TRACE ELEMENT STUDIESON KARACHI POPULATIONS, PART I: NORMAL RANGES FOR BLOOD COPPER, ZINC AND MAGNESIUM FOR ADULTS

Pages with reference to book, From 43 To 49

William W.T. Manser (Departments of Biochemistry & Pathology, The Aga Khan University Medical College, Karachi.)
M. Altaf Khan (Department of Microbiology, Karachi University, Karachi.)

Abstract

Normal ranges in whole blood were established for copper, zinc and magnesium for a Karachi. population. For copper, it is 71 — 116 ug/dl (mean 93$), there being no significant difference between the sexes; for zinc, males 602.5 — 850 ug/dl (mean 726), females 519 —853 (686),P C 0.01 for males+ females 563 —859.5 (711),; and for magnesium, males 2.97 — 4.80 ug/dl (mean 328), females 2.65 — 4.66 (3.50), 0.05 > P > 0.01; for males + females 2.75 — 4.80 (3.61). There were weak correlations only between pairs of blood metal levels for the population (JPMA 39 43 1989).

INTRODUCTION

Several research projects are underway to investigate the effects of environmental pollution on the levels of metals in body fluids and, where. ever possible, the effects on health. Because of the lack of suitably sensitive instrumentation the work will be confined to the levels of copper, zinc, magnesium and lead and perhaps selenium initially. The establishment of blood normal ranges for copper, zinc and magnesium was necessaw bpcause the ranges for blood copper and zinc and for serum magnesium vary considerably between global populations[1-13] but no information is available on magnesium levels in whole blood. In the establishment of normal ranges for clinical chemistry tests, it was found that the nonnal ranges for a typical patient population was different, in some cases considerably so, from the oft-quoted values in Western literature (Unpublished results). Hence. it was clear that normal ranges in blood for Karachi-ites would have to be established before other work could proceed.

MATERIALS, METHODS & EQUIPMENT

Water

All water used was doubly deionised distilled water stored in a previously acid soaked and well-washed polythene container. It was tested for zero response by atomic absorption spectrophotometry for each element under test. Glassware, blood sample tubes, and pipette tips

All were soaked for at least 24 hours; in most cases for several days, in 20% nitric acid to ensure metal-free surfaces, After washing at least six times in doubly deionized distilled water and oven drying, items were stored in polythene bags tied at the top. Blood sampling Blood samples were taken by ordinary 5 or 10 ml disposable plastic syringes with steel needles. Samples were immediately transferred to acid washed glass containers containing 200 ul of 10% EDTA. The EDTA solutions had been previously tested for the absence of the metals under test. Blood was not allowed to come into contact with the cap of the container as the caps are unstable to acid treatment and could therefore, not be rendered metal-free. Samples were stored at -20°C.

Chemicals

“Spectrosol” grade cupric nitrate, zinc nitrate and magnesium nitrate were purchased from BDH Chemicals Ltd., Poole, Dorset, U.K. Concentrated acids and all other chemicals used were also of

“Spectrosol” grade from B.D.H. Atomic Absorption Spectrophotometry Standards, samples and blanks on estimation for copper, zinc or magnesium were aspirated into a Pye-Unicam SP2900 Atomic Absorption Spectrophotometer with the following settings

Filter clear

Monochromator bandpass 0.4 nm
Reading 4 second integration
Fuel : acetylene 1.0 L/min
Air: 5.OL/min For the estimation of — Copper 324.8 nm at 5 mA
Zinc: 213.9 nm atlOmA
Magnesium 285.2 nm 4 mA

METHOD

As very small concentrations of metals were being estimated care was taken to ensure a clean, dust-free environment and that all surfaces with which solutions under test were likely to come into contact, to be meticulously metal-free. Treatment of samples standards and blanks Each sample and blood standard was digested with an equal volume of concentrated nitric acid initially for 24 hours at room temperature and then under gradually increasing heat on a sand-bath until a clear yellow solution was obtained. Most of the acid was then removed, and the residual solution diluted with a suitable volume of water. Blanks, each consisting of an equivalent volume of acid, were evaporated and diluted as for the samples. Each batch of samples, standards and blanks took about two weeks to process. At this stage, each solution was ready for aspiration into the atomic absorption spectrophotometer for estimation of the metal.

Aqueous “standards” and controls

Aqueous solutions are not acid digested as above and neither can they be used as standards. When a solution is aspirated into the atomic absorption spectrophotometer, the actual volume taken in per unit time and the resulting signal is influenced greatly by viscosity. Differences in matrix between an aqueous solution and a treated blood sample probably also contribute towards spurious results. For a given metal, absorbances may be linearly related to concentrations in treated blood but not in water, or vice versa. It was found that using an aqueous standard, blood samples (after treatment, see above) appeared to have approximately 78% of the true value for Oopper, 85% for zinc and 88% of the true value for magnesium. Hence, it is essential to use a standard of similar medium to that of the sample. Aqueous solutions may be used as controls after being calibrated about 30 times each against a blood standard. A test run is in control if the value of the control falls with the mean ± 2 SD (standard deviation) of those 30 results.

Preparation and Calibration of Blood Standards

Aliquotes of a pooled blood volume are stored at —20° C until required: This standard was calibrated for each metal under investigation by the method of standard additions in which zero and three different volumes of “Spectrosol” grade cupric nitrate, zinc nitrate or magnesium nitrate solution diluted 1:10 with water were added to four different aliquotes. A blank was run concurrently. Alter acid digestion and dilution, the absorbance was taken for each solution at least in triplicate (often in quadruplicate or even quintuplicate) the first often being abortive due presumably to carry over. After making an allowance for the blank reading, the mean value of the absorbances for each treated aliquote was plotted graphically against added concentration of metal. For copper and zinc straight lines were obtained, for magnesium a curve. From these, the concentration in the original blood was found For each metal, the procedure was carried out 30 to 45 times and the concentration of a metal in the pooled blood standard was taken as the mean. The S.D., the C.V. (coefficient of variation) and the mean of the percentage recoveries were all calculated for that metal.

Determination of normal ranges

Thirty three non -fasting clinically healthy males and twenty-nine females were chosen as subjects for the normal range estimations from among the teaching staff and laboratory technologists of The Aga Khan University. Both parents of each subject were of Indo-Pak origin and each subject was of high socioeconomic status and lived in an area of relatively low atmospheric pollution in Karachi. Each sample was estimated for copper, zinc and magnesium as described above. Each run for copper and zinc was carried out with sufficient volumes of standard and blank so that the absorbance of the latter two could be found before and after each run and after about every 15 samples. As absorbance versus concentration for these two elements is linear, the concentration of each in each sample is given by direct proportions with the absorbance of the corresponding standard. Absorbance versus concentration for magnesium is non-linear and the plotting of a calibration curve is necessary. Three standard solutions were prepared consisting of the blood standard and the additions of zero and two different volumes of added "Spectrosol" magnesium nitrate solution diluted 1: 10 with water. These were digested in the same way as the samples under test as described above. During each run on the atomic absorption spectrophotometer two previously calibrated aqueous controls of different concentrations (not acid digested) were aspirated into the instrument after aspiration of the treated blood standards. If the calculated value of each canre within the pre-determined mean value ± 2SD, then that part of the run was in control. Interferences Lead absorbs at 217.0 nm, which is close to that of zinc, 213.9 nm. Estimation of blood lead in the presence of varying amounts of zinc and vice versa showed that there was no inference between these metals. Estimations of blood copper, zinc and magnesium in the presence of varying amounts of iron also showed that iron does not interfere.

RESULTS

The results obtained for the estimation of copper, zinc and magnesium in the blood standard are given in Table 1.

TABLE 1. Copper, Zinc and Magnesium Levels in our Blood Standard and Other Data.

	Copper	Zinc	Magnesium
No of times estimated	30	30	45
Mean Value	81.81 ug/dl	653.75 ug/dl	3.49 mg/dl
S.D.	2.26 ug/dl	18.42 ug/dl	0.12 mg/dl
C.V.	2.76 %	2.82 %	3.44%
Mean Recovery	99.7 %	96.3 %	99.8%

In each case, the coefficient of variation is satisfactory and the percentage recovery is good. The normal ranges for each metal in blood were calculated by a non-parametric method as the distribution

of results in each case was, at best, only close to a normal Gaussian distribution. For this reason, a significant difference between the sexes for the normal range of each metal was looked for using the Wilcoxon Rank Sum Test. Normal range results are given in Table II.

TABLE II. Normal Ranges for Copper, Zinc and Magnesium in Whole Blood
33 Males and 29 Females.

Metal	Sex	Median	Mean	Normal Range	P
Copper	M	90.0 ug/dl	91.9 ug/dl	70 – 114 ug/dl	
	F	96.5	96.7	70 – 124	>0.05
	M+F	94.5	93.5	71 – 116	
Zinc	M	726.0 ug/dl	726.0 ug/dl	602.5 – 850 ug/dl	
	F	695.5	685.9	519 – 853	<0.01
	M+F	705.0	711.0	563 – 859.5	
Magnesium	M	3.80 mg/dl	3.78 mg/dl	2.97 – 4.80 mg/dl	
	F	3.515	3.50	2.65 – 4.66	<0.05 ; >0.01
	M+F	3.60	3.61	2.75 – 4.80	

Normal Ranges for Copper in Whole Blood

The distribution of results was non—Gaussian for females, approximately normal-Gaussian for males and close to normal for males and females combined (Figure 1).

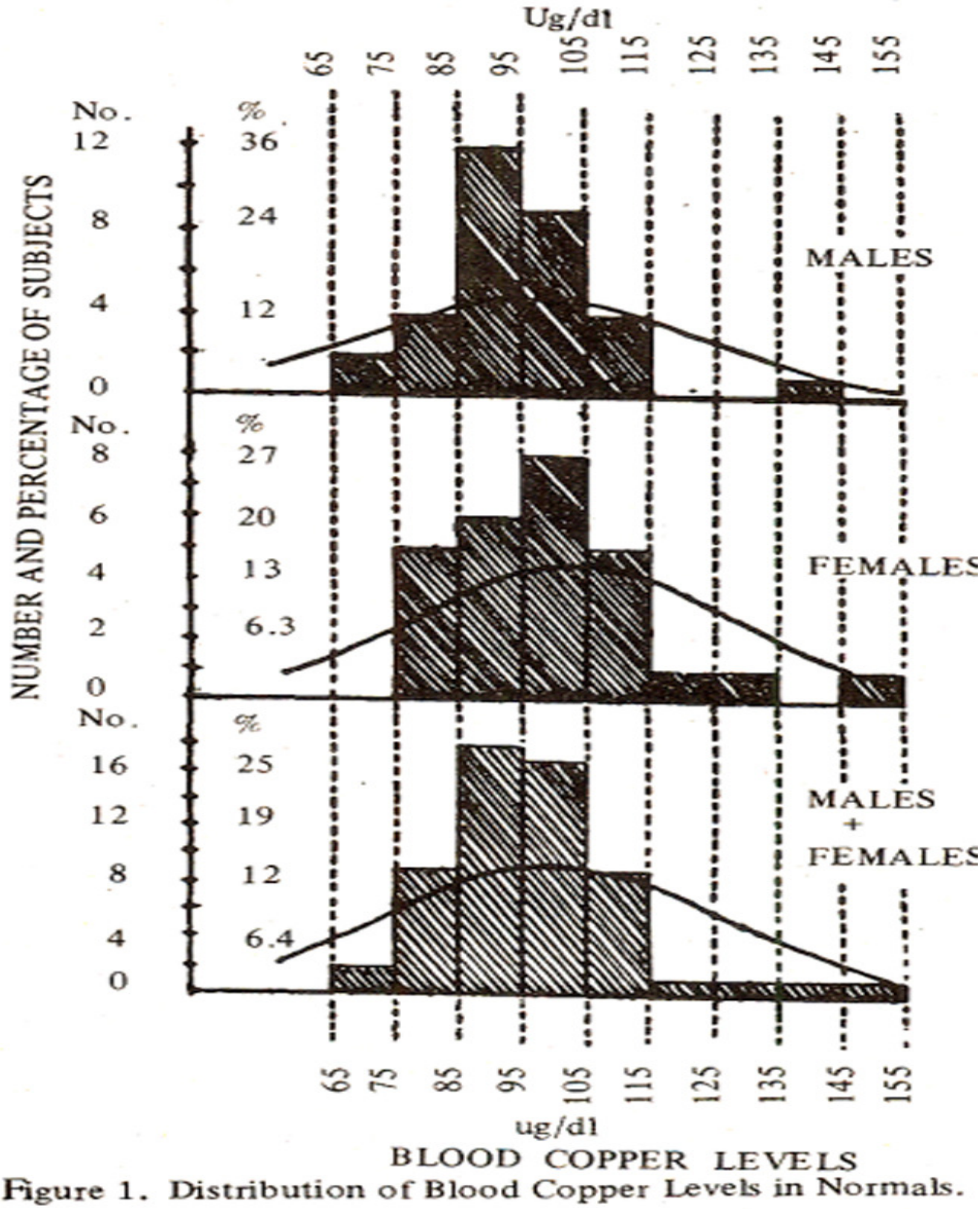

Figure 1. Distribution of Blood Copper Levels in Normals.

There was no significant difference between the blood normal ranges for males and females and combination of the results for the two sexes gave a normal range of 71 — 116 ug/dI (mean 91.5) for blood copper.

Normal Ranges for Zinc in Whole Blood

Normal ranges for blood zinc are significantly different between the sexes (P< 0.01). The distribution of values is given in Figure 2,

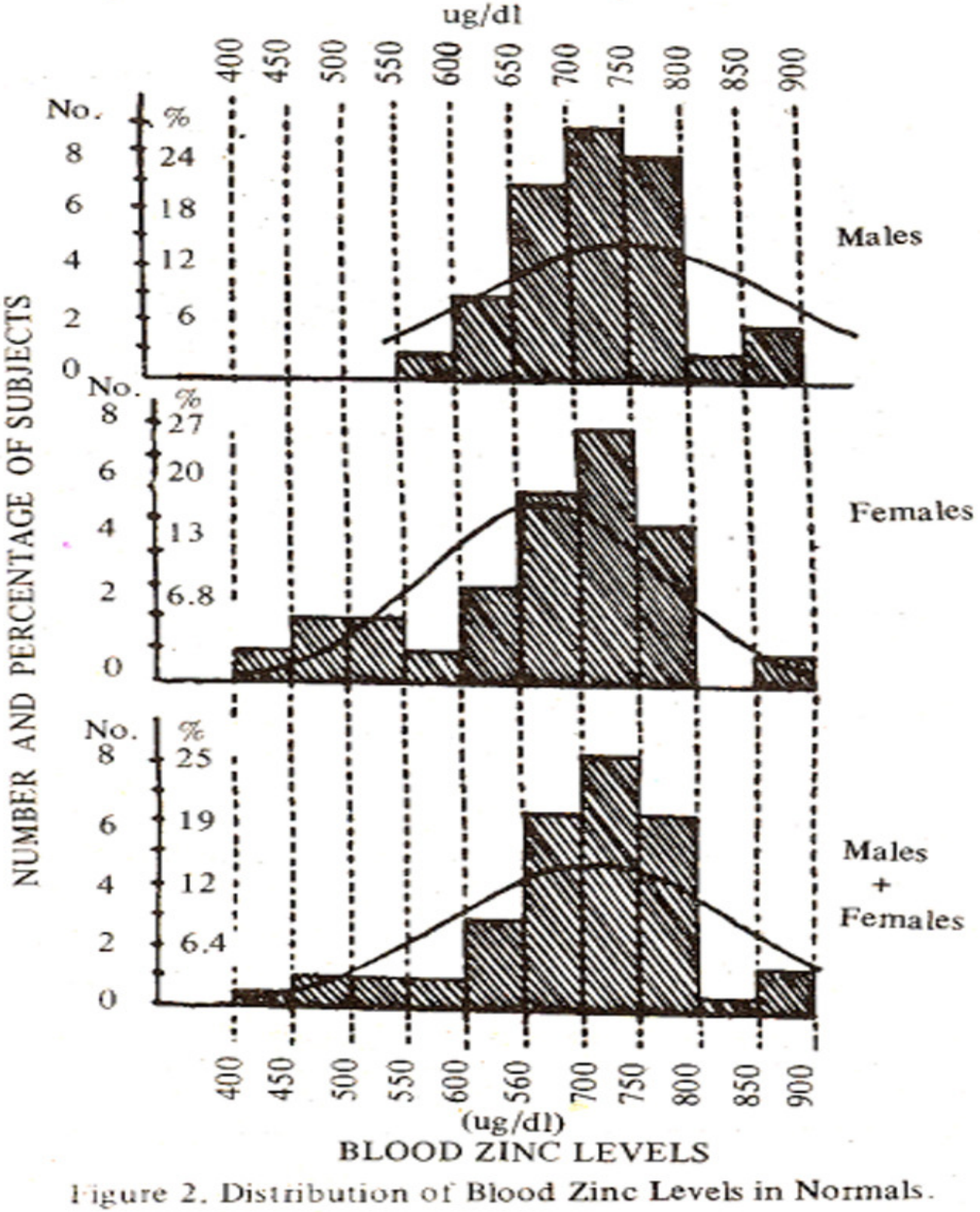

Figure 2. Distribution of Blood Zinc Levels in Normals.

being approximately normal—Gaussian for males and for males and females combined, but non-Gaussian for females. The normal ranges for blood zinc for males were 602.5 - 850 ug/dl (mean 726),

females 5 19 - 853 (686) & males + females 563- 8595 (711).

Normal Ranges for Magnesium in Whole Blood

As for zinc, normal ranges for blood magnesium were significantly different between the sexes, ($0.05 > P > 0.01$), the distribution of results being approximately normal-Gaussian for each sex and fairly close to normal for males and females combined (Figure 3).

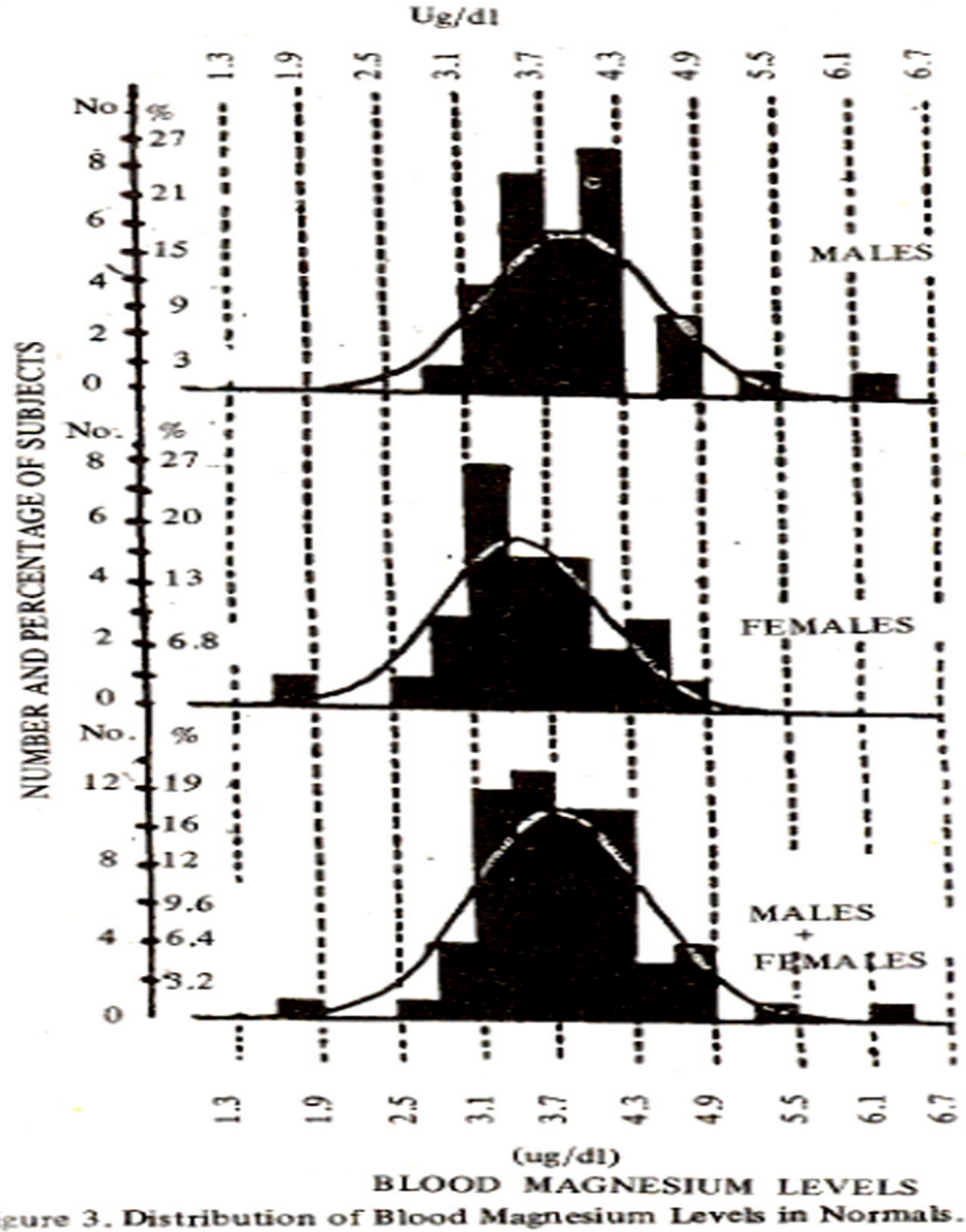

Figure 3. Distribution of Blood Magnesium Levels in Normals.

The normal ranges for blood magnesium for males were 2.97 — 4.80 mg/di (mean 3.78), females 2.65 - 4.66 (3.50) males ÷ females 2.75 - 4.80 (3.68).

Regression Analysis

The correlation coefficient between the results for pairs of metals for our normal population was

calculated according to the formula given and derived as follows:

$$y = Bx + A$$

Where

$$B = \frac{n \Sigma xy - \Sigma x \Sigma y}{n \Sigma x^2 - (\Sigma x)^2}$$

$$A = \frac{\Sigma y - B \Sigma x}{n}$$

The correlation coefficient

$$r = \frac{n \Sigma xy - \Sigma x \Sigma y}{\sqrt{n \Sigma x^2 - (\Sigma x)^2 \; n \Sigma y^2 - (\Sigma y)^2}}$$

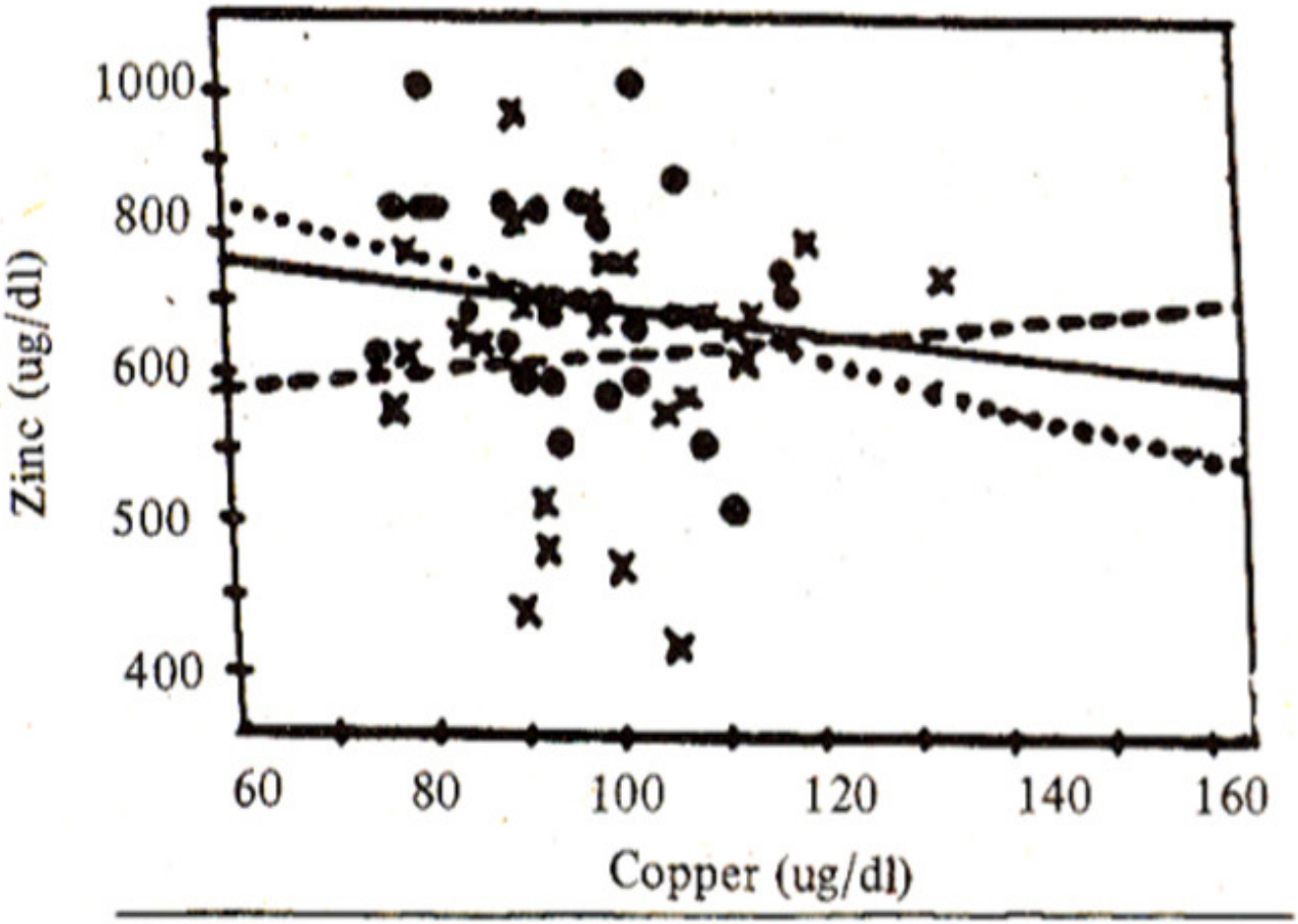

Figure 4. Blood Zinc against Copper Levels in Normals

●Males..... x Females -------- Total ——

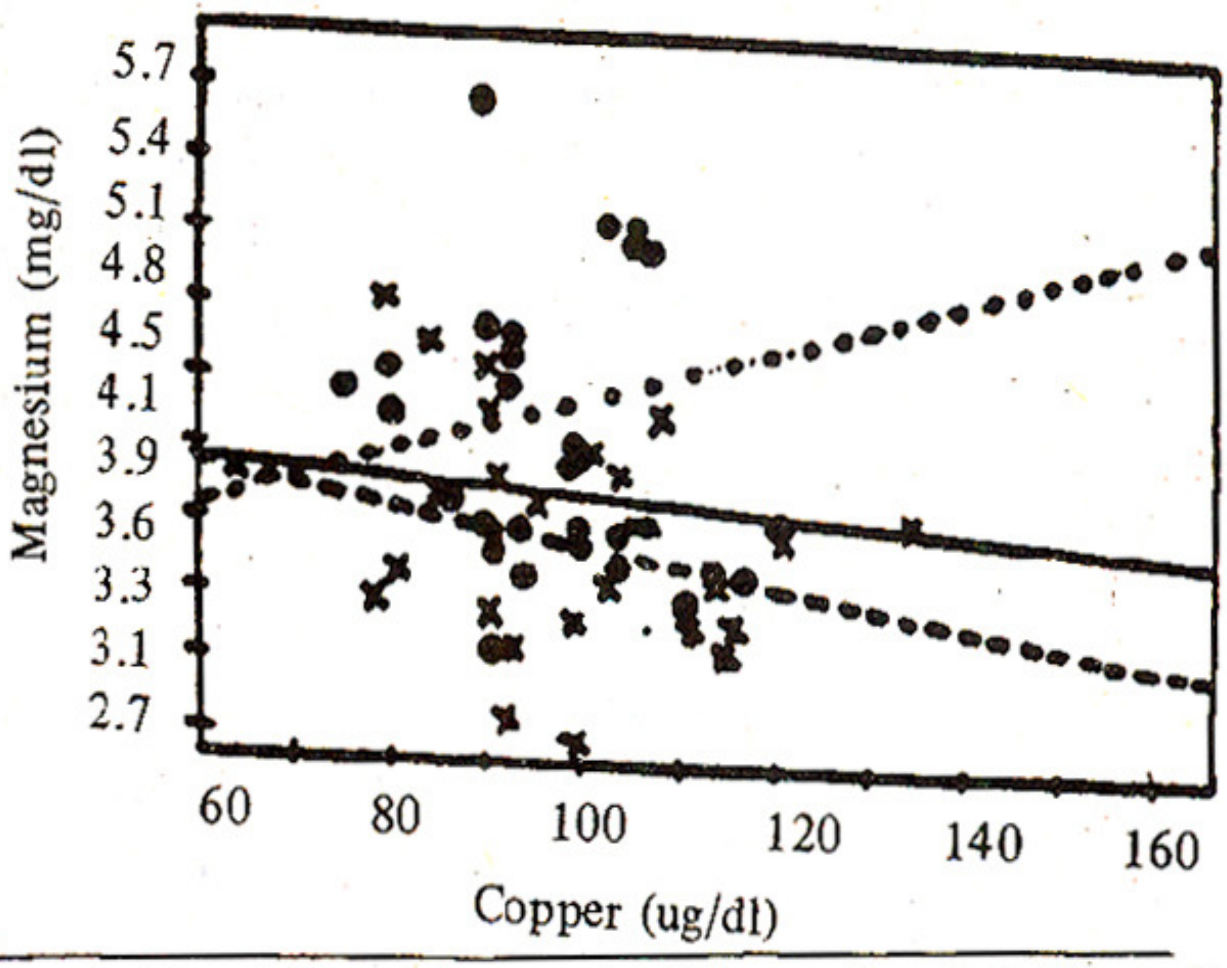

Figure 5. Blood Magnesium against Copper Levels in Normals

● Males ..... x Females ------- Total ——

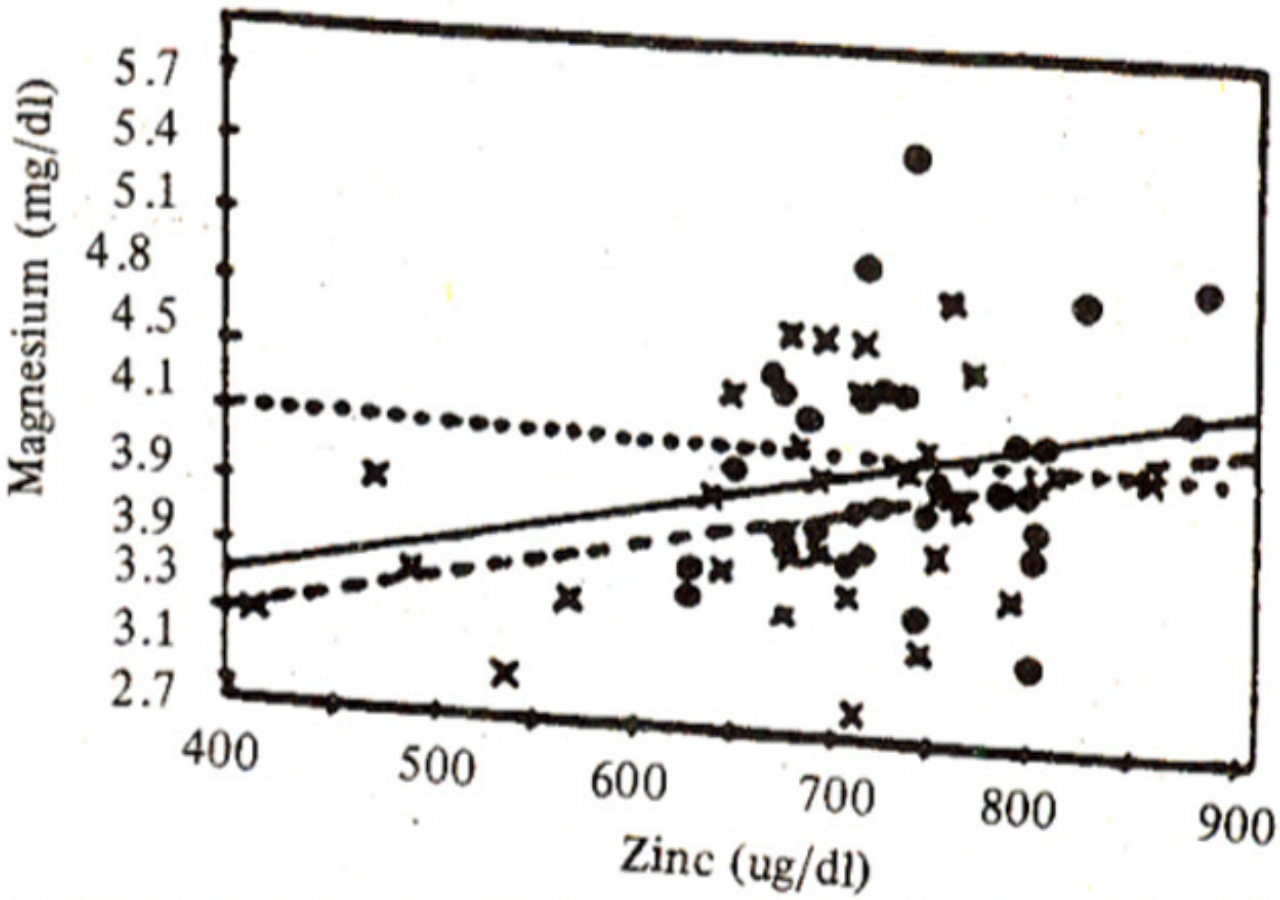

Figure 6. Blood Magnesium against Zinc Levels in Normals.
● Males..... x Females -------- Total ——

The plots are given in Figure 4-6 and the regression straight line equations and the correlation coefficients, r, are given in Table III.

TABLE III. Intermetallic Regression Equations and Correlation Coefficients for Normals.

Matals	x	y	Sex	Regression Equation	Correlation Coefficient(r)
Zn V. Cu	Cu	Zn	M	$y = -1.580x + 871.1$	– 0.247
			F	$y = 0.658x + 611.0$	0.095
			M+F	$y = -0.746x + 772.4$	– 0.108
Mg V. Cu	Cu	Mg	M	$y = -0.0107x + 2.90$	0.320
			F	$y = -7.63 \times 10^{-3} x + 4.28$	– 0.209
			M+F	$y = -2.77 \times 10^{-3} x + 3.98$	– 0.042
Mg V. Zn	Zn	Mg	M	$y = -3.08 \times 10^{-4} x + 3.95$	– 0.0479
			F	$y = 1.92 \times 10^{-3} x + 2.25$	0.367
			M+F	$y = 1.75 \times 10^{-3} x + 2.52$	0.249

DISCUSSION

It should be pointed out that none of the highly sophisticated instrumentation is yet available to us for work on body fluids, only a basic and reletively crude instrument not capable of the mesurement of elements in blood and urine below 10 ug/dl. Hence, work is restricted to copper, zinc, magnesium and lead and possibly selenium also. According to the coefficients of variation and the percentage recovery for each element, our method of analysis is satisfactory. It should be emphasised that strictest precautions should be taken against contamination and only the purest chemicals and reagents should be used.

Normal Range for Blood Copper

No significant difference was found between the ranges for males and females and hence 71 11 6 ug/di (mean 93.5) as being quoted as the normal range in whole blood for our normal population. Some normal ranges quoted in dif ferent parts of the world are given in

TABLE IV. Regional Values for the Normal range for Copper in whole Blood.

Country	Sex	Normal Range (ug/dl)	Mean (ug/dl)	Reference
Spain	M	80 – 125	105	1
	F	97 – 137	117	
Japan	M	50 – 113	82	2
	F	70 – 114	86	
Bangladesh	M+F	40 – 276	98	3
Denmark	M	71 – 255	83	4
Sweden	M+F	50 – 250	100	5
Canada	M+F	78 – 160	111,122	6
U.S.A.	M+F	31 – 281	100	7
Venezuela	M+F	43 – 260	99	7

Table IV and it is seen that there is a very wide variation. Only Japan2 and Spain1 reported differences in sex, the range for females being greater than that for males. Our mean value is less than some and greater than others of the world-wide values, but on the whole, out range is much narrower. There is

not a great difference in values for compared with serum as there is relatively little copper in red blood cells. About 75 - 88% of zinc in blood is found in the red-blood cells and therefore whole blood levels are much higher than plasma or serum levels. Levels tend to vary with time of day and after fasting are higher. Blood samples were taken mostly in mid-morning but not after fasting. Evidence seems to be conflicting whether levels in whole blood, serum, red-blood cells, leucocytes or in hair should be taken to reflect zinc status[14,15]. Serum levels may not reflect red blood cell levels[16-17]. Normal range was significantly different between the sexes, P<0.01: For males it was, 602.5 - 850 ug/dl (mean 726),females : 519 - 853 (686), males + females: 563 - 859.5 (711). Normal ranges in whole blood vary considerably world-wide and lower levels may reflect zinc deficiency in a population e.g., because of low levels in the soil. Some values are given in Table V,

TABLE V. Regional Values for the Normal Range for Zinc in whole Blood.

Country	Sex	Normal Range (ug/dl)	Mean (ug/dl)	Reference
Japan	M	420 – 720	620	2
	F	430 – 800	580	
Bangladesh	M+F	300 – 630	440	3
Sweden	M+F	400 – 800	600	5
	F	400 – 760	590	9
Canada	M+F	200 – 1039	620 – 660	6

and only from Japan2 were sexual differences reported. Like ours, the mean level for females was lower than that for males, although the range was higher. From Spain it was reported that females (normal range 462 - 708 ug/dl, mean 585) had lower levels than males (502 -7l2,mean 607) but the difference was not significant8. On the whole, zinc levels in Pakistan are higher than elsewhere as may be seen from the table.

Normal Ranges for Blood Magnesium

Normal range for males were significantly higher than that for females, 0.05 > P> 0.01 For males the values, were 2.97 - 4.80 mg/dl (mean 3.78), females : 2.65 - 4.60 (3.50),males + females: 2.75 -4.8 (3.61). A literature survey indicates no references for normal ranges in whole blood. Levels in serum are much less as only about 30% of blood magnesium is in the serum or plasma, a typical European normal range being10 L56 — 2.52 mg/dl. Black and white American males have higher normal ranges in serum than females (cf. our ranges in whole blood) and for each sex and in total , the ranges are higher for whites than blacks[11] Around the world, serum levels for males are higher than for females[11] except amongst Danes[12] and Eastern Indians[13]. Also, they vary with race[11].

Regression Analysis (Table III)

In the case of zinc versus copper, there was virtually no correlation for females and that for males and

males plus females was weak and negative. For magnesium versus copper for males there was a weak positive correlation, for females a weak negative and for the two combined, almost zero. There was virtually no correlation in the case of males for magnesium versus zinc, and weak positives for females and males plus females. The only correlations which one can comment on are those for zinc versus copper. The negative correlations obtained are to be expected. it is known that zinc tends to block the uptake of coppór from the gastrointestinal tract[18] possibly by inducing the synthesis of a copper-binding ligand in the mucosal cells thus making the copper less available for transfer to the serum[19]. Klevay proposed that copper deficiency may be an aetiological factor in the development of cardiovascular disease and certainly rats fed a diet high in zinb and low in copper developed hypercholesterolaemia and cardiac abnormalities. There is evidence of this happening in humans too[20].

ACKNOWLEDGEMENTS

We wish to thank the Aga Khan University Medical College for a grant in support of the research, and to thank Ms. Seema Mohamedi for typing this report.

REFERENCES

1. Buxaderas, S.C. and R. Farre-Rovira. Whole blood and serum copper levels in relation to sex and age. Rev. Esp. de FisioL, 1986; 42 213.
2. Satoh, Y. and Yazawa, A. Contents of heavy metals in the blood of inhabitants in Yokohama city in Japan. Yokohama Eiken Nenpo, 1978;17: 63.
3. Khan, A.H., Khaliquzzam, M., Zaman, M. B., Husain, M., Abduila, M. and Akhtar, S. Trace element composition of blood in adult population in Bangladesh. 3. Radioanal. them., 1980; 57: 157.
4. Clausen, J. and Rastogi, S.C. Heavy metal pollution among autoworkers. Br. 3. lad. Med., 1977; 34:216.
5. Brune, D., Frykberg, B., Samsahi, K. and Wester, P.O. Determination of elethents in normal arid leukaemic human whole blood by NAA Report AE—60. Stockhohn, Sweden, 1962.
6. Ward, N.I., Stephan, R. and Ryan, D.E. Comparison of three analytical methods for the determination of trace elements in whole blood. Anal. Chim. Acta, 1979; 11th 9.
7. Hecker, L.H., Allan, N.E., Dinman, B.D. and Neel, J.V. Heavy metal levels in acculturated and unacculturated populations. Arch. Environ. Health, 1974; 29: 181.
8. Buxaderas, S.C. and Farre - Rovira, R. Whole blood and serum zinc in relation to sex and age. Rev. Esp. Fisiol., 1985; 41 463.
9. Abdulla M.,Jagerstad, M., Kolar, K., Nordern, A., Schutz, A: and Svenson, S. Essential and toxic elements in prepared meals; 24 hour dietary sampling employing the duplicate sampling technique, Trace Element Analytical Chemistry in Medicine & Biology, K Bratter & P. Schramel, (Eds.) 1982, p. 75.
10. Tietz, N.W.ed. Fundamentals of clinical chemistry Philadelphia, Saunders, 1976.
11. Lowenstein, F. W. and Stanton, ME. Serum magnesium levels in the United States, 1971 — 1974. 3. Am. Coil. Nutr., 1986; 5:399.
12. Peterson, B., Scholl, M., Christansen, C. and Transbol, I. Serum and erythrocyte magnesium in elderly Danish people. Relationship to blood pressure and serum lipids. Acta. Med. Scand., 1977; 201 : 31.
13. Chawla, L.S., Verman, P.N. and Pun, ViC. Study of trace elements (Zinc, Iron, Copper and Magnesium) in normal healthy population. J. Assoc. Physicians India, 1983; 3 :141.
14. Gibson, R.S., Anderson, B.M. and Scythes, C.A. Regional differences in hair zinc concentra tions; a possible effect of water hardness. Am. J. Clin. Nutr., 1982; 37: 37.

15. Danks, D.M. Diagnosia of trace metal deficiency with emphasis on copper and zinc. Am. J. Clin. Nutr., 1981; 34: 278.
16. Kosman, OJ. and Henkin, RI. Erythrocyte zinc in patients with taste and smell dysfunction. Am. 3. CUn. Nutr., 1981; 34: 118.
17. Robson, J.R.K. and Spell, L. Erythrocyte zinc. Am. 3. Clin. Nutr., 1981; 34:1983.
18. Van Campen, D,R. and Scaife, P.U. Zinc interference with copper absorption in rats. J. Nutr., 1967;91 :473.
19. Fischer, P.W.F., Giroux, A. and L'Abbe, M.R. The effect of dietary zinc on intestinal copper absorption. Am. J. Clin. Nutr., 1981; 34 :1670.
20. Medeiros, D.M. The copper; zinc hypothesis and cardiovascular disease. Biochem. Arch., 1985; 1: 67.