NUTRITION PROBLEMS OF THE URBAN FAMILY

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I. INTRODUCTION

During the recent years, there has been a surge of interest in the nutritional metabolic role of the "trace" mineral elements. Part of this interest is due to improved case in the quantitative analyses of these elements by the introduction of atomic absorption spectrophotometry. However, it has been the studies on zinc, in particular, that have fueled much of this interest when it was discovered that naturally-occurring zinc-deficiency could happen in man. It was reported that a syndrome consisting of dwarfism, iron-deficiency, anemia hepato-splenomegaly occurs in poor villages in Egypt and Iran. This syndrome was accompanied by low plasma and hair zinc levels, and decreased urinary excretion of zinc. Prior to this report, it has been tacitly assumed that since many of the "trace" elements occur in fairly abundant quantities in food, and because they are present in very small quantities in the tissues of man, dietary deficiency of the trace minerals was unlikely to occur in man. It has become obvious that difficulty in absorption or poor availability of the trace elements from the diet may prove to be an important etiologic mechanism in the causation of deficiencies of these elements. In the case of zinc, a study in the United States reported cases of children with low hair zinc concentration and with low taste acuity (hypogeusia). The condition responded to treatment with zinc supplementation. It was also reported that zinc-deficiency is secondary to intestinal malabsorption. The possibility that zinc-deficiency might accompany protein-calorie malnutritions had been suggested by some investigators who found low plasma zinc levels in children with kwashiorkor. Since protein-calorie malnutrition is a world-wide problem and is particularly common in developing countries, it appears important that the nutritional status with respect to zinc should be included in the nutrition research programs of such countries.

In this study made on apparently normal adults, the zinc levels in serum and hair were examined. Current studies made on pregnant women will be the subject of a subsequent report.

II. MATERIALS AND METHODS

The subjects consisted of healthy medical and graduate students at the College of Medicine, University of the Philippines, enrolled from 1972 to 1974, and male blood donors to the blood bank of the University of the East, Ramon Magsaysay Memorial Medical Center. Only the first-time donors to the blood bank with negative VDRL tests and hemoglobin levels of 12 grams or more per 100 were examined.

Blood samples from students were collected by veinpuncture using plastic syringes and stainless needles which were previously soaked overnight in 2% disodium ethylenediamine tetra-acetic acid (Na_2EDTA). Initial blood withdrawals on 68 students enrolled in 1972 using unwashed plastic syringes showed somewhat higher blood values than samples collected with pre-washed syringes. This suggested possible contamination of the syringes with zinc, thus the data obtained were discarded. Blood samples from donors were obtained from the blood which remained in the plastic tube at the termination of the blood donation. Approximately 5 ml of blood were obtained from each subject. The blood was allowed to clot at room temperature and the serum was carefully separated for zinc analyses. Sera showing hemolysis were not included.

Hair samples amounting to 100-200 mg were obtained by cutting samples from the suboccipital region. The hair was washed initially twice with 1-ml aliquots of hexane, twice with 1-ml aliquots of 95% ethanol, and thrice with 1-ml aliquots of deionized water. The hair samples were dried in the oven at 100°C overnight. They were placed in a desiccator, brought to room temperature, and approximately 100 mg aliquots were weighed for zinc analyses.

Serum zinc analyses. Zinc was determined in 1 ml serum to which 4 ml of zinc-free 6.7% trichloracetic (TCA) solution was initially added to precipitate the proteins. The TCA solution was rendered zinc-free by prior extraction with 1 mg% dithizone solution in chloroform, which extraction was done repeatedly until no change in color of the dithizone solution was noted. The samples were centrifuged and supernates analyzed for zinc concentration in an atomic absorption spectrophotometer (Varian Techtron Model 1000) using blanks and standard zinc solutions containing concentrations of TCA similar to the samples. The readings were made up at the scale expansion of 4.5 times. The zinc standards were made from metallic zinc (analytical grade) which was dissolved in minimal amounts of 6N hydrochloric acid solution. A typical standard curve for serum zinc analysis is shown in Figure 1. The validity of the procedure was established by recovery experiments (Table I).

Recoveries of zinc added at two different levels of 12 different polled serum samples, when analyzed by this method gave an overall mean recovery of 101±4% (S.D.)

Hair Zinc analyses. Previously washed hair samples containing approximately 100 mg hair were digested in 30-ml micro Kjeldahl digestion flasks using 1 ml of a 17:3 mixture of nitric-perchloric acid (vol:vol) as digestant. The solutions were heated until the resulting digest was almost colorless. The samples were diluted with appropriate volumes of deionized water (usually 10 ml) and were read against zinc standards in water. The standards contained concentrations of 1, 2 and 3 mg zinc per ml. The samples and standards were read in the atomic absorption spectrophotometer without any scale expansion.

III. RESULTS AND DISCUSSION

Serum Zinc Levels. The serum zinc concentrations of 209 apparently healthy adult students (203 medicine and 6 female graduate students) are given in Table II, and the frequency distribution of the zinc values are shown in Figure 2. Although the mean value for females (107 mg zinc/100 ml) is slightly lower than that for males (111.9 mg zinc/100 ml), statistical analyses revealed that the difference between means is not significant (t=1.66, p is greater than .05).

The zinc concentrations (ug zinc/100 ml serum) of 189 male adult blood donors gave a mean of 87.5 mg, range of 52.5-140 mg, standard deviation of 20.3 mg, standard error of the mean of 1.48 mg, and a median value of 85 mg. The mean zinc concentration is statistically significantly lower than the mean of 108.8 mg for students (t=10.31, p is less than .001). The frequency distribution of zinc values from blood donors (Figure 3) as well as that for students (Figure 2) showed a very slight kurtosis to the right.

A detailed distribution of zinc values for the blood donors' group according to occupation is presented in Table III as it is possible to show its relation to the students' group who belong to a higher income level. Although the detailed comparison for a number of cases were few for so many occupational categories, the data suggested the possibility of serum differences for different categories of occupation. It is interesting to note, for example, that subjects who worked as "office employees" yielded the highest mean serum zinc levels, while "street vendors" gave the lowest mean value.

When the serum zinc values obtained from Filipino students were compared with values obtained from other workers abroad, the mean values which used atomic absorption spectrophotometry for zinc determination, as well as the standard deviation, were similar (Table IV). Even when compared against values published abroad, the mean value for the blood donors' group appeared lower.

The blood samples in the different subjects were drawn at various times during office hours (8 AM to 4 PM) and were not timed in relation to meals. There were earlier reports that no differences in plasma zinc levels occurred one hour before and after breakfast. Recent studies, however, indicate that a diurnal or circadian variation in serum zinc levels exists although the evidence is conflicting regarding the direction of change of the levels at different times of the day. It occurred to us, therefore, to compare the serum zinc concentration before breakfast and 30 minutes after lunch. The data obtained from 28 medical students (four females and 24 males) and two male laboratory technicians who volunteered for the study are shown in Table V. The post-lunch samples were lower by a mean of 18 ug zinc and statistical analysis (paired samples) showed this difference is highly significant ($t=4.74$, p is less than .001). When the per cent change of the zinc values of post-lunch samples were calculated from the corresponding fasting values, twenty-one subjects showed decreases of from 9 to 44.6%, four showed no change (0%), and only five showed increases of from 5.5 to 17.9%. The mean change in all 30 subjects was a decrease of 15.1%. The results clearly showed that differences in the time of withdrawal of blood samples, or that an antecedent meal shortly before the sampling, may cause significant variation of serum zinc levels in normal adults.

The availability of hemoglobin data which was determined in the screening of blood donors gave an opportunity to examine a possible relationship between serum zinc and hemoglobin levels. The data shown in Table VI showed no apparent change in serum zinc levels at different concentrations of hemoglobin.

Hair Zinc Levels. The hair zinc levels obtained for different groups of medical students, blood donors, and some values by other authors abroad who had used atomic absorption spectrophotometry for zinc determination, were compared in Table VII. Medical students ('73-'74) and blood donors showed higher zinc levels than medical students ('74-'75). We had no adequate explanation for this difference. Although hair samples from medical students ('73-'74) and the other group, on the one hand, were determined by different analysts, the possibility that seasonal variations in hair zinc levels might occur could not be excluded. Nonetheless, the values for hair zinc of medical students ('74-'75) were comparable to the mean values reported by other studies abroad. Although female medical students ('73-'74) had statistically higher hair zinc concentrations than their male co-students, this sex difference was not obvious in the group of medical students ('74-'75).

A study of the possible correlations between serum and hair zinc levels of blood donors and medical students ('73-'74) (Table VIII) showed no apparent relationship. Similarly, possible variations of serum and hair zinc with age in these groups of subjects (Table IX) also showed no apparent relationship.

IV. SUMMARY AND CONCLUSIONS

A procedure for serum zinc determination whose accuracy had been validated by recovery studies was presented.

The serum zinc of healthy adult Filipino students was 108.8±20.8 (Mean ± S.D.) per 100 ml. This mean was comparable to those of normal healthy subjects from abroad in which atomic absorption had been used as the method of determination.

A group of 189 male blood donors gave lower zinc values when compared to the group of students or to some published values from abroad. A study of the differences in occupation of the different donors and their corresponding zinc values in serum suggest the possibility that occupations likely to be associated with low income tend to have lower serum zinc values.

Serum zinc levels determined before breakfast decreased by a mean of 15.1% when compared to values determined 30 minutes after lunch.

There was no apparent correlation between serum zinc and hair zinc values, so that in adults, hair zinc levels did not have any predictive value on the serum zinc level.

Serum zinc levels showed no apparent relation to changes in hemoglobin levels.

Within the age ranges of 19 to 49 years, serum and hair zinc showed no variations with age.

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Table I

Recovery of Added to Different Pooled Serum Samples

Pooled Sample No.	Zn (ug/100 ml)	Sample + 50 ugZn /100 ml.	% Recovery	Sample + 100 ug Zn/100 ml.	% Recovery
1	124	172.5	99.1	225	100.4
2	116	164.5	99.1	217	100.5
3	116	168.5	101.5	217	100.5
4	116	172.5	103.9	221	102.3
5	148	197.0	99.5	245	98.8
6	140	168.5	89.5	233	97.1
7	120	181.0	106.4	233	106.9
8	124	184.5	105.0	237	105.8
9	120	172.5	101.4	221	100.5
10	140	189.0	99.4	241	100.4
11	128	184.6	103.6	245	107.4
12	124	189.0	108.6	221	98.7
Mean Recovery (%)			101.4		101.5

Table II

Serum Zinc Values of Medicine and Graduate Students

(ug Zn/100 ml)

Parameters	Males	Females	Both Sexes
n	79.0	130.0	209.0
Mean	111.9	107.0	108.8
Median	109.0	103.0	107.0
Range	63-167.0	73-170.5	63-170.5
S.D.	22.3	19.6	10.8
S.E.	2.5	1.7	1.4

Table III

Serum Zinc Concentrations in Blood Donors
According to Occupation

Occupation	No. of Subjects	Mean Serum Zinc (ug/100 ml)
"Jobless"	58	84.9
"Laborers"	57	91.9
Students	22	91.6
Drivers	10	82.8
Office Employees	8	102.3
Carpenters	5	89.0
Security Guards	4	83.8
Painters	3	82.7
Street Vendors	3	71.3
Race Bookies	3	83.7
Locksmith	1	92.0
Fisherman	1	85.0
Baker	1	72.5
Manual Workers	8	74.2
Others*	5	77.7

Table IV

Comparison of Serum Zinc Levels of Filipino Students with Others

AUTHORS	Refrenece No.		SUBJETCS	Zn (ug/100 ± S.D.)
Pekarek, *et al.*	(1)	99	Males	102 ± 17
Sinha & Cabrioli	(2)	100	Males	121± 18
		100	Females	118 ± 21
Fuwa, *et al.*	(3)	9	Adults	122 ± 3*
This study	(4)	79	Males	112 ± 22
		130	Females	107 ± 20

* Standard error of mean.

References:

(1) Pekarek, R.S. and Beisel,, W.R. *Appl. Microbiol.* 18:482 (1969).

(2) Sinha, S.E., Gabrioli, E. R. *Am. Jr. Clin. Pathol 54:* 570 (1970).

(3) Fuwa, K., Pulido F., Mckay, R. and Vallee, B. L. *Anal. Chem. 36:* 2407 (1964).

* Statistical comparison of the difference between means (18 ug Zn/100 ml) showed: S. E. of mean difference = 3.8, t = 4.74, p. $<$ .001, using paired samples.

Table V

Effect of Meals on Serum Zinc Levels of Thirty Adult Students Zn (ug/100 ml)

Time Sampling	Range Mean	Mean*	S.D.
Before Breakfast	87-180	119	22
30 minutes after lunch	62-180	101	24

Table VI
Relation Between Hemoglobin and Serum Zinc Levels
In Blood Donors

No. of Subjects	Hemoglobin (u/100 ml)	Zinc (ug/100 ml ± S.D.)
40	12-12.9	85 ± 13
52	13-13.9	89 ± 20
41	14-14.9	89 ± 20
34	15-15.9	86 ± 19
22	16-16+	87 ± 17

Table VII
Hair Zinc Levels in Filipino Subjects
Compared with Subjects Abroad
(ug Zn/1 g hair)

Subjects	No.	Range	Mean	S. D.
A. Filipino Subjects:				
1. Medicine ('73-74)*				
Females	68	85-638	300	126
Males	42	132-501	230	104
ALL	110	85-638	274	122
2. Medicine ('74-75)**				
Females	58	80-330	183	55
Males	37	77-355	165	66
3. Blood Donors				
Males	189	88-573	242	105
B. Other Studies Abroad:				
McBean, et al.[1]				
Adults	14	—	176	37
Schroeder and Nason[2]				
Males	82	—	167	5 (SE)
Females	47	—	172	9 (SE)

* Females vs. Males: $t = 3.05, p < 105$

** Females vs. Males: $t = 1.38, p > 05$

[1] McBean, L. D. and Halsted, J. A. *J. Clin Pathol.* 22:623, 1969.

[2] Schroeder, H. A. and Nason, A.P. *J. Invest. Dermatol.* 55:71, 1969.

Table VIII

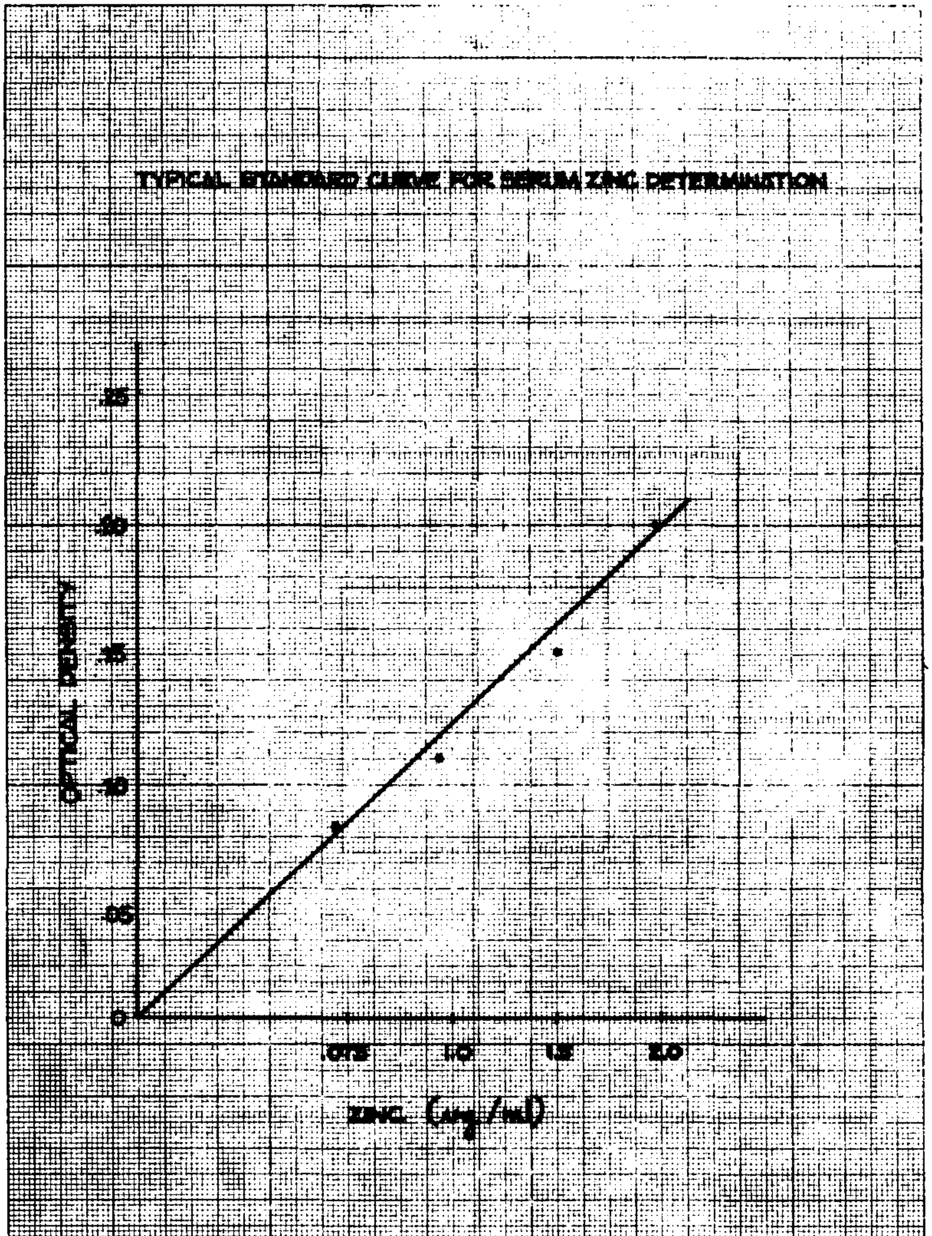
Relation Between Serum and Hair Zinc Levels in Filipinos

No. of Subjects	Serum Zn (ug/100 ml)	Mean Hair Zn (ug/g)
	FEMALES	
15	90 + <	305
18	91 - 100	286
23	101 - 110	286
13	111 - 120	319
11	121 - 130	316
19	131 - >	253
	MALES	
18	70 + <	228
20	71 - 80	265
36	81 - 90	237
28	91 - 100	224
23	101 - 110	255
19	111 - 120	225
14	121 - 130	185
28	131 - >	226

Table IX

Relation Between Age and Zinc Levels in Serum and Hair in Filipinos

Age (Years)	No. of Subjects	Serum Zn (ug/100 ml) ± S.D.	No. of Subjects	Hair Zn (ug/g) ± S.D.
19 & <	6	94 ± 17	12	238 ± 119
20 - 29	239	96 ± 21	286	250 ± 100
30 - 39	37	88 ± 18	26	252 ± 110
40 - 49	7	89 ± 30	5	210 ± 33


TYPICAL STANDARD CURVE FOR SERUM ZINC DETERMINATION
OPTICAL DENSITY
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.20
.15
.10
.05
0
1.0
1.5
2.0
ZINC

FIG. 1

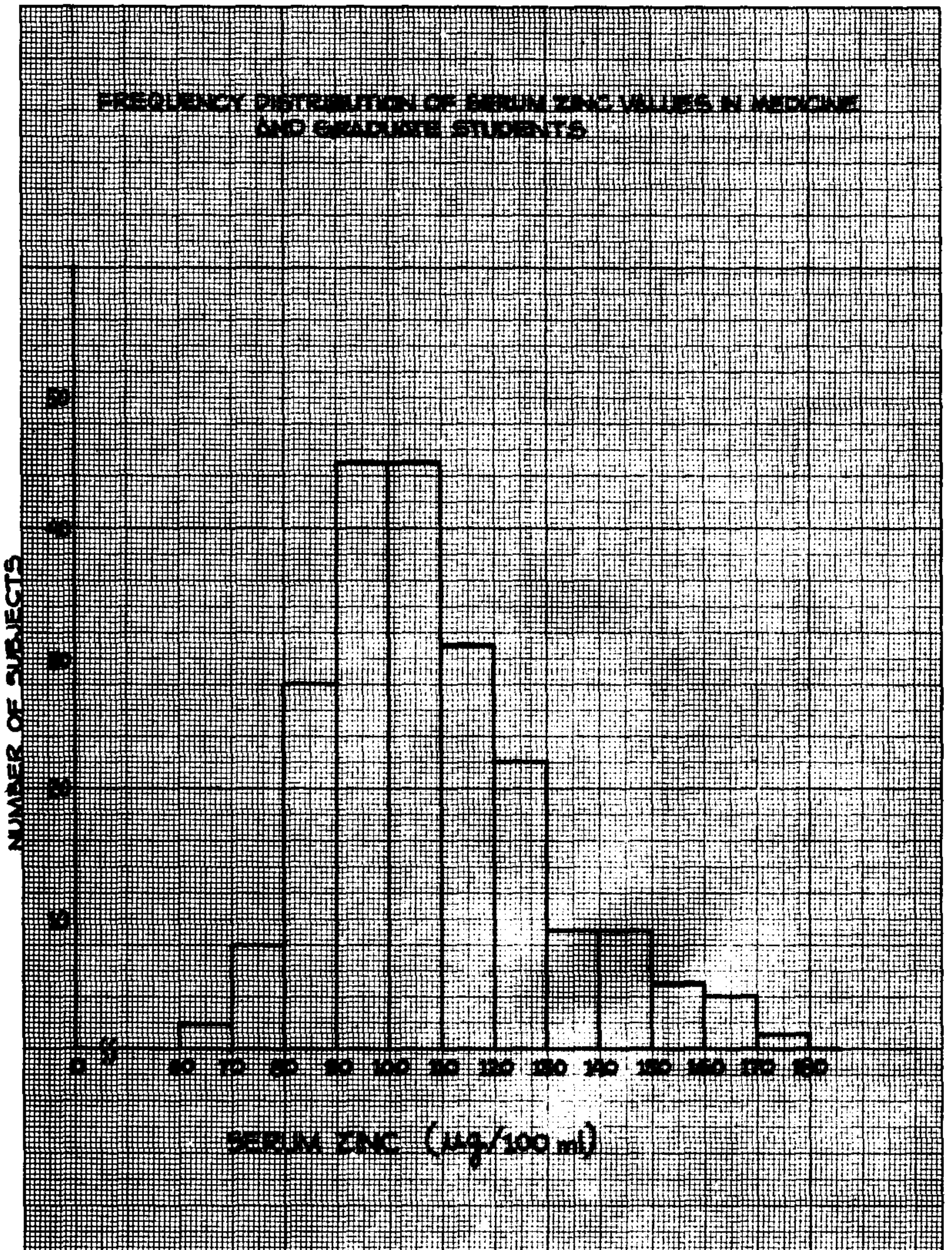
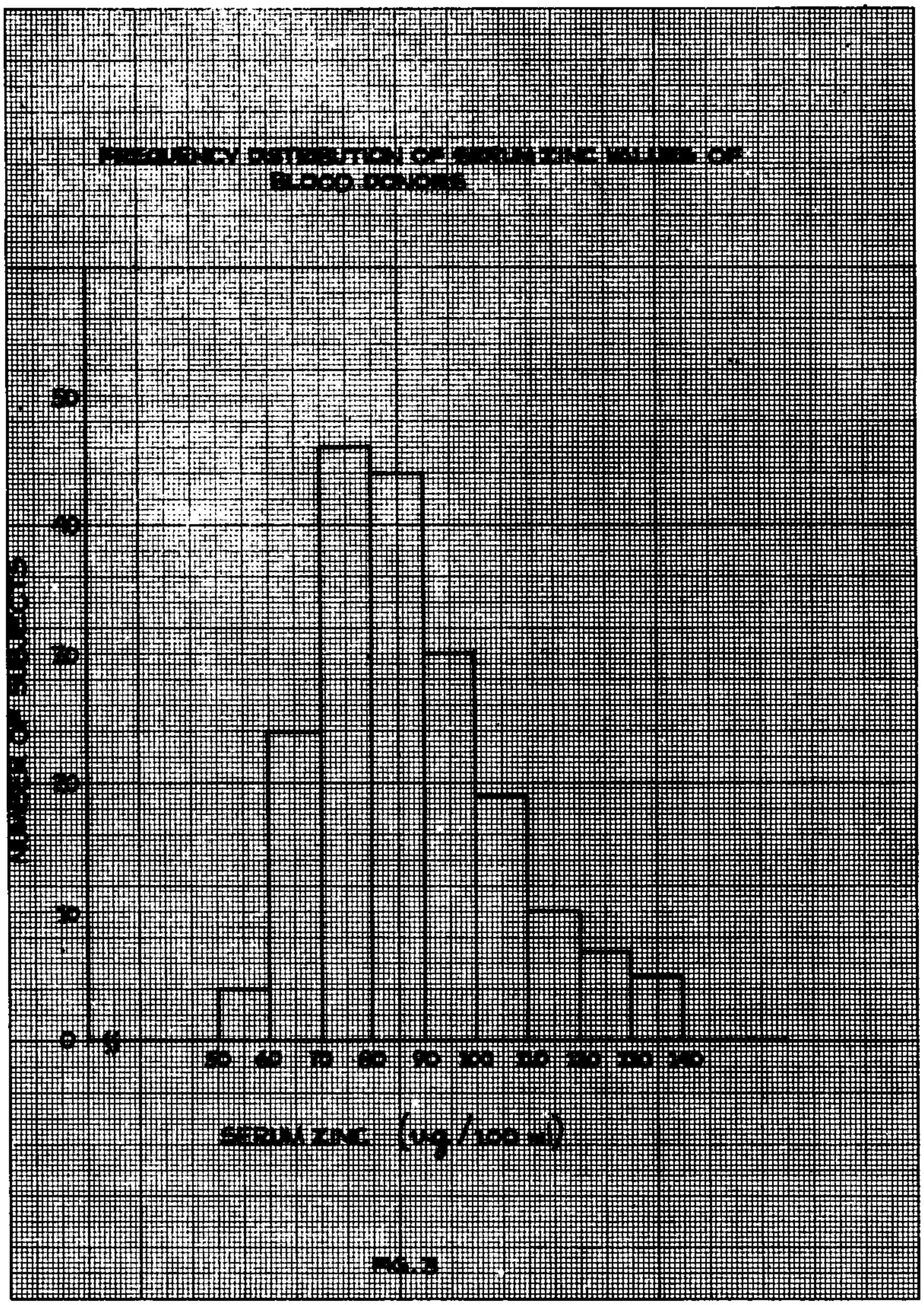

FREQUENCY DISTRIBUTION OF SERUM ZINC VALUES IN MEDICINE AND GRADUATE STUDENTS
NUMBER OF SUBJECTS
0
60 70 80 90 100 110 120 130 140 150 160 170 180
SERUM ZINC (μg/100 ml)

FIG. 2


FREQUENCY DISTRIBUTION OF SERUM ZINC VALUES OF
BLOOD DONORS
SERUM ZINC (ug/100 ml)