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Measurements of plasma zinc

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Part I In health and disease

SYNOPSIS Zinc is an essential trace element. Previous methods of measuring zinc in clinical material have been difficult and reported findings must be treated with caution. Using atomic absorption spectroscopy it has been established that plasma zinc is one of the most uniform biochemical characteristics of normal adult blood. Sex and age differences in adult life are insignificant. Increased metabolic activity, on the other hand, induces a marked, immediate fall in plasma zinc level. The possible implications of this are discussed. Zinc levels in patients with diabetes mellitus, cardiovascular disease, and anaemia due to acute blood loss have been within normal limits. Plasma zinc is low in certain types of liver disease.

Zinc in trace concentrations is essential for growth and health. The first metalloenzyme to have been identified, carbonic anhydrase (Keilin and Mann, 1940), is a zinc compound, and several others have been described since (Mathies, 1958; Vallee and Hoch, 1956; Li, 1966). Among zinc-deficiency syndromes in animals porcine parakeratosis has been successfully treated with zinc supplements (Tucker and Salmon, 1955; Underwood, 1962), and in man a syndrome of anaemia, hypogonadism, hepatosplenomegaly, and dwarfism, prevalent in parts of Iran and Egypt, has been ascribed to lack of zinc in the diet (Prasad, Miale, Faid, Sandstead, and Schulert, 1963; Sandstead, Shukry, Prasad, Gabr, El Hifney, Makhtar, and Darby, 1965). Zinc is essential for the growth and propagation of all cell cultures, and recent clinical studies suggest that it may be a limiting factor in normal wound healing (Savlov, Strain, and Huegin, 1962; Pories, Henzel, Rab, and Strain, 1967).

Blood-zinc levels in health and in various diseases have been measured by a number of workers, notably by Vallee and Gibson (1948) and by Vikbladh (1950, 1951). The dithizone method used in these early studies is difficult and the potential sources of error are numerous. Both on account of these difficulties and because of the relatively small numbers of measurements performed many other of these early reports must be discounted and conclusions based on them treated with reserve. Atomic absorption spectroscopy, introduced into biological work during the past decade, is particularly suited to the estimation of zinc (Willis, 1963; Zettner, 1964; Prasad *et al.*, 1963), but, though the technique is simple and quick, the rigorous avoidance of contamination and the elimination of other interfering factors is no less essential. We report here a study of plasma zinc levels in healthy adults and in patients based on over 1,000 measurements.

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MATERIAL

Our normal adult range of plasma zinc was established in a series of 67 healthy doctors and nurses between the ages of 19 and 58. Physiological variables were studied both in these normals and in 200 hospital patients. Possible pathological changes were investigated in groups of diseases in which abnormal plasma zinc levels have been reported in the past. These included liver disease, acute gastrointestinal haemorrhage, malabsorption, cardiovascular disease, diabetes, and malignant disease. The last group will be discussed separately.

METHOD

Venous blood for plasma zinc estimations was collected into disposable plastic tubes containing 8·4 mg dilithium EDTA for 10 ml blood. The time relation to the last meal was noted. Disposable plastic syringes and needles were used. The blood was spun within an hour of collection, and specimens showing even minimal signs of haemolysis were discarded. When necessary, the plasma was stored at 4°C. The estimations were performed on a Unicam S.P.90 atomic-absorption flame spectrophotometer, equipped with a propane burner head with a slot width of 10 × 0·1 cm and using propane as fuel and air

as oxidiser. Deionized water was used for dilution and for the final rinsing of specially cleaned glassware. The danger of contamination was reduced by reducing the steps in the preliminary processing of the samples. Equal volumes of 10% (w/v) trichloracetic acid (certified highest reagent grade, British Drug Houses Ltd) and plasma were thoroughly mixed in sterile, chemically clean disposable bottles and allowed to stand for 10 minutes. After centrifugation at 3,000 r.p.m. for 10 minutes the supernatants were transferred into another set of chemically clean plastic containers and then aspirated into the burner. Standard solutions were prepared from the highest purity zinc obtainable from Koch-Light Laboratories Ltd and concentration-absorbance curves were constructed from standards containing 5% (w/v) trichloracetic acid. The settings on the instrument were as follows: wavelength 2139 Å; air pressure 30 lb/sq in; flow rate 5 litres on the flowmeter; propane, 10 lb/sq in and a setting of 450 ccm on the flowmeter; burner height 15 mm; slit width 0·25 mm; lamp current 10 mAmp.

RESULTS

Figure 1 shows the distribution of normal plasma zinc values in 31 normal women and 36 normal men. The specimens were collected during the latter part of a normal working day, one to three hours after the last meal. The mean value for women is 96 μg/100 ml (SD 10·5). The mean value for men is 95 μg/100 ml (SD 13). For our studies of physiological and pathological variations we have accepted 76 to 125 μg % as our normal adult range for either sex. Under the conditions specified only about 3% of normals can be expected to fall into the 70 to 75 or 126 to 130 μg % range and less than 0·1% can be expected to have plasma zinc values of under 70 or over 130 μg %.

Figure 2 shows the results in our normals and

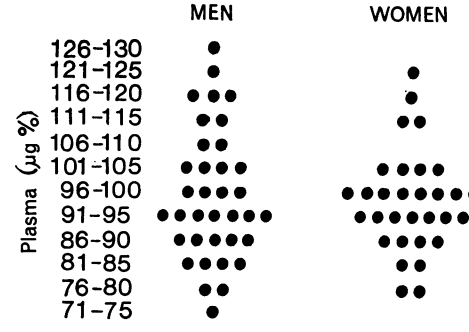

FIG. 1. *The distribution of non-fasting plasma zinc levels in 67 normal controls.*

patients, excluding cases of malignant disease, grouped according to age in decades. There is no significant change in plasma zinc attributable to age between 20 and 60 years.

Preliminary observations of diurnal and postprandial variations (both in normals and in patients) led us to investigate the effect of changes in overall metabolic activity on plasma zinc. Figures 3 and 4 illustrate the effect of standardized glucose loads by mouth and intravenously. Within 10 minutes of drinking 50 g glucose in 100 ml (zinc-free) water, the plasma zinc began to fall in all our normal controls and in almost all patients. Lowest levels were reached within 40 to 60 minutes, and there was a return to near fasting levels within two hours. After intravenous glucose, 0·5 g per kilogram body weight, there was a similar but even steeper fall and recovery. Parallel measurements of changes in total plasma osmolality, other plasma electrolytes and plasma

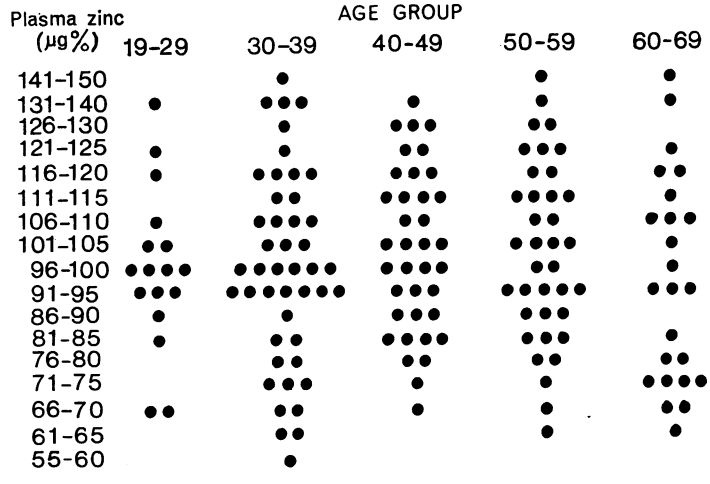

FIG. 2. *The non-fasting plasma zinc levels in 67 normal controls and 104 patients (excluding cases of malignant disease) grouped according to age in decades.*

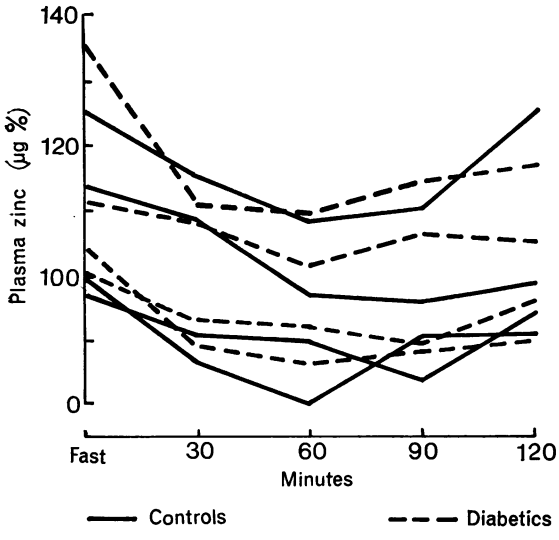

FIG. 3.

FIG. 3. *The changes in plasma zinc levels in three normal controls and in three mild diabetics (not on insulin) after 50 g glucose by mouth.*

FIG. 4. *Changes in plasma concentrations expressed as a percentage of the fasting concentrations (100%) in three normal controls after an intravenous glucose load of 0·5 g glucose per kilogram body weight.*

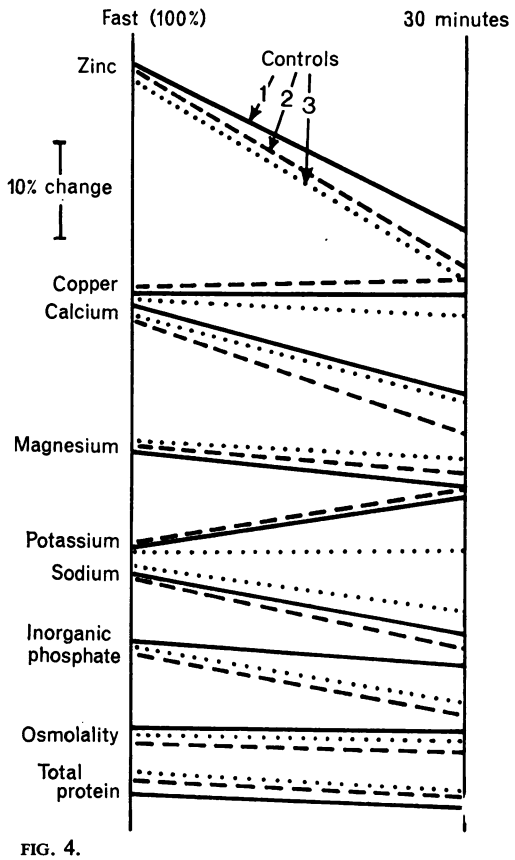

FIG. 4.

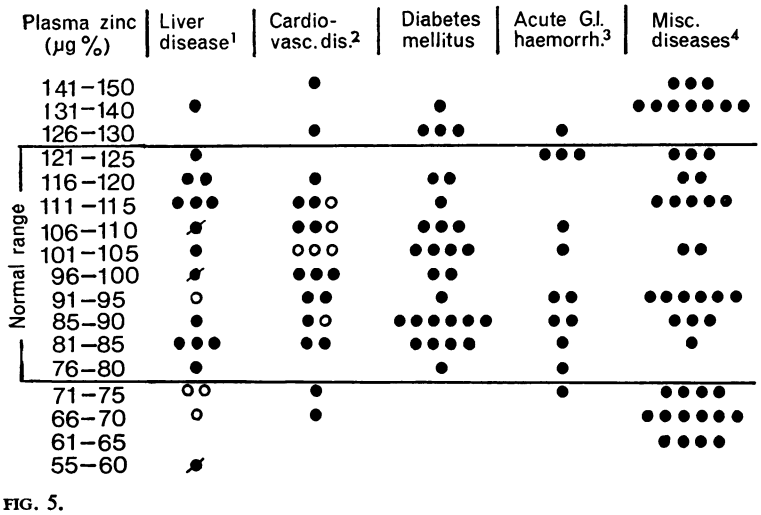

FIG. 5.

FIG. 5. *Non-fasting plasma zinc levels in disease.*

1 ○ = *alcoholic cirrhosis;* ● = *infectious hepatitis;* ◉ = *the same patient, a case of intermittent porta-systemic encephalopathy two years after a shunt operation.*

2 ○ = *patients within two weeks of coronary infarction.*

3 Patients within a week of a major acute gastrointestinal haemorrhage.

4 Patients admitted for chronic bronchitis (3 cases), rheumatoid arthritis (3 cases), malabsorption (5 cases), chronic renal disease (4 cases), degenerative nervous disease (4 cases), pyrexia of unknown origin (3 cases), chronic skin ulceration (6 cases), and single cases for other medical disorders. Patients with malignant disease are not included.

proteins showed that the fall and rise in plasma zinc could not be accounted for by dilution.

Figure 5 shows the distribution of plasma zinc levels in patients with liver disease, cardiovascular disease, acute gastrointestinal haemorrhage, diabetes, and a miscellaneous group of illnesses.

DISCUSSION

Our results show that in health plasma zinc is one of the most uniform biochemical characteristics of blood, individual variations being very much smaller than variations in plasma copper, magnesium, or calcium. Its remarkable constancy is maintained despite a continuous and relatively massive zinc turnover both within the body and between the body and its environment. That this turnover is an immediate and essential requisite of normal metabolic activity is suggested by the striking fall in plasma zinc after physiological loads of oral glucose. (The possibility that such a fall might reflect a state of temporary depletion due to loss in the exocrine pancreatic juice, a body fluid particularly rich in zinc, can be excluded by the even more marked fall after intravenous glucose.) It may be noted that plasma copper shows no such acute metabolic fluctuations.

The plasma zinc response to glucose does not, of course, give any indication as to the primary sites where the metal is required either in metabolic or in anatomical terms. A certain amount of indirect evidence suggests, however, that the immediate need for extra zinc arises from a rise in the nucleic acid and protein turnover rate rather than from changes in carbohydrate and fat metabolism (Winder and Denneny, 1959; Underwood, 1962). In this connexion it may be relevant that in our series of diabetics, many with grossly abnormal glucose tolerance curves and free fatty acid and insulin responses to glucose, the fall in plasma zinc was of the same order as in our normal controls. In terms of body systems we have seen an abnormal flat response in one patient with recurrent subacute pancreatitis and pancreatic insufficiency. It seems reasonable to suggest that a more detailed study of the plasma zinc response after different kinds of metabolic loads in different groups of people may provide a clue, hitherto lacking, to the main primary sites of zinc utilization.

At the clinical level the plasma zinc response to food raises the question of whether zinc estimations should be confined to fasting plasma samples. (Statistically fasting plasma zinc values give an approximately 12% higher normal mean.) At this stage of clinical pilot studies this would seem to us a mistake since it would seriously limit the number

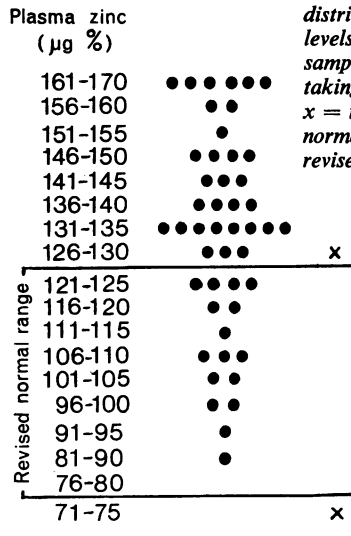

FIG. 6. *The 'normal' distribution of plasma zinc levels based on blood samples collected without taking special precautions. x = two out of 67 normals falling outside the revised normal range.*

of acceptable specimens. For routine estimations we would suggest that specimens should be collected at any time of the day but not within an hour after the last meal. In patients whose random plasma zinc estimation is within 5 μg % of the upper or lower limit of normal, the estimation should be repeated on two further blood samples, the first fasting and the second taken 40 to 60 minutes after a standard glucose load.

Routine zinc estimations, whether fasting or random, will be of no value unless a few simple but stringent technical rules are observed. In formulating a dependable routine, two points in particular need to be remembered. First, zinc is an ubiquitous contaminant of glass, lead, and rubber piping, water, and of many chemicals even of the highest analytical grade. Second, the zinc content of all cells (including blood platelets) is many times that of plasma. During the early stages of the present study we performed over 100 zinc estimations on blood samples collected with ordinary sterile glass syringes into 'chemically clean' glass bottles with metal caps. A number of these specimens were allowed to stand unspun for several hours. Although specimens showing obvious haemolysis were discarded, the rule was not applied to all plasmas showing faint discoloration. Apart from these preliminary steps the technique of estimation was the same as in the later series. The results in a series of normal controls, in every way comparable to the series used for establishing our revised normal range, are shown in Figure 6. Such a discrepancy inevitably raises doubts about the reliability of many zinc studies reported in the literature.

Among the diseases which we have studied, our series do not confirm that plasma zinc undergoes characteristic changes in diabetes, after acute gastrointestinal haemorrhage, in congestive heart failure, or after cardiac infarction. On the other hand, unexpected and significant changes in some forms of malignant disease and of superficial skin ulceration have led us to a more detailed investigation of these groups of patients. Our patients with liver disease formed a somewhat heterogeneous group and their number is still too small for statistical analysis. Vallee and others (Vallee and Hoch, 1956; Vallee, Wacker, Bartholomay, and Hoch, 1957; Vallee, Wacker, Bartholomay, and Robin, 1956), have reported a significant lowering of plasma zinc in alcoholic cirrhosis. In three of our five cases the plasma zinc was consistently below the lower limit of normal; in one it was repeatedly normal; and in one (two years after a portacaval shunt operation) it showed great fluctuations. Patients at various stages of infective hepatitis (all jaundiced) had normal plasma zinc levels, and, contrary to some earlier reports, we have found no correlation between plasma zinc levels and serum enzyme activities. In two patients with a clear history of chronic alcoholism but with little demonstrable impairment of liver function the plasma zinc was normal.

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Part II In malignant disease

SYNOPSIS The plasma zinc and plasma copper was estimated in 81 patients with malignant disease. There was a striking decrease in plasma zinc levels in carcinoma of bronchus compared both with normals and most other types of malignancy.

Studies on the relation between zinc and malignant disease are numerous but, on the whole, desultory and inconclusive. Low zinc concentrations have been reported in leukaemic cells compared to normal white blood cells (Gibson, Vallee, Fluharty, and Nelson, 1950; Dennes, Tupper, and Wormall, 1961); ^{65}Zn studies suggest that the zinc turnover rate in malignant prostatic tissue is considerably slower than in the normal gland (Prout, Sierp, and Whitmore, 1959; Hoare, Delory, and Penner, 1956), and whole blood zinc was depressed in most of the 31 patients with carcinoma investigated by Addink and Frank (1959). On the other hand, high zinc concentrations have been reported in some experimentally induced tumours (Cristol, 1927). The present study was prompted by the unexpected finding of extremely low plasma zinc levels in some patients with carcinoma of the bronchus.

MATERIAL AND METHODS

Non-fasting plasma zinc was measured in 88 patients with malignant disease. Doubtful cases or samples from patients in whom insufficient clinical or laboratory data were available were excluded. In view of suggestions that several trace metals may be involved in an abnormal metabolic pattern, the estimation of plasma copper was added to the list of routine investigations. The same blood samples, processed in parallel, were used for measuring the two metals. A preliminary study of plasma copper in 70 healthy adults established our normal range as 80 to 160 μg %. The technique for collecting and preparing the plasma and the method of