (36). However, additional studies are needed for the precise mechanism of these interrelations to be clarified. The human and animal studies have demonstrated clearly that trace metals may interact at every stage during absorption, storage, transport, and metabolism. Besides, it is also probable that variations in serum concentration of these trace metals with age and with sex might be related to physiological adaptations along this growth period and to nutritonal changes.

TABLE 4
Backward stepwise regression analysis for serum zinc, serum copper, and serum iron in healthy schoolchildren.

Variables in the equation	ß	*standard error*	*«t» value*	*Significance* level[1]
Serum zinc				
Age	3.27	0.28	11.80	<0.05
Sex	3.22	1.33	2.42	<0.05
SCu	0.31	0.02	13.78	<0.05
SFe	0.23	0.01	1.78	ns
Serum copper				
Sex	-0.98	1.82	-0.54	ns
Age	0.56	0.46	1.29	ns
SZn	0.56	0.07	8.10	<0.05
SFe	0.99	0.06	15.81	<0.05
Serum iron				
Age	1.13	0.30	3.78	<0.05
Sex	2.30	1.21	1.89	ns
SZn	0.06	0.05	1.13	ns
SCu	0.45	0.03	15.89	<0.05

SFe= serum zinc. SCu=serum copper. SFe= serum iron
ns= statistically not significant
n= 320

In summary, the serum concentration of Zn, Cu and Fe detected in our schoolchildren, with certain differences, are very similar to those reported in other countries. The present study has shown that there is a complex interaction between SZn, SCu, and SFe and age and sex of the children. The results reinforced the importance of being able to identify the detailed biological interrelations of the factors involved (age, sex and serum metal concentration). On the other hand, our observations suggest that more detailed studies of these metals should be done, and that the study should include metabolic balances and associations between serum zinc, serum copper, serum iron and anthropometric variables (height, weight, body mass index and skinfold thickness).

REFERENCES

1. Aggett PJ. Physiology and metabolism of essential trace elements.: An outline. Clin Endocrinol Metabol 14:513-543, 1985.
2. Cousins RJ. Nutritional Regulation of Host Defense Systems: Emphasis on trace minerals. In: Mineral Homeostasis in the Elderly. Alan R. Lis, Inc.pp207-222. 1989.
3. Ronnlund DR., Suskind RM. Iron, zinc, and other trace elements' effect on the immune response. J. Pediat Gastroenterol Nutr. 2:5172-5180. 1983.
4. Burguera JL., Burguera M., Alarcón OM. Levels of some elements in human blood serum of residents of Merida, Venezuela. Trace Elem Med 3:117-120. 1986.
5. Versieck J., Cornelis F. Normal levels of trace elements in human blood plasma or serum. Anal Chim Acta 116:217-254. 1980.
6. Iyengar V., Woittlez I. Trace elements in human clinical specimens: Evaluations of literature data to identify reference values. Clin Chem 34:474-481. 1988.
7. Delves HT., Clayton BE., Bicknell J. Concentration of trace metals in the blood of children. Br J Prevent Soc Med 27:100-107, 1973.
8. Carpentieri V., Myers J., Thorpe L., Daeschner III CW. Haggard ME. Cooper, zinc and iron in normal and leukemic lymphocytes from children. Cancer Res 46:981-984, 1986.
9. Rocks BF., Sherwood RA., Bayford LM., Riley C. Zinc and copper determination of microsamples os serum by flow injection and atomic absorption spectroscopy. Ann Clin Biochem 19:338-344, 1982.
10. Rocks BF., Sherwood RA., Turner ZJ., Riley C. Serum iron and total iron-binding capacity determination by flow-injection analysis with atomic absorption detection. Ann Clin Biochem 20:72-76, 1983.
11. Burguera M., Burguera JL., Garaboto F. AM., Alarcón OM. Determination of iron and copper in infant formula powdered milks by flow inyection atomic absorption spectrometry. Quim Anal 6:227-233, 1987.
12. Butrimowitz GP., Purdy WC. Resolutions of age-dependent reference intervals: polynomial regression methodology with applicability to plasma zinc levels in a childhood population. Clin Biochem 12:33-36, 1979.
13. Chuwa LM, Mwiruki G., Bilal MG., Mnubhi EK., Swai AB. Serum iron, zinc, copper and bromine in malnourished children in Dar es Salaam, Tanzania. East Afr Med J 73 (5 Supple):S21-S23. 1996.
14. Udomkesmalee E., Dhanamitta S., Yhoung-Aree J., Rojroongwasinkul N., Smith Jr. JC. Biochemical evidence suggestive of suboptimal zinc and vitamin A states in schoolchildren in Northeast Thailand. Am J Clin Nutr 52:564-567. 1990.
15. Ohtake M., Tamura T. Serum zinc and copper levels in healthy Japanese children. Tohoku J Exp Med 120:99-103. 1976.
16. Bekaroglu M., Aslan Y., Gedik Y., Deger O., Mocan H., Erduran E., Karahan C. Relationships between serum free fatty acids and zinc and attention deficit hyperactivity disorder: a research note. J Child Psychol Psychiatry 37:225-227, 1996.
17. Bucaderas SC., Farré-Rovira R. Whole blood and serum zinc levels in relation to sex and age. Rev Esp Fisiol 41:463-470. 1985.
18. Kosielec T., Kasczyk-Kaczmarek K., Kotkowiak L., Pozniak J., Nocen I. The level of calcium, magnesium, zinc and copper in blood serum in children and young people between 5 and 18 years of age. Przegl Lek 51:401-405. 1994.
19. Fons C., Brun JF., Fussellier M., Cassanas G., Bardet L., Orsetti A. Serum zinc and somatic growth in children with growth retardation. Biol Trace Elemn Res 32:399-404. 1992.
20. Varavithya W., Porananout P., Srianujata S., Thongonopakul W. Zinc status in normal Thai infants and children. Southeast Asian J Trop Med Public Hlth 10:534-539. 1979.
21. van Wouwe JP., Waser I. Comparisson between total and ultrafiltrable serum zinc as test to diagnose zinc deficiency in infants and children. Biol Trace Elem Res 40:203-211. 1994.
22. Malvy DJ., Arnaud J., Burtschy B., Richard MJ., Favier A., Houot O., Amedee-Manesme O. Reference values for serum zinc and selenium of French healthy children. Eur J Epidmeiol 9:155-161. 1993.
23. Brun JF., Dieu-Cambrezy C., Charpiat A., Fons C., Fedou C., Micallef JP., Fussellier M., Bardet L., Orsetti A. Serum zinc in highly trained adolescent gymnasts. Biol Trace Elem Res 47:273-278. 1995.
24. Tessmer CF., Krohn W., Johnston D., Forrest TB., Hrgovcic M., Brown B. Serum copper in children (6-12 years old). An age-correction factor. Am J Clin Pathol 60:870-878. 1973.
25. Ogihara H., Ogihara T., Miki M., Yasuda H., Mino M. Plasma copper and antioxidant status in Wilson's disease. Pediatr Res 37:219-226. 1995.
26. Sass-Kortsak A. Copper metabolism. In: Sobotka, Stewart CD (eds) Advances in Clinical Chemistry, Vol 8. Academic Press; New York pp1-67. 1965.
27. Hrgovcic M., Tessmer CF., Mickler TM., Mosier B., Taylor GH. Serum copper levels in limphoma and leukemia. Special reference to

Hodgkin's disease. Cancer 21:743-755. 1968.

28. Seltzer CC., Wenzell BJ., Mayer J. Serum iron and iron-binding capacity in adolescents I. Standard values. Am J Clin Nutr 13:343-353. 1963.

29. Preziosi P., Hecberg S., Galan P., Devanlay M., Cherouvrier F., Dupin H. Iron status of a healthy French population: factors determining bichemical markers. Ann Nutr Metab 38:192-202. 1994.

30. Koerper M.A., Dallman PR. Serum iron concentration and transferrin saturation in the diagnosis of iron deficiency in children: Normal developmental changes. J Pediaitrics 91:870-874. 1977.

31. Woodruff CW. Iron deficiency in infancy and childhood. Pediatr Clin N Am. 24:85-105. 1977.

32. Wadsworth GR. Nutritional factors in anaemia. World Rev Nutr Diet 21:75-85. 1975.

33. Card RT., Brown MG., Yalbeerg LS. Serum iron and iron-binding capacity in normal subjects. Canad Med Ass J 90:618-622. 1964.

34. DalhstromKA., Ament ME., Medhin MG., Meurling S. Serum trace elements in children receiving long-term parenteral nutrition. J Paediatr 109:625-30. 1986.

35. González-Silva M., Beranl MD., Cabezón I. Valores hematológicos y niveles férricos en una población escolar rural. Sangre 39:99-103. 1994.

36. Laitinen R., Vuori E., Dahlstrom S., Akerblom HK. Zinc, copper, and growth status in children and adolescents. Pediatr Res 25:323-326, 1989.

Recibido: 08-04-1996
Aceptado: 24-02-1997