DETERMINATION OF SERUM ZINC LEVELS IN NORMAL MALTESE ADULTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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Introduction

The importance of zinc as one of the essential trace elements in the living organisms is becoming increasingly recognised. Raoult and Breton (1877) were the first to report its presence in the human organism, having detected it in the liver. Because of difficulties in methodology, investigations were for a long time of a purely qualitative nature. Keilin and Mann (1944) isolated and purified the first metalloenzyme carbonic anhydrase, containing 0.33 per cent zinc as part of its molecule. This enzyme is found in high concentration in red blood cells, gastric acid-secreting cells and in renal tubular cells. Zinc was shown to be essential to the mechanism of action of this enzyme, which catalyzes the reaction: $CO_2 + H_2O \rightleftharpoons H_2CO_3$. Zinc was thus confirmed as an essential trace element and a significant role in metabolic processes assigned to it.

In the process of carrying out measurements to establish normal reference values for our laboratory, it was noticed that the values being obtained, generally appeared to be higher than those published in the

Table I

Normal Values for Zinc in Serum or Plasma

Reference		No. of subjects	Sample	Mean ± S.D. (μg Zn/100 ml)
Prasad *et al.*	(1965)	14 men	plasma	104 ± 14
Davies *et al.*	(1968)	30 men	plasma	96 ± 12
		30 women	plasma	97 ± 11
Pekarek *et al.*	(1972)	99 men	serum	102 ± 11
Oon *et al.*	(1974)	33	serum	102 ± 9
Beeley *et al.*	(1974)	57	serum	89 ± 11
Thind and Fescher	(1974)	15	plasma	113 ± 4

literature where similar techniques had been employed (Table I). Results obtained from a few North European (Scandinavian and British) medical students clerking in Malta fell within the range of published normal values. It was thus decided to obtain samples from other North European students attending a Summer course at the University, and to compare the results with those of the local population.

Subjects and methods

Altogether 61 subjects were studied. Venous blood was obtained with minimal venostasis, the blood being withdrawn into a plastic syringe. The blood was allowed to clot, after which the supernatant was removed, and centrifuged at 1500 revolutions per minute for an hour, in order to remove any remaining blood cells. Serum specimens in which haemolysis had occurred were discarded. Plasma proteins were precipitated with 10% trichloracetic acid (T.C.A.) as described by Davies, Musa and Dormandy (1968). The specimens were again centrifuged to remove the resultant precipitate.

The zinc concentration in the protein-free supernatant was determined using a Unicam S.P. 90A atomic absorption spectrophotometer with an air-acetylene flame. Standard curves (10, 20, 30, 35, 40, 45, 50, 55, and 60 μg/100 ml) were obtained using a standard zinc solution (1 mg/ml, Analar) diluted with deionized water before each series of measurements. The standards were made in sodium chloride solution (50 m mol/1), which is the approximate final sodium concentration in the diluted serum, as it has been reported that sodium reduces absorbance readings for zinc (Prasad, Aberleas and Halsted, 1966). Equal amounts of T.C.A. were added to the standard solutions and the serum samples. Individual aliquots of serum were diluted 1 : 3 with deionized water, Samples of deionized water and T.C.A. showed that no extraneous zinc was present either in the reagents or in the centrifuge tubes. Serum samples from Maltese subjects were run concurrently with those obtained from foreigners. Significance was tested by Student's paired t-test (Snedecor & Cochran, 1971). All computations were performed on

Table II

Serum Zinc levels in Maltese and Foreign Subjects

Nationality	Sex	No. of subjects	Mean ± S.D. (μg Zn/100 ml)
Maltese	males	31	124 ± 16
Maltese	females	12	117 ± 13
Foreign	males	11	107 ± 9
Foreign	females	7	110 ± 15

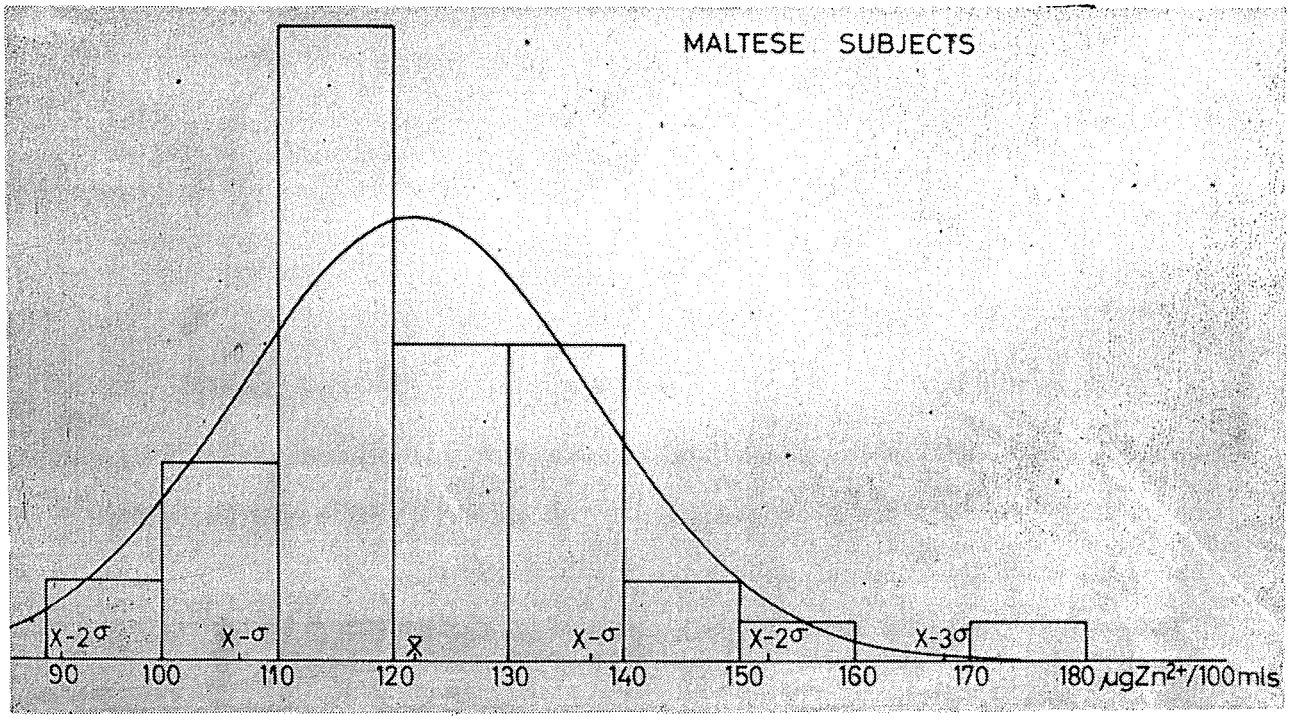

Fig. 1 Histogram and distribution curve for serum zinc values in Maltese subjects. (X = mean, δ = 1 standard deviation).

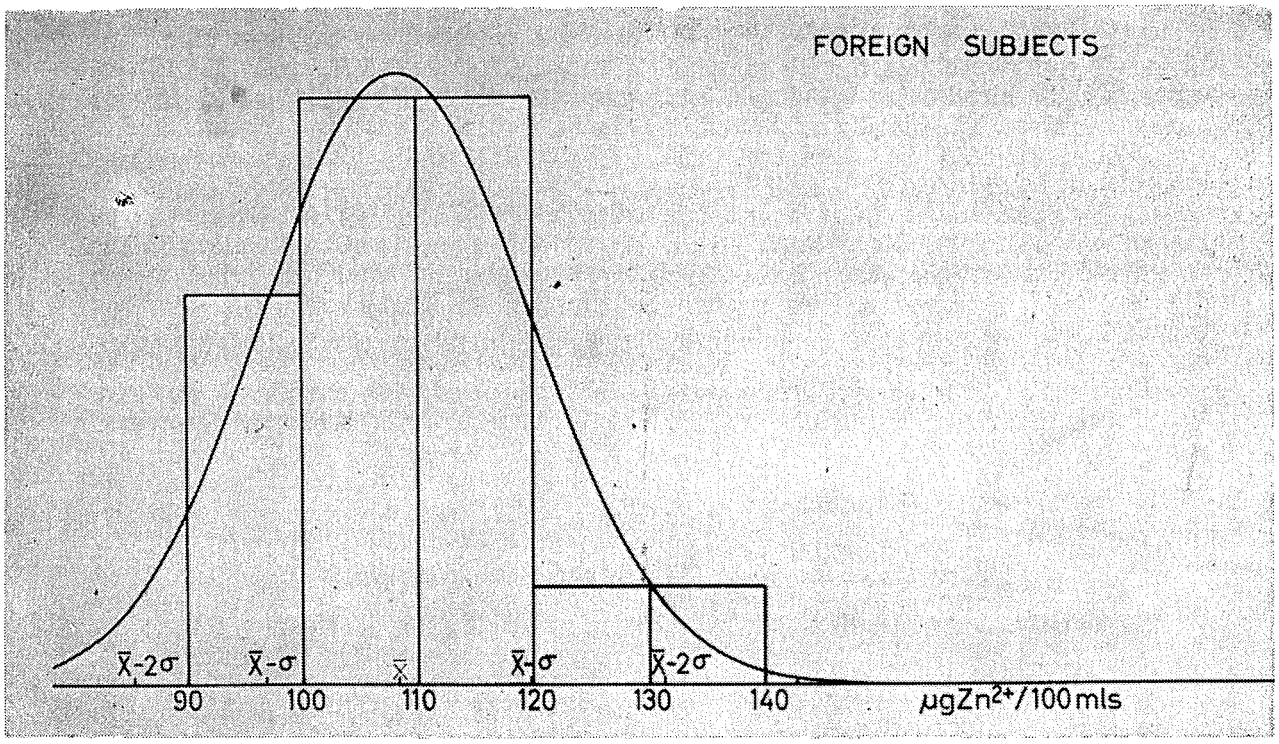

Fig. 2 Histogram and distribution curve for serum zinc values in foreign subjects. (X = mean, δ — 1 standard deviation).

a Hewlett-Packard 9100B Calculator fitted with a 9101A Extended Memory.

Results

The distribution of serum zinc levels in Maltese and in foreign subjects as shown in Table II and figures 1 and 2. The mean value for Maltese males was 124 μg/100 ml, for Maltese females 117 μg/100 ml. The corresponding values for foreign subjects were 107 μg/100 ml and 110 μg/100 ml. No statistical sex differences in serum zinc concentrations were present, either in the local ($P < 0.10$) or foreign ($P < 0.35$) subjects. This is in agreement with previous published data (Davies, Musa & Dormandy, 1968). Maltese subjects showed significantly higher serum zinc levels, 122 μg, than the values obtained from foreign subjects, 108 μg ($P < 0.01$).

Discussion

Zinc has now been detected in all the cells and fluids of the human organism and the zinc content of the human body is equal to that of iron and about ten times higher than the copper content (Mikac-Devic, 1970). Zinc is now recognised to be an integral constituent and cofactor of a number of enzymes. The presence of zinc is required for collagen synthesis (Fernandez-Madrid, Prasad and Oberleas, 1971), and alkaline phosphatase, which is a zinc-containing metallo-enzyme, is essential for normal bone formation. Recent evidence indicates that it may have a fundamental role in DNA, RNA and protein synthesis.

True zinc-deficiency states, although reported, are rare (Prasad, Halsted & Madami, 1961). Plasma or serum zinc concentrations have been measured in a large number of different clinical states. Reduced levels have been reported in patients with chronic pulmonary infection (Halstead & Smith, 1970), bronchial carcinoma (Beeley, Darke, Owen & Cooper, 1974) leprosy (Oon, Khong, Greaves and Plummer, 1974), chronic renal failure (Mahler, Walsh and Haynie, 1971), in hypertensive patients with renal artery stenosis (Thind and Fescher, 1974) and in epilepsy (Barbeau and Donaldson, 1974), amongst other conditions. The significance of the change in zinc concentration in these conditions remains undetermined.

It is important to gain further understanding of this metal in both health and disease as it appears that zinc is essential to an important variety of metabolic processes in man. Under normal conditions zinc maintains a fairly stable consistency in the body fluids and tissues, in spite of a rapid and continuous turnover (Pekarek, Beisel, Bartelloni and Bostian, 1972). However, Kubota and his associates in 1968 reported a 5-fold difference in zinc values measured in blood from 243 subjects from 19 different locations in the United States. They suggested that this may be due to the influence of air contaminated with zinc and to different dietary conditions. We are unable to offer a satisfactory explanation for the difference in serum zinc levels in the two groups reported in this paper. Local water does not appear to have a high zinc content and Maltese soil is known to be zinc deficient. It is known however that local farmers use a zinc containing fungicide (Zineb) on a large scale. 'Zineb' is zinc ethylene 1, 2 bis-dithiocarbamate and is sprayed on potato and onion crops and on vines for foliage protection. Serum zinc values increased to the reported levels appear to have no untoward effect and no clinical condition has as yet been reported in which zinc levels are elevated. This, of course, excludes cases of zinc toxicity as a result of zinc oxide fumes inhalation, where levels of serum zinc are markedly increased. 'Zineb', however, is a carbamate and therefore has goitreogenic effects, although these are reported to be very small when it is used in the recommended concentrations. It is perhaps important that even greater emphasis should be laid on explaining to local farmers the possible dangers of this fungicide when recommended dosage schemes are exceeded.

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