AETIOLOGICAL AND PATHOPHYSIOLOGICAL IMPLICATIONS OF ZINC IN DIABETES MELLITUS : A STUDY OF PLASMA AND URINARY ZINC LEVELS IN INDIGENOUS DIABETIC ZAMBIANS.

BY

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APPROVAL

This dissertation of Dorothy Kalangu Mulalami Kasonde is approved as fulfilling part of the requirements for the degree of Master of Medicine by the University of Zambia.

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A STUDY OF PLASMA AND URINARY ZINC LEVELS IN INDIGENOUS DIABETIC ZAMBIANS.

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I - INTRODUCTION

1.1 Objectives of the study

Normal nonfasting plasma zinc levels are reported to fall within the range 70 -240 microgram 100 ml[1,2]. In man the principal path way of zinc excretion as determined by Zn^{65} studies appears to be via faeces[3]. The daily urinary excretion of zinc has been described to lie in the range of 300 - 500 µg per day[4,5]. In diabetes mellitus there is as yet unexplained disorder of the zinc metabolism demonstrated by hyper zincuria. Reports on plasma zinc in diabetics have been conflicting[2]. Sullivan et al found that plasma zinc levels in diabetics tended to be lower than normals but not significantly so,[6] other workers found levels in plasma or serum to be higher in diabetics than normals.[7,8] Meltzer et al appear to be the first to have studied the pattern of trace elements in diabetes mellitus,[9] they showed that diabetics excreted more zinc than non diabetics. Further studies on zinc excretion have confirmed and have failed to confirm the work of Meltzer.[10,11]

These conflicting reports prompted me to undertake this study. The aim of the study is to document the plasma zinc and twenty four hour urinary zinc levels in the Zambian diabetic patients and to see if any

relationship exists between the plasma glucose level, the duration of diabetic state, serum cholesterol level, various diabetic complications and type of treatment with the level of plasma and urinary zinc levels.

To the best of my knowledge this type of work has not been done before in this group.

1.2 Brief description of Diabetes Mellitus

DIABETES MELLITUS

Diabetes mellitus is a clinical syndrome characterized by hyperglycaemia due to deficiency or diminished effectiveness of insulin. The disease is chronic and affects the metabolism of carbohydrate, protein, fat, water and electrolytes sometimes with permanent and irreversible functional and structural changes in the cells of the body, those of the vascular system being particularly susceptable. The changes lead in turn to the development of well defined clinical entities, the so called 'complications of diabetes', which most characteristically affect the eye, the kidney and the nervous system.[12] The disease is the most common of the serious metabolic diseases of the humans. The true frequency in the general population is difficult to ascertain because of widely differing standards of diagnosis, but probably is somewhere between two and six percent.[13] In Zambia prevalence studies have not been done. In the University Teaching Hospital in Lusaka where this study was carried out, an average of 9.900 adult patients are admitted to the medical emergency admission ward for various medical problems every year, out of these 336 are admitted for diabetes

mellitus giving a 3.4 percent of the total admissions per year. The diabetic clinic at UTH has 814 diabetics[14] on its list.

The aetiology of diabetes is complicated. In the great majority of cases there seems to be no single cause of the condition. This is hardly surprising since diabetes itself is not a discrete and specific disorder, rather it is a departure from normality[13].

Among the factors which we think are important in the aetiology of diabetes mellitus are:

- age
- sex
- body weight
- inheritance
- virus infection
- immune deficiency
- trauma
- alcohol

and recently workers have focused their attention on the effects of zinc ion on the confirmation of antigenic determinants on insulin as a possible cause of altered insulin activity in the diabetic.

1.3 Brief description of relationship of zinc ion to Insulin molecule.

Zinc is an essential trace element that is directly involved in the physiology of insulin. Dorothy Hodgkin a nobel prize winner was the first to elucidate the three dimensional structure of insulin. She showed that six molecules of insulin are joined together with two atoms of zinc to give a stable crystalline substance which is presumed to be present as the storage form of insulin in the Beta cells of the islets of Langhan[13]. This is the active form of insulin that is released in the portal venous system at the time of Beta -cell degranulation in response to increased blood sugar level in the blood. Structurally, the addition of zinc to insulin results in conformation changes which have been detected by ultra violet circular dichroic spectroscopy[15]. There are several reasons to suspect that abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus and some of its complications.[16] A correlation has been shown between zinc and insulin concentration in the diabetic pancreas,[17] the diabetic pancreas containing some fifty percent less zinc than the non diabetic and some twenty five percent less insulin. Following this study it was found that experimental diabetes could be induced by treating rabbits with

certain chelating agents in particular alloxan[18]. It was suggested that alloxan produced its effects either by an action on the insulin/zinc complex or on the islet cells of the pancreas . It has been shown that pretreatment of insulin with zinc causes an accelerated and increased magnitude on the binding and simultaneously inhibits the degredation of insulin by the liver plasma membrane[19].

II - MATERIALS AND METHODS

2.1 Selection of cases

Confirmed diabetic patients were entered in the study. The patients were selected from two sources.

a) patients attending the diabetic clinic at this hospital.

b) patients with florid symptoms of diabetes who were admitted to the emergency admission ward of the University Teaching Hospital.

The study was fully explained to the patients and only those who gave verbal consent were entered in the study.

Sixty controls were selected from adult healthy Zambians attending medical clinic for minor ailments or for medical check up, in some instances patients recovering from malaria or pneumonia were included. None of these subjects were on drugs or were suffering from disease which affects the blood sugar. None of the controls had positive family history of diabetes. Controls were not matched for age or sex because earlier studies have shown that there is no age or sex effect on the level of zinc in plasma or urine.[20]

The diabetic state was defined as any patient showing a fasting blood sugar of more than 7 mmol/L on more than three occassions. For borderline cases, WHO

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expert committee on diabetes[21] recommendations were used and patients showing more than 10 mmol/L after 75 g of glucose load were accepted as diabetics. The concentration of glucose in venous whole blood estimated by a specific enzyme assay should be as follows:- (values for plasma are 15% higher than those for whole blood) -

	Normal	Diabetic
Fasting	$<$ 5.5 mmol/L	$\geq$ 7.0 mmol
2 hr. post prandial	$<$ 7.0 mmol/L	$\geq$ 10.0 mmol

The period of study ranged from January 1985 to September, 1985. A total of 160 subjects were entered in the study 100 diabetics and 60 controls. Of the diabetics sixty seven (67%) were males and thirty three (33%) were females. For the controls twenty two (36.7%) were females and thirty eight were males (63. (63.3%). All subjects were residents of Lusaka. For proper supervision of twenty four hour urine collection and uniformity of diet before collection of samples all subjects were admitted to one of the medical wards of the University Teaching Hospital, for a minimum of thirty six hours. Anthropometric data was collected from all subjects, for diabetics the number of years since diagnosis, present symptoms, present therapy, family history, history of alcohol ingestion and

history of viral infections were noted. A full physical examination which included measurement of height, weight, pulse rate, blood pressure in recumbert and erect position, and examination of cardiovascular respiratory, gastrointestinal and central nervous systems, with particular attention in elucidation of signs pertaining to complications in the four target organs namely:- the eye, nervous system, kidney and the vascular bed.

The eye was examined for evidence of cataract and retinopathy, fluorescin angiography facilities are not available so this was omitted. Ischaemic heart disease was excluded by E.C.G. Chest x-ray was done to exclude chronic lung diseases. Pancreatic calcification was looked for in the plain x-ray of the abdomen.

2.2 Exclusion criteria

Extreme care was taken to exclude diabetic patients with other concomitant conditions known to alter plasma zinc levels like:-

> Laenec's cirrhosis, acute and chronic alcoholism, multiple myeloma, lymphoma, pernicious anaemia, pregnancy, epilepsy, women on contraceptive pills[2] and skin diseases[22].

2.3 Collection of samples

All subjects were bled between 08.00 hours and 09.00 hours in a fasting state approximately twenty four hours after admission to hospital. Twenty milli litres (cc) of non occluded blood was collected in two ten milli litre disposable (Heliti Pharmaceutical GMBH) plastic syringes, with disposable needle (Tuttling GMBH). The blood was divided as follows:-

i) - 10 cc was transferred in 10ml plastic bottle containing Lithium heparin Brunswick LH/10 for plasma zinc estimation.

ii) - 2cc of blood was transferred in a 5 ml tube containing fluoride oxalate (Sterilin Fx/2.5) for glucose estimation[23].

iii) - other estimations were:-

i) blood urea nitrogen[24]

serum cholesterol[25]

serum triglyceride[26]

Haemoglobin and erythrocyte sedimentation rate[27].

Samples for plasma were centrifuged at 3,000 r.p.m for 10 minutes within three hours of collection and the plasma pipetted into a metal free plastic tube. Plasma specimens showing even minimal signs of haemolysis were discarded because it has been shown that blood cells and platelets contain significant amounts of zinc.[28] Plasma specimen were stored at -20°C until zinc analysis. The rest of the samples were analysed on the same day.

Urine was collected in a two and half litre glass bottle, patients were asked to collect twenty four hour samples and the importance of avoiding contamination of the sample with dust and tap water was stressed, the total volume was measured and twenty millilitre sample was stored in a plastic metal free bottle at -20° until zinc analysis. The samples were kept in this frozen state for a period ranging from two weeks to seven months while awaiting analysis. Total protein, urinary creatinine sodium

and patassium was also estimated on the twenty four hour urine sample. Direct urine microscopy and urinalysis using 'Ames' multistix reagent strips was carried out on a fresh sample of urine, any subject with more than 5-10 pus cells per high power field was excluded from the study.

III - CLEANING OF EQUIPMENT

Trace element analysis is subject to contamination from a number of sources and for this reason all glass polyethylene equipment was leached in potassium perchlorate overnight to remove contaminating cations and finally rinsed in distilled water and dried in the oven or allowed to drain dry prior to collection or analysis of samples. Disposable equipment like syringes, needles, blood collection tubes and separating apparatus were checked for contamination by filling with distilled water standing overnight and then analysing for zinc.

Analyses

There are a number of ways of preparing plasma for atomic absorption spectrophotometry (AAS). Piper and Higgins[29] found that a simple dilution of one part plasma to four parts deionized water was quite satisfactory. Parker et al[30] are in agreement with this method but, in addition, found that for accurate results the standards should be of the same viscosity as the unknowns. They therefore, made up their standards in three percent (3%) bovine albumin.

The other commonly used method is that described by[28] Prasad et al. In this method two millilitres plasma is lyophilized to concentrate zinc in the final

solution. To this one millilitre of 10 percent trichloro acetic acid (TCA) was added, the sample is then allowed to stand for ten minutes, after which it is heated to 90^{o}C for five minutes and then centrifuged at 3,000 r.p.m for twenty minutes. The supernatant is decanted and saved. The precipitate is washed with one millilitre of ten percent TCA heated again to 90^{o}C for five minutes and centrifuged as outlined above. The supernatants were combined and read for zinc concentration in the AAS. Davis I.J.T. et al 1968[1] found this method cumbersome and more liable to contamination, so they eliminated the preliminary processing of the sample and just mixed equal volumes of ten percent TCA and plasma thoroughly and allowed it to stand for ten minutes. After centrifugation at 3,000 r.p.m for ten minutes the supernatants were transferred into metal free plastic containers and aspirated into the burner. The modern AAS instrumentation has the sensitivity which enables copper and zinc to be measured in up to a twenty fold of serum dilution[31]. Such a dilution eliminates interference by proteins[32]. In this study simple dilution method[31] was employed and the samples were prepared as follows:-

a) Plasma

One millilitre of plasma was pipetted into a ten millilitre volumetric flask and made to the mark

with distilled water giving a one in ten (1:10) dilution, after thorough mixing the solution was ready for reading on the AAS. Samples were prepared in duplicates.

b) Urine

Samples were prepared in a similar way.

Repeatability of the method was checked by recovery of added zinc to the plasma. Zinc in varying amounts from 0.1 g to 1 g per millilitre of plasma was added prior to dilution and the zinc content of the plasma determined. As the plasma content prior to the addition of zinc was known, percentage recovery was calculated.

PREPARATION OF CALIBRATION SOLUTIONS

Zinc stock solution was prepared by dissolving 100 g pure zinc metal (analytical grade) in 10 cc of 5 N hydrochloric acid and made up to one litre with distilled water and stored in a plastic bottle. From this 10 cc of zinc stock solution was pipetted into 100 cc volumetric flask and made up to 100 cc mark with distilled water this gave 10 mg per litre zinc. Finally 10 cc of zinc stock from step two was pipetted into a 100 cc volumetric flask and made up to the mark with distilled water giving a solution of 100 g/100 cc zinc.

Sodium stock solution of 140 mmol per litre Na^+ was prepared by dissolving 8.2 gramme dry sodium chloride (zinc free analytical grade) in water and made up to one litre with water and stored in a plastic bottle. The calibration solutions were then prepared as follows:- Into three hundred millilitre volumetric flasks 0, 10, 20, cm^3 of zinc stock solution (100 µg/%) was pipped. Ten millilitres of sodium stock solution was added to each flask and made up to the mark with distilled water. These solutions contain 0, 10, 20 g zinc per hundred millilitres corresponding to 0, 100, 200 µg per hundred millilitre in the sample solution. Fresh calibration solutions were made up every day and a calibration curve (Fig V) was plotted for each batch of the samples, with absorbance on 'Y' axis and zinc concentration in micrograms per hundred millilitre plasma or urine on 'X' axis. The unknown values were read out from the curve.

Random samples of University Teaching Hospital tap water were also analysed for zinc.

Operation of AAS

The machine used was a Techtron AA 475 Atomic Absorption Spectrophotometer. The setting of the instrument were as follows:- wavelength 213.9 nm, air pressure 2.1 bar (30 psi) 4.5 - 5.5 flow (litre/ minute) acetylene pressure 0.7 bar (10 psi) 1.0 - 1.5 flow (litre per minute) no filter was used

observation height was 0.5 - 1.5 cm, burner type angle was 10 cm air/C_2H_2 in line lamp current was 10 mA with a slit width of 0.1 mm and an integration time of four seconds. Five readings were taken for each sample and the mean recorded as the absorbance value. To test the accuracy of the readings recovery tests were performed on five randomly selected samples per batch.

Statistics

Standard statistical methods were employed for analysis of the data.[33,34] The results as shown in table I are expressed as mean $\pm$ standard error of the mean. For comparison of two means paired t test was used using the formular

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

taking $n_1 + n_2 - 2$ as degree of freedom, t value was read from the tables. A range of not more than two standard errors was taken as implying "No difference".

The correlation coefficient was limited to a straight line, where it was obvious from the scatter diagram that there was no straight line $r = 0$.

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The actual r for doubtful case were worked out using the formula

$$r = \frac{\Sigma(X - \bar{X})\Sigma(Y - \bar{Y})}{\sqrt{\Sigma(X - \bar{X})^2 \ \Sigma(Y - \bar{Y})^2}}$$

Standard error of coefficient correlation is approximately $\frac{1 - r^2}{n}$

$$t = r \frac{n - 2}{1 - r^2}$$

3. - RESULTS

3.1 General consideration

Table I is a summary of basic data expressed as mean plus or minus standard error of the means. Where some values had to be excluded for various reasons, like spillage, haemolysis, inadequate sample, the appropriate number of estimated samples is given in brackets.

The age of diabetics ranged from sixteen to seventy seven, only two patients were over sixty years of age. The mean age was 41.1 for males and 44.1 for females. For controls the age ranged from 16 to sixty with a mean age of 35.7 for males and 36.0 for the females. The mean height and weight for the four groups is shown in Table I. Earlier workers[1,20] have shown that there is no statistically significant differences in plasma zinc levels attributable to age sex and surface area and therefore no further computation was done on this data.

3.2 Plasma zinc levels in diabetics and controls

Histogram of plasma zinc concentration for both groups showed a skewed distribution so a logarithmic transformation has been used to normalize the data. Fig. I show the histogram on this diagram the mean plasma zinc level appears to be higher for diabetics than the controls. The subjects where further subdivided according to sex and mean plasma zinc level worked out in each group. (See table I) The male diabetic group had the highest value 2.38 ± 0.027 SEM. A t test on the most widely differing of the means ie male diabetic and male controls revealed no statistical significant difference $p > 0.05$.

3.3 Plasma zinc levels in relation to duration of diabetic illness in years.

The duration of diabetic illness ranged from six days to eighteen years. 84% had the illness for up to 10 years, 16% had the illness for more than ten years. Table II shows mean plasma zinc levels as $\log_{10}$ microgram/100 ml in diabetics grouped according to duration of illness. The lowest mean 2.37 was recorded in the 6-10 year group, while the highest mean 2.41 was recorded in the 1-5 year group. Scatter diagram did not show any linear regression therefore $r = 0$ indicating that there is no correlation between the duration of illness and the plasma zinc level.

3.4 Plasma zinc levels in relation to degree of hyper glycaemia.

The fasting plasma glucose level for the diabetics ranged from 4 mmol/L to 24.5 mmol/L. A frequency distribution of seven classes was drawn with class interval of 4 mmol. The mean value of plasma zinc as $\log_{10}$ microgram per 100 ml $\pm$ standard error of the mean was worked out for each class. Correlation of degree of hyperglycaemia with the mean plasma zinc level showed no statistically significant correlation, $r = 0$.

3.5 Mean plasma zinc levels in patients with raised serum cholesterol and those with normal serum cholesterol.

In our laboratory the normal range of serum cholesterol is 3.6 - 6.5 mmol/L.86 diabetics had their serum fasting cholesterol levels estimated, 73 of these (84.9%) had a normal serum cholesterol level (range 3.2 - 6.4 mmol/L)and 13 (15.1%) had raised serum cholesterol level (range 6.7 - 14 mmol/L). The mean plasma zinc level as $\log_{10}$ microgram per 100 ml was 2.43 $\pm$ 0.23 for patients with normal cholesterol level and 2.44 $\pm$ 0.60 for patients with hypercholesterolaemia. A t test on the difference between means shows that the difference is not significant $t = 0.017$ $p > 0.05$.

3.6 Correlation between plasma zinc level as $\log_{10}$ microgram/100 ml with various diabetic complications.

Seventy eight patients had some form of complication at the time of examination. The main complications appeared to be acidosis, neuropathy, retinopathy and hypertension (see table V). Ten patients had combination of acidosis and neuropathy, twelve had retinopathy and neuropathy, four patients had hypertension and retinopathy. Two patients had significant proteinuria, one had full blown picture of nephrotic syndrome. Cardiovascular complications were very few. Of the eight patients with hypertension only two showed significant left ventricular hypertrophy on electrocardiogram and chest x-ray, none of the 78 patients showed any ischaemia heart changes on electrocardiogram. Others include gangrene 2 patients, cataracts 4 patients, infected wounds 2 patients, there was no statistical significant difference between the mean plasma zinc level in various groups.

$p > 0.05$ at 95% confidence limit.

3.7 Difference between plasma zinc level in various treatment groups

Table VI shows the frequency distribution of various types of treatment and a mean plasma zinc level as $\log_{10}$ microgram per 100 ml. 42% of patients were receiving soluble insulin, 26% lente insulin, 28% were on oral therapy of either chlorpropamide or Glibeclamide (Daonil). One patient was on combination of lente and soluble insulin (1%). The remaining three patients (3%) were on diet control. Mean plasma zinc levels could only be computed for 3 groups. The reason for this subdivision was to investigate the effect of additional zinc intake in the form of various injected insulin preparations on the quantitative aspects of plasma zinc levels. The total zinc/100 i.u. of soluble insulin is 20 micrograms and lente preparation (insulin zinc suspension) 390 microgram. These amounts are highly insignificant compared with the 10-15 milligram intake per day with food[35]. These groupings refer only to treatment at the time of blood collection. There was no significant difference among the various groups ($p > 0.05$.)

3.8 Comparison of mean plasma zinc levels as $\log_{10}$ microgram/100 ml from this study with the other workers[36]

The mean plasma zinc values from my work are higher than those reported from the West. Ette S.I. reports mean plasma zinc levels of 107.4 $\pm$ 15.8 in normal Nigerians, a t test on mean plasma zinc level on all controls from this study and that from Ette, S.I.[35] shows value $p < 0.05$, therefore the difference is highly significant.

b TWENTY FOUR HOUR URINARY ZINC LEVELS

3.9 Comparison of the 24 hour urine zinc levels in micrograms/100 ml between diabetic and the controls.

Patients with urine volume of less than 500 mls per twenty four hours were excluded from the study. The mean 24 hour urine volume, creatinine and zinc levels in various groups are shown in Figure I. The urinary creatinine levels in 24 hour urine collection was taken as a measure of accuracy of the total urinary volume. Female diabetics excreted more zinc than male diabetics but there is no statistical significant difference p 0.05. Female controls excreted more zinc in the urine than male controls, again there is no statistical significant difference. Overall diabetics excreted more zinc than none diabetics. Figure IV shows scatter diagrams of 24 hour urinary zinc in micrograms for the two groups, the range was from 179 - 5383 microgram/24 hours with a mean of 2.273 $\pm$ 158.9. For the controls the values ranged from 140 to 5270 with a mean of 1500.24 $\pm$ 218.3. A t test on the difference between the means was t 3.3 at 114 degree of freedom $p < 0.05$, therefore the difference is highly significant. This is in agreement with earlier reports of hyperzincuria in diabetic patients.[20]

3.10 Correlation of degree of glycaemia with mean urine zinc levels (Fig. III).

Further subdivision was done to find out whether the degree of hyper glycaemia had some bearing on the level of 24 hour zinc levels. The correlation coefficient was not significant ($p \geq 0.05$).

3.11 Relationship between mean 24 hour urinary zinc and type of therapy.

The diabetic group has been further subdivided according to therapy Table VIII, this was to investigate the effect of additional zinc intake in the form of various insulin preparations. The groupings refer only to the treatment at the time of urine collection. There were three patients on diet control and the mean zinc urinary level was 1054.33 microgram per 24 hours. The patients on oral hypoglycaemic drugs show a urinary zinc level of 1684.52 per 24 hours. The highest mean was recorded in patients taking Lente Insulin, the mean was 2951.75, the group taking soluble insulin the mean was 2132.67. A t-test was used to find whether there is significant difference between the Lente Insulin group and the oral therapy group and also the lente groupe and soluble insulin group. The difference between oral therapy group and lente insulin was significant ($t = 2.6$ $p < 0.05$.)

In the diet control group, the number was too small for statistical computation. Patients on oral hypoglycaemic therapy showed lower levels than those of the other two groups, this is expected since these patients have a mild to moderate form of diabetes mellitus. The group on soluble insulin has lower readings than that on lente insulin. Most patients on soluble insulin are young brittle diabetics in whom overall control is better achieved with soluble

insulin. The high zinc urinary levels in the group receiving lente insulin can be due to the following reasons:-

1. The amount of zinc in lente insulin is higher than in soluble insulin although not statistically significant.

2. Control of diabetes is poor with lente than soluble insulin.

The diabetics were further subdivided into ketotics and non ketotics. The term ketotic was used to include all patients showing ketonuria at the time of urine collection. The results were as follows:

Ketotics:- Mean urinary zinc 2752 $\pm$ 1346 SD

Non ketotics - Mean urinary zinc 2194 $\pm$ 1467 SD.

The difference between the two means attained no statistical significance $p > 0.05$.

TABLE I SUMMARY OF BASIC DATA

	MALE DIABETIC	FEMALE DIABETIC	MALE CONTROL	FEMALE CONTROL
NUMBER OF SUBJECTS	67 (67%)	33 (33%)	38 (63.3%)	22 (36.7%)
AGE IN YEARS	41.08 ± 1.7	44.13 ± 2.8	35.7 ± 2.1 *(38)	36.0 ± 3.2
WEIGHT (kg)	62.27 ± 1.8 SEM *(65)	63.01 ± 2.5	58.9 ± 1.5 *(27)	56.1 ± 2.9 (17)
HEIGHT (cm)	168.55 ± .20 (65)	160.85 ± 1.2	171.1 ± 1.7 *(20)	160.2 ± 1.9 (11)
LOG_{10} Zn Microgram/100 ml plasma.	2.38 ± 0.02 *(59)	2.35 ± .035 *(23)	2.32 ± 0.027 *(34)	2.34 ± 0.033 (20)
GLUCOSE mmol/L	12.47 ± .75	13.98 ± 1.2	4.36 ± .1	4.56 ± .12
24-HOUR URINARY ZINC IN MICROGRAMS.	2214.54 ± 169.8 (59)	2265.32 ± 554.6 (20)	1168.93 ± 115.8 (23)	1315.1 ± 200 (13)
Mean 24 hour urine creatinine level mmol/L	5789 ± 934 (59)	6193.6 ± 157 (20)	5870 ± 250 (23)	5610 ± 216 (13)
Mean 24 hour Urine volume	1963 ± 181 (59)	1485 ± 232 (20)	977 ± 77 (23)	901 ± 63 (23)

* ( ) Actual number of samples estimated

± Standard error of the mean.

TABLE II

RELATIONSHIP OF DURATION OF DIABETES MELLITUS WITH PLASMA ZINC LEVELS.

DURATION	No. of DIABETICS	% of Total	Mean Plasma Zinc ± SEM
0 to 1 yr	18	18%	2.39 ± 0.60 (16)**
1 - 5 yrs	45	45%	2.41 ± 0.30 (41)**
6 - 10 yrs	21	21%	2.37 ± 0.43 (19)**
11 - 15 yrs	14	14%	2.40 ± 0.64 (9)**
16 - 20 yrs	2*	2%	-

* Number too small for statistical computation.

** Actual number of samples estimated

SEM : Standard error of the mean.

TABLE III

RELATIONSHIP OF DEGREE OF HYPERGLYCAEMIA IN mmol/L WITH THE MEAN LEVEL OF PLASMA ZINC AS LOG_{10} MICROGRAM/100 ml

RANGE OF PLASMA SUGAR	No. of Diabetics	% Total	$\bar{X}$ Plasma ± SE
0.5 - 4.5	5	5%	2.40 ± 0.055 (4) *
4.5 - 8.5	33	33%	2.32 ± 0.34 (30)*
8.5 - 12.5	13	13%	2.43 ± 0.7 (11)*
12.5 - 16.5	24	24%	2.42 ± 0.38 (24)*
16.5 - 20.5	10	10%	2.46 ± 0.62 (9) *
20.5 - 25.5	15	15%	2.39 ± 0.51
Total	100	100	

* Actual number of samples estimated

SEM : Standard error of the mean.

TABLE IV

MEAN PLASMA ZINC LEVEL IN PATIENTS WITH RAISED CHOLESTEROL LEVEL AND THOSE WITH NORMAL CHOLESTEROL LEVEL

Group	Number of cases	% Total	Mean cholesterol level	Mean plasma zinc lo
Normal serum cholesterol level	73	84.9%	4.84 mmol/L	2.43 ± 0.23
Hypercholesterolaemia	13	15.1%	8.10 mmol/L	2.43 ± 0.60

* Standard error of the mean.

TABLE V

DIFFERENCE BETWEEN PLASMA ZINC LEVELS AND VARIOUS DIABETIC COMPLICATIONS

TYPE OF COMPLICATION	NUMBER OF PATIENTS	MEAN PLASMA ZINC Log_{10} mg/100 ml
Acidosis	10	2.38 $\pm$ 0.57 SEM
Neuropathy	13	2.40 $\pm$ 0.58 SEM
Retinopathy	9	2.40 $\pm$ 0.8 SEM
Hypertension	8	2.40 $\pm$ 0.62 SEM
Acidosis & neuropathy	10	2.41 $\pm$ 0.58 SEM
Retinopathy & neuropathy	12	2.41 $\pm$ 0.55 SEM
Hypertension & neuropathy	* 2	-
Hypertension & retinopathy	* 4	-
Others	10	-
Total	78	

* Number not statistically significant for computation.

SEM Standard error of the mean.

TABLE VI

DIFFERENCE IN MEAN PLASMA ZINC LEVELS IN VARIOUS TREATMENT GROUPS.

TYPE OF Rx	NUMBER OF PATIENT	% OF TOTAL	MEAN PLASMA ZINC
Soluble Insulin	42	42%	2.41 ± 0.30
Lente Insulin	26	26%	2.36 ± 0.34
Lente and Soluable Insulin	* 1	1%	-
Oral therapy (Sulphonylureas)	28	28%	2.39 ± 0.35
Diet alone	* 3	3%	-
Total	100	100%	

* Number not statistically significant for computation

TABLE VII

COMPARISON OF MEAN TWENTY FOUR HOUR ZINC EXCRETION IN VARIOUS CLASSES.

Groups compared	Difference between means	P value	Confidence limits
Male diabetics with male controls	1030	<0.05	95%
Female diabetics and Female controls	990	<0.05	95%
all diabetics with all controls	700	<0.05	95%

TABLE VIII

RELATIONSHIP BETWEEN MEAN 24 HOUR URINARY ZINC LEVELS AND TYPE OF THERAPY.

TYPE OF Rx	No. OF SUBJECTS	MEAN 24 HOUR URINARY ZINC IN MICROGRAMS/DAY.
Diet Control	3	1054.33 ± 190.4 SEM
Oral hypoglycaemic	28	1684.52 ± 274.8 SEM
Lente Insulin	26	2951.75 ± 299.2 SEM
Soluble Insulin	42	2132.67 ± 226 SEM

SEM - Standard error of the mean.

FIGURE I : FREQUENCY DISTRIBUTION HISTOGRAM FOR PLASMA ZINC FOR ALL DIABETICS AND ALL CONTROLS

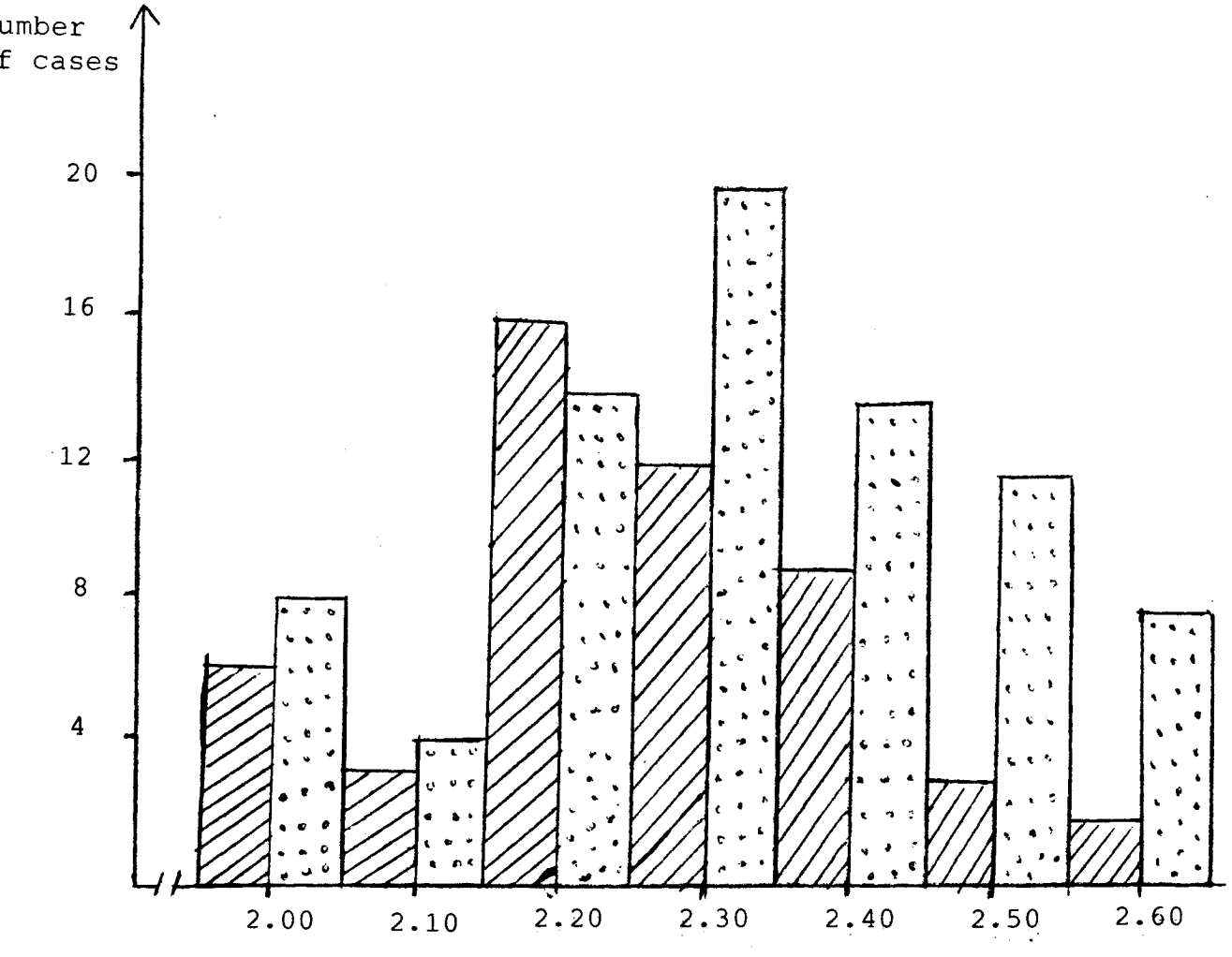
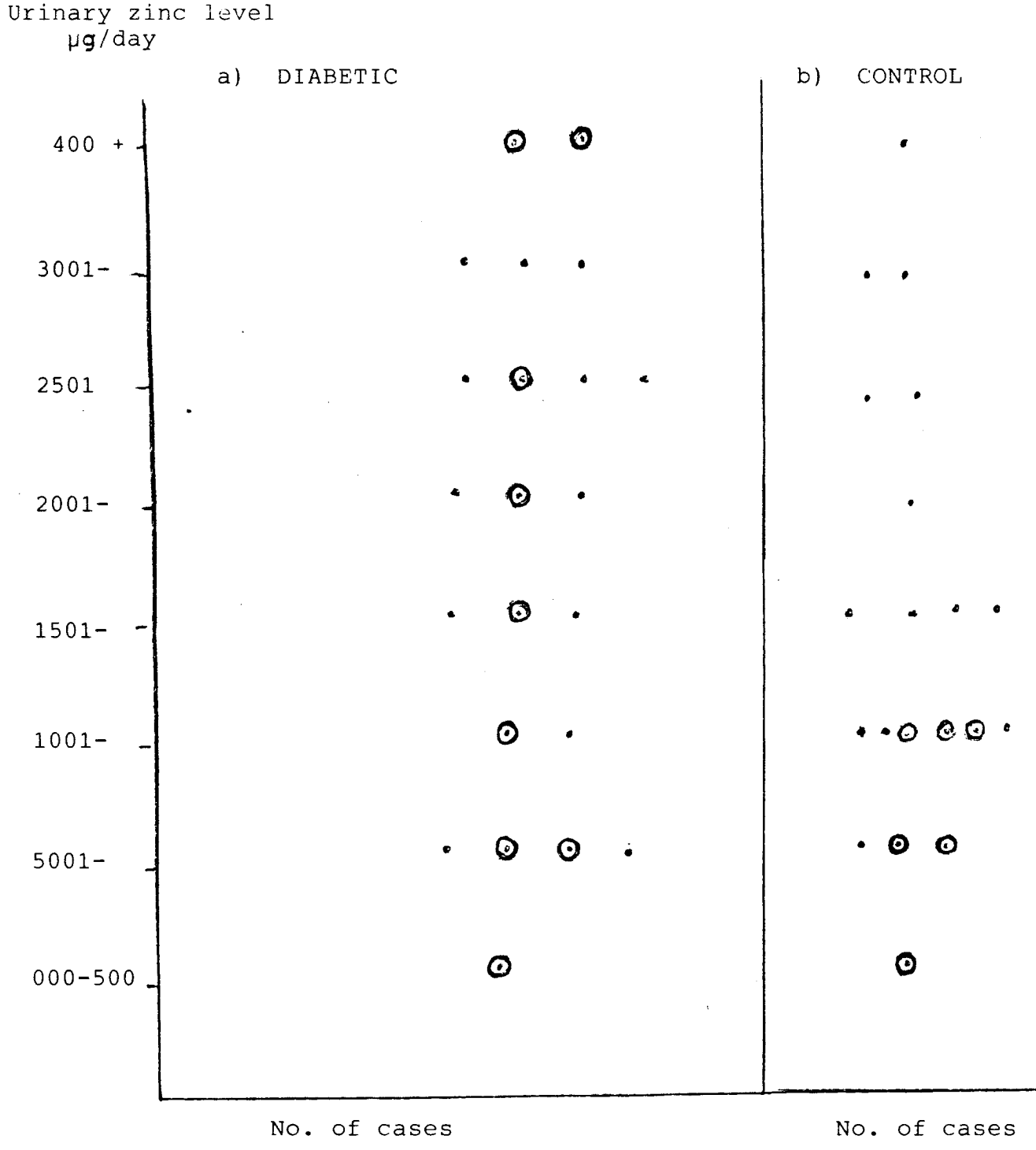


FIGURE II SCATTER DIAGRAM OF FREQUENCY DISTRITION OF 24 HOUR URINARY ZINC LEVEL IN MICROGRAMS/24 HOURS FOR DIABETICS AND NORMALS.



KEY ⊙ = 5
• = 1

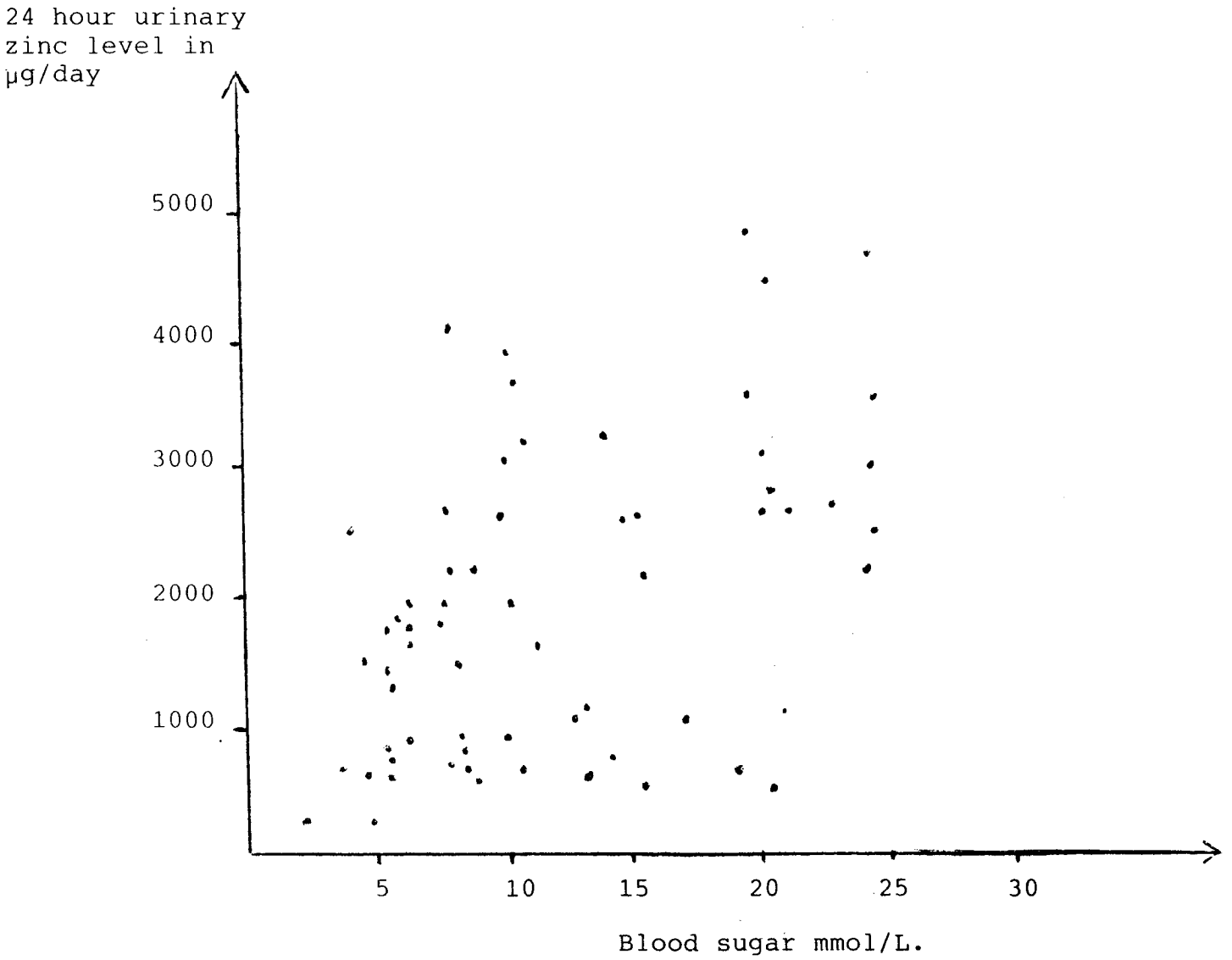
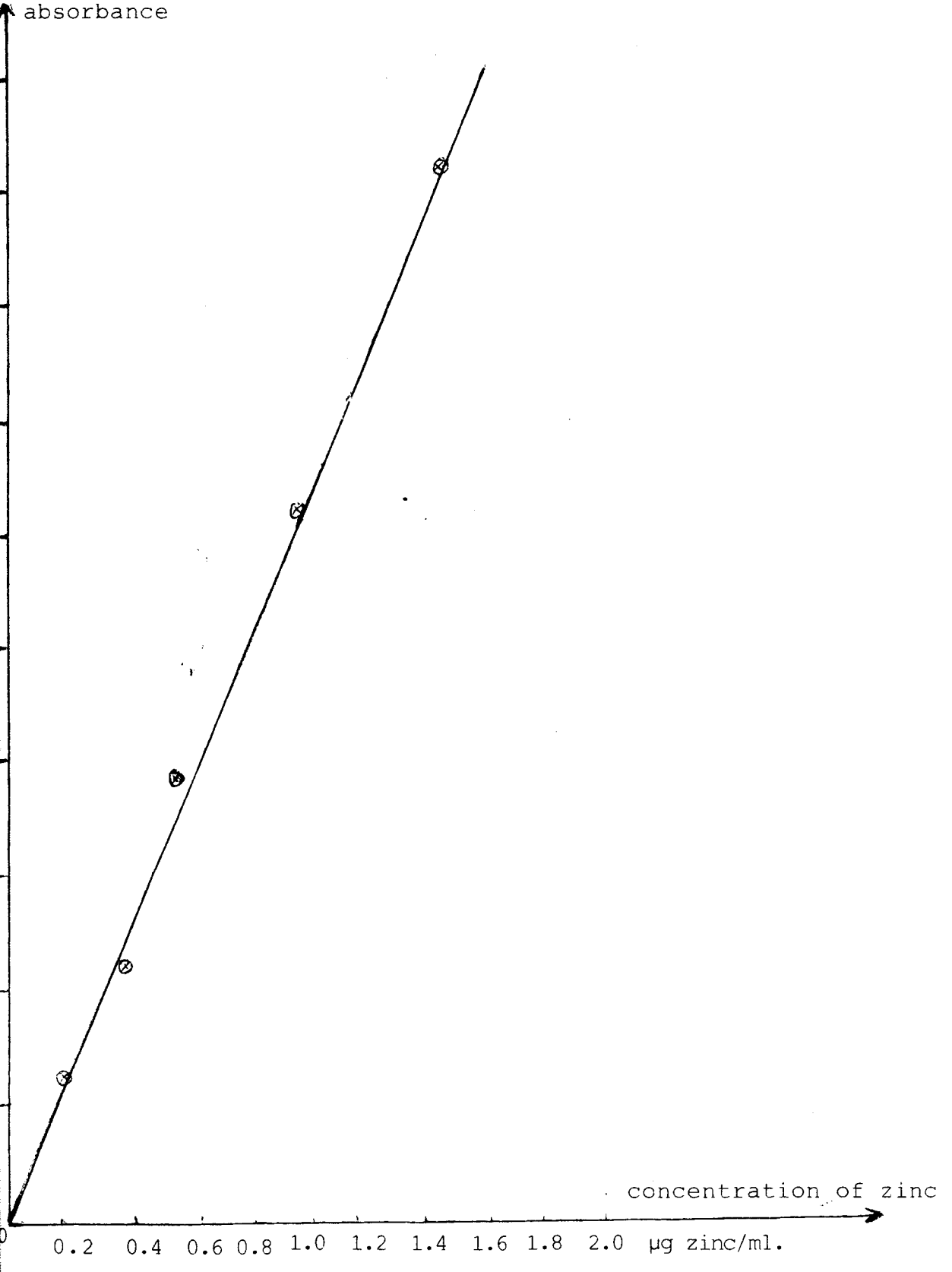

24 hour urinary zinc level in µg/day
5000
4000
3000
2000
1000
5
10
15
20
25
30
Blood sugar mmol/L.

FIG. IV

TYPICAL STANDARD CALIBRATION CURVE USED TO DETERMINE ZINC CONCENTRATION IN PLASMA AND URINE



4. DISCUSSION

My results show that the zinc level estimation in plasma and urine are higher than those reported by othe workers. The reasons for higher zinc levels than previously reported could partially be due to following reasons:

1. As shown by this work the tap water from University Teaching Hospital, contained 3 microgram/ml zinc, this is tentimes higher than zinc water levels reported from the European series. Moreover distilled (which contains some trace ions) and not deionized water was used for dilution and cleaning of equipment.

2. The zinc estimations from this study were done on fasting plasma samples, statistically fasting plasma zinc values give an approximately 12% higher normal mean.[1]

The following conclusions can be made from this study:

1. There is no significant difference in plasma zinc levels between diabetics and normals, in Zambia.

2. There is a highly significant difference in the urinary zinc excretion between the diabetics and control group.

3. Insulin therapy particularly Lente does seem to increase nearly two fold the Zinc urinary output as compared to dietary control group, this difference does not seem to be a consequence of sex, age, level of hyperglycaemia, ketonuria, or proteinuria.

The reason for hyperzincuria in the diabetics is obsure, McCance and Widdowson have reported the average daily intake of zinc with the focd to be 10 to 15 mg and also showed that there was no increase in urinary zinc output when the dietary zinc intake was approzimately doubled in human beings, and that qualitatively the excess was eliminated via the faeces.[35]

Diabetics are on a high protein low carbohydrate diet. Protein rich foods such as eggs, milk and beef contain high zinc levels than high carbohydrate foods. Thus it would seem that the differing carbohydrate to protein ratio in normals and diabetics diets would be insignificant from this point of view, since the plasma zinc level in the two groups is the same.

Mateo Martin M.C. et al have looked at the metabolism of zinc metalloenzymes, namely lactic dehydrogenase and alkaline phosphatase and were able to show statistically significant correlation between the serum concentration of lactic dehydrogenese and hyperzincuria in females.[36] So some workers are of the

opinion that hyperzincuria of diabetes is due to defective metabolism of zinc metalloenzymes.

Tauri has described zinc metabolism in depancreatized dogs and alloxan diabetic rabbits.[5] Alloxan is chelating substance for zinc, and using this substance rabbits were rendered diabetic and in this state showed hyperzincuria. In a later paper Tauri 1963 has demonstrated the correlation between severity of diabetic state and degree of hyperzincuria and that following treatment the hyperzincuria is reduced. Experiments with radiozinc have shown that the principal zinc elimination occurs via the pancreas or the alimentary tract.[5] An increased urinary excretion of zinc would result in a decrease elimination from the principal route. This is the most valid reason in explaining cases in which hyperzincuria is not accompanied by a significant decrease in plasma zinc levels. The assumption made from Tauri's work is that, the changes observed in a diabetic pancrease are due to longstanding diabetic condition rather than an aetiological factor. Since several chelating agents were designated as diabetogenic substances,[38] attention should be paid to the evidence of accumulation of metablic products which possess chelating properties following metabolic derangements in diabetes.

Summary

A review of literature indicated some disagreement concerning plasma zinc levels in diabetes mellitus.

One hundred diabetic patients have been studied and compared to sixty controls. Plasma zinc concentration was performed using simple dilution method and read on atomic absorption spectrophotometer. The results show that there are no significant differences in the fasting plasma zinc levels between diabetics and controls. Similarly a study of the urinary zinc excretion pattern of diabetic and controls was undertaken, the results show a significant hyperzincuria in diabetics as compared to the controls. The reason for this hyperzincuria is still obscure, further studies have to be done to try and isolate endogenous chelating substances for zinc which may appear subsequent to a metabolic derangement in diabetes mellitus, which could be responsible for hyperzincuria.

REFERENCES

1. DAVIS, I.J., Musa, M., Dormandy, T.L., : Measurement of plasma zinc I - In health and diseas
II - In malignant disease
J. Clin. Path. 21 : 359 1968.

2. ROSNER, F., Gorfien, P.C., : Erythrocyte and plasma zinc concentration in liver diseases comparison with normal controls and certain other chronic diseases. Gastroenterology 54 : 1098, 1968.

3. SPENCER, H., Rosoff, B., Lewin, I. and Samachson, J., : Studies of zinc^{65} metabolism in man. In Prasad, A.S., : Zinc metabolism Springfield III, Charles C. Thomas Chapter 19 1966.

4. VALEE, B.L., Wacher, W.E.C., Bartholomy, A.F., and Hock, F.L., : Zinc metabolism in hepatic dysfunction New England J.Med Vol 257 : 1055 1957.

5. TAURIS, S., : Studies on Zinc metabolism I. The influences of chelating agents on urinary excretion of metals. Med J Osaka Univ. 10 : 499, 1960.

6. SULLIVAN, J.F., Parker, M.M., and Boyeti, J.D. : Incidence of low serum zinc in non cirrhot patients. Proc. Soc. Exp. Biol. Med. 130 591, 1969.

7. HALSTEAD, J.A., Hackley, B., Rudzki, C., and Smith, J.C : Plasma zinc concentration in liver diseases. Comparison with normal controls and certain other chronic diseases. Gastroenterology 54 : 1098, 1968.

8. BUCZKOWSKI, M., Slominska-Petelenz, T., and Perlinksi, K. : Serum zinc levels in patients with primary diabetes. Pol. Tyg. Lek. 23 : 90, 1968.

9. MELTZER, L.E., Rutman, J., George,P., Rutman, R., and Kitchell, J. : The urinary excretion pattern of trace elements in diabetes mellitus. American J. of Med.Sci Vol 244 : p 282 1962.

10. CONSTAM, G.R., Leeman, W., Almasy, F., and Constam, A.G : Weitere Beobachtungen iiber elen Zinkstoff Wechsel bei diabetes mellitus. Schweiz med. Wschr 94 : 1104, 1964.

6. SULLIVAN, J.F., Parker, M.M., and Boyeti, J.D. : Incidence of low serum zinc in non cirrhot patients. Proc. Soc. Exp. Biol. Med. 130 591, 1969.

7. HALSTEAD, J.A., Hackley, B., Rudzki, C., and Smith, J.C : Plasma zinc concentration in liver diseases. Comparison with normal controls and certain other chronic diseases. Gastroenterology 54 : 1098, 1968.

8. BUCZKOWSKI, M., Slominska-Petelenz, T., and Perlinksi, K. : Serum zinc levels in patients with primary diabetes. Pol. Tyg. Lek. 23 : 90, 1968.

9. MELTZER, L.E., Rutman, J., George,P., Rutman, R., and Kitchell, J. : The urinary excretion pattern of trace elements in diabetes mellitus. American J. of Med.Sci Vol 244 : p 282 1962.

10. CONSTAM, G.R., Leeman, W., Almasy, F., and Constam, A.G : Weitere Beobachtungen iiber elen Zinkstoff Wechsel bei diabetes mellitus. Schweiz med. Wschr 94 : 1104, 1964.

11. SCHROEDER, H.A., Nason, A.P., Tipton, I.H., and Balassa, J.J. : Essential trace metals in man : Zinc relation to environmental cadium. J. Chronic Dis. 20 : 179, 1967.

12. JOYCE, D. Baird : Diabetes mellitus, Davidson's Principles and practice of medicine; John Macleod.; Thirteenth edition 1981. Churchihill Livingstone Great Britain p. 500.

13. DANIEL, W. Foster Diabetes mellitus; Harrison's Principles of Internal medicine. Isselbacher, Kurt, J., Adams Raymond, D., Braunwald Eugene., Petersdorf Robert, G., Wilson Jean D., 9th Edition 1980. Mac Graw Hill Kogausha Ltd. Tokyo p 1741 - 1154.

14. MEDICAL records University Teaching Hospital registry.

15. GOLDMAN, J., Carpenter, F.H. : Zinc binding, circular dichroism and equilibrium sedimentation studies on insuline (bovine) and several of its derivatives : Biochem. 13 : 45 66 - 74, 1974.

16. WILLIAM, B. Kinlaw., Allen, S. Levine., Morley, John, E McClain Graig, I. : Abnormal zinc metabolism in Type II diabetes mellitus; American J. of med Vol 75 p 177 - 376 August 1983.

17. SCOT, D.S., Fisher, A.M. : The insulin and zinc content of normal and diabetic pancrease. J. of Clin. inv. vol 17, P 725 ; 1983.

18. ALLEN, S. Levin., McClain., Handwerger, Barry, S., Brown David, M., and Morley John, E. : American J. of clinical nutrition vol 37 March 1983 pp 382 - 386.

19. ARQUILLA, E.R., Thiene, P., Muyamoto, S., Dial, L. : Modulation by zinc of binding and degradation by two liver plasma receptors. Proceedings of the 9th Congress of the International Diabetes Federation. Amsterdam : Excerpta media International congress series No. 413.

20. PIDDUCK, H.G., Wren, Peter, J.J., Evans David, A. Price : Hyperzincuria of diabetes mellitus and possible genetic implications of this observation; Diabetes vol 19 No. 4 : 240 - 47, April 1970

21. WHO expert Committee on Diabetes mellitus, WHO technical report series 646 1980.

22. GREAVES, M., and Boyde T.R.C. : Plasma concentrations in patients with psoriasis other dermatoses and venous leg ulceration. Lancet 2 : 1019, 1967.

23. SOMOGY, Nelson : Estimation of blood sugar J. Biol Chem. 4 : 190, 1967.

24. NELSON, S., Scot M.L., and Jaffe, C. : J. of American Clin. path. 21 : 275 1951.

25. ZLATKIS, A., Zak, B., and Boyle, A.J. : Estimation of serum cholesterol Journal Lab. Clin. method 14 : 486, 1953.

26. TODD, Sanford : Clinical Diagnosis by Laboratory methods 14th Edition.

27. ERYTHROCYTE sedimentation rate Wintrobe.

28. PRASAD, A.S., Oberleans D., and Halstead, J.A. : Determination of zinc in biological fluids by atomic absorption spectrophotometry in normal and cirrhotic patients. J Lab. Clin. method vol 166 No. 3 p 510.

29. PIPER, K.G., and Higgins, G. : Estimation of trace elements in biological materials by AAS. Proc. Ass. Clin. Biochem. 4 : 190 1976.

30. PARKER, M.M., Humoller, F.L., and Mahler, D.J. : Determination of copper and zinc in biological material. Clinical Chem. 13 : 40, 1967.

31. PYEN Uncam Ltd. : Atomic absorption methods Serum Copper and Zinc.

32. DAWSON, J.B., and Walker, B.E. : Direct estimation of zinc in whole blood plasma and urine by AAS. Clin. Chim. Acta., 26; 465 1969.

33. STATISTICS at square one : Swinscow, T.D.V., (From the BMJ.) British medical association Tavistock Sq London 1980 p 1 - 24 p 62.

34. FISHER and Yates, : Table III Statistical tables for biological, agricultural and medical research Longman Group LTD London.

35. MAC CANCE, R.A. & Widdowson, E.M., : The absorption and excretion of zinc, Biochemistry Journal 34; 1163, 1940.

36. ETTE, S.I., Ofodile, F.A. and Oluwasanmi, J.O. : Vitamin and trace element profiles in chronic leg ulcers in Nigerians 73 - 75 Tropical and Geographical Medicine vol 34 No. 1 1982.

37. MATEO MARTIN, M.C., Bustamante Bustamante, J., Quiros, J.B., Fernandez., Machando, O. Ortiz : A study of the metabolism of zinc its metalloenzymes in Diabetes mellitus. Biomedicine : 23, 134 - 136 1975.

38. OKAMOTO, K. : Experimental studies on the pathogenesis of diabetes mellitus (zinc theory of diabetes mellitus by Okamoto) Acta Sch. Med. Univ. Kioto 27 : 43, 1949.