DETERMINATION OF TRACE ELEMENTS IN RELATION TO DIABETES MELLITUS IN MAN

by

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CHAPTER I

I INTRODUCTION

Living organisms have an essential requirement for inorganic material without which they could not sustain life. These inorganic constituents can be divided into two major groups:

Macroelements	Trace Elements
Carbon	Iron
Hydrogen	Iodine
Oxygen	Copper
Nitrogen	Zinc
Sulphur	Manganese
Phosphorus	Cobalt
Calcium	Nickel
Potassium	Molybdenum
Sodium	Selenium
Chlorine	Chromium
Magnesium	Fluorine
	Tin
	Silicon
	Vanadium

All the above are essential to the well being of the individual (plant or animal) and they fulfil many roles. The four main roles are:

(a) Structural

(b) Electrophysiological

(c) Metalloenzymes

(d) Catalytic

The macroelements combine to form the structural components of cellular material and the composites of intra and extracellular fluids. As analytical techniques have developed the full realisation of the potential of trace elements has been established. Most of them exist in quantities less than one part per million and if their concentration should rise to any great extent above this they will become toxic to the cells. As evidence of essentiality of the trace elements grew it became apparent that elements observed in isolation did not provide an adequate picture of many situations and the importance of mineral interactions was realised. It is now known that elements with similar valence shell structures are likely to be biological antagonists e.g. Fe^{3+} and Co^{3+}, Cu^{2+} and Zn^{2+} and may compete for uptake by the tissues. Also some elements are known to protect the body against the effects of another more toxic element, for example selenium which is known to counteract the effects of methylmercury, however, arsenic, in contrast, will potentiate the toxic effect of mercury. Altered trace element metabolism may be the primary cause of certain disease states as seen in patients with endemic goitre due to iodine deficiency, where dietary replacement of iodine will alleviate the symptoms and lead to remission of the goitre. Whereas a second type of trace element disturbance may occur as a consequence of an infectious disease, prolonged parenteral

nutrition or the effects of drug therapy. The topic under investigation in this study was the relationship of trace elements to the condition, Diabetes mellitus. Diabetes is unlikely to be a simple case of a primary trace element deficiency as individuals suffering from this disease also show disturbed enzymic and hormonal systems. However, once diabetes is established the affected metabolism of these patients may be enhanced by trace element anomalies. The multielemental technique of neutron activation analysis allowed simultaneous analysis of several trace elements implicated in this disease in order to clarify the discrepancies which exist in the present data. These conflicting results may be due to inadequate experimental design possibly coupled with ineffectual control of sample contamination, so all aspects of the experimental procedure to overcome these problems will be outlined.

The metabolic problems of Diabetes mellitus will be discussed along with the possible relationship of obesity to non-insulin dependent diabetes and how trace elements may affect this progression. As trace element deficiencies can often be masked by drug or diet therapy subjects taking part in this study were fasted and relieved of all diabetic drugs, hence, any abnormal response to a controlled situation, with respect to trace elements, would surely be observed.

I.1 Diabetes Mellitus

1.1 Introduction

The growing incidence of Diabetes mellitus throughout the western world appears to be a result of an increasing dependence on a highly refined diet and consequently characterised as a disease of modern civilisation. However, as long ago as the second century A.D. a clinician, Aretus the Cappadocian, described a condition known to be Diabetes mellitus.

".... a melting down of the flesh and limbs into urine.... Emaciation proceeds quickly and death occurs rapidly." (Reed 1954).

Knowledge of this condition existed down the centuries (Frank 1957) in many cultures but it was not until the discovery of the actions of insulin by Banting and Best in 1921 that any real steps forward were made in treating diabetes.

In 1979 the National Diabetes Data group (an international group of clinicians) produced a system of classification of Diabetes mellitus and in the light of present knowledge of the disease agreed the following definition:

Diabetes mellitus is a genetically and clinically heterogeneous group of disorders that share glucose intolerance as a common factor.

Genetic heterogeneity is known to be associated with the major histocompatibility complexes at loci HLA-B8 and HLA-B15. If these two are present at the same time the risk of diabetes is dramatically increased (Rotter and Rimoin 1981).

1.2 Classification

Diabetes mellitus can be classified into five distinct groups each one possessing its own clinical characteristics.

(1) Type I Insulin-dependent Diabetes mellitus (I.D.D.M.)

Patients in this group are dependent on injected insulin to prevent ketosis and sustain life.

(2) Type II Noninsulin-dependent Diabetes mellitus (N.I.D.D.M.) sub-divided into:-

(a) Nonobese N.I.D.D.M.

(b) Obese N.I.D.D.M.

Onset of this type of diabetes is usually after the age of forty and 60-90% of the patients are obese. Some patients are characterised by hyperinsulinemia and insulin resistance.

(3) Gestational Diabetes mellitus This condition is thought to be due to the complex metabolic and hormonal changes occurring during pregnancy. It can be associated with increased perinatal complications and may progress to diabetes 5-10 years after parturition.

(4) Diabetes associated with malnourished populations Severe malnutrition due to any cause will depress pancreatic function leading to diabetes.

(5) Other types of Diabetes mellitus Diabetes may be seen as a secondary condition to certain diseases e.g.

(a) pancreatic disease

(b) endocrine disease

It may also be associated with certain genetic syndromes e.g.

(a) Down's syndrome

(b) Glycogen storage disease

1.3 Normal Glucose Metabolism in Man

An adequate supply of dietary carbohydrate is necessary to maintain blood glucose concentrations in the range 4.2 - 6.3 $m.mol.L^{-1}$ to allow for normal daily activity (recommended daily allowance is 50-100g of digestible carbohydrate). Throughout the day the body's needs for metabolic fuels may increase due to increased exercise or stress and so the stored product of glucose metabolism i.e. glycogen, is recycled to release glucose. At the same time the ingestion of food at intervals throughout the day ensures that these stores can be replaced. The whole process of ingestion, uptake, tissue metabolism and/or storage is controlled by various neurological and endocrine systems to enable the body to supply a steady state of glucose to all the tissues. The individual processes involved will be discussed in the following sections.

(i) Diurnal Glucose Mobilisation

In the post absorptive state there is a baseline concentration of circulating insulin (10-20 $mU.L^{-1}$) which results in almost total cessation of glucose uptake by insulin-dependent tissues. In the noninsulin dependent brain and erythrocytes, glucose uptake continues at the rate of 150-200 $mg.min^{-1}$. Glucose homeostasis is maintained by hepatic release of glucose following glycogenolysis and gluconeogenesis. Glycogenolysis being the major pathway stimulated although there is mobilisation of precursor amino acids and fatty acids for gluconeogenesis.

Ingestion of food provides the necessary stimulation for insulin release by the β cells and glucose uptake of insulin-dependent tissues increases. Felig, Wahren and Hendler (1975) studied the passage of glucose into the portal system following a 100g glucose load in man and showed the progress of glucose disposal. The peripheral insulin-dependent tissues receive 15% of the glucose, the non-insulin dependent tissues 25% and hepatic tissue receives 55%. Hepatic metabolism especially glycogen synthesis, triglyceride formation and glycolysis are greatly increased to replace stores depleted whilst sleeping or fasting. Any changes in the relative concentrations of the glucose disposal routes may result in hyperglycaemia especially if the proportion of glucose going to the peripheral insulin-dependent tissues increases along with peripheral insulin resistance. This point will be discussed further in section 1.4.(ii).

Any alterations in the balance between glucose production/ingestion and tissue utilisation will lead to a situation of glucose intolerance which will upset the entire metabolic balance of the individual.

(ii) Glucose Regulation

The regulation of glucose metabolism in the human body has been quite extensively studied and glucose levels are known to be controlled by several neurological and endocrine systems.

a. Insulin

Insulin is a polypeptide hormone (molecular weight (min)=5734D) produced by the βcells of the pancreas which is released in response to circulating glucose. Insulin permits glucose uptake

by insulin responsive tissues and subsequent intracellular utilisation of glucose. The β cell is unable to respond to hyperglycaemia if there is a lack of Ca^{2+} or c.AMP, hence, glucose uptake is influenced by intracellular events (Cerasi, Luft 1970).

b. Glucagon

A decrease in circulating glucose stimulates the release of glucagon (a polypeptide hormone - molecular weight = 3485 D) from the α cells of the pancreas. Glucagon stimulates hepatic glycogenolysis and gluconeogenesis to re-establish basal glucose concentration (4.2-6.3 $mmol.L^{-1}$). Glucagon production is stimulated by the action of the autonomic nervous system which innervates the α cells.

c. Growth Hormone

The concentration of growth hormone in plasma falls after oral administration of glucose and recovers two - five hours later. The mechanism for this phenomenon is known to be insulin antagonism and is obviously important in glucose regulation because insulin-induced hypoglycaemia is a potent stimulator of growth hormone release. An increase in plasma growth hormone produces an increased glucose output from the liver and decreased uptake by the periphery, so enabling increased glucose uptake in noninsulin dependent tissues.

d. Gastrointestinal Hormones

The presence of intraduodenal glucose leads to an increase in the peripheral concentration of plasma insulin. The stimulation of insulin production by the gastrointestinal hormones (secretin, gastrin, gastric inhibitory polypeptide and cholecystokinin-

pancreozymin) in turn stimulates somatostatin release which acts as a positive-feedback mechanism between the gut and the pancreatic islets by decreasing the secretion of gut hormones (Unger 1981). Somatostatin may also be inhibiting cellular communication between the α and β cells of the islets of Langerhans (Kohen et al 1983). Cellular communications by gap junctions within the pancreas are important to protect against large unnecessary rises in the hormone i.e. 'fine tuning' in the regulatory process.

e. Autonomic Nervous System

Autonomic innervation of pancreatic tissue allows stimulation or suppression of pancreatic function in response to the central nervous system. There are two types of pancreatic nerve connections:-

(1) Cholinergic neurones and vagal nerve impulses increase stimulation of insulin production in response to food intake and are important in normal nutrition.

(2) Adrenergic neurones inhibit insulin production via an α receptor on the pancreatic β cells. This type of innervation is important in the response to stress. An increase in circulating adrenalin results in a decreased production of insulin and stimulates the production of glucagon; there is a resulting increase in circulatory glucose (glucagon stimulates hepatic glucose release) to the brain and muscles.

1.4 Obesity

The obesity syndrome cannot be studied as a single pathophysiological condition however results of experiments with obese mice and rats have led workers to believe that there is a general trend in the events leading to obesity. Obesity is known to lead to an increase in circulating free fatty acids, triglycerides, cholesterol and very low density lipoproteins (V.L.D.L.) above the levels of lean individuals (Nestel and Goldrick 1976) and is often associated with diseases such as coronary artery disease and Diabetes. Obese persons have an increased likelihood of glycosuria and an increasing prevalence of diabetes (Kannel, Gordon and Castelli 1979). The connections between obesity and diabetes will be discussed in the following sections.

(i) Possible schemes for development of obesity

The hypothalamus is known to play a major role in food intake and regulation, and in 1961 Anand showed that the hypothalamus contained two interacting areas. The 'feeder centre' located in the ventrolateral area (VLH) and a 'satiety centre' located in the ventromedial area (VMH) of the hypothalamus, were shown to act as glucoreceptors and with the VMH exerting an inhibitory influence on the VLH.

Feeder Centre (VLH)	← inhibition	Satiety Centre (VMH)
(i) glucose sensitive		(i) glucose and insulin sensitive
(ii) parasympathetic innervation		(ii) sympathetic innervation

Experimental lesions of the VMH are followed by hyperphagia and the development of obesity whereas lesions of the VLH lead to aphagia.

These observations led to the development of the first hypothesis.

A A primary lesion of the hypothalamus leading to hyperphagia would result in hyperactivity of pancreatic β cells so inducing hyperinsulinaemia and the resulting obesity.

The possibility of a defect in the β cells themselves cannot be ruled out and as some individuals do not show hyperphagia a second hypothesis was put forward by Goldman, et al(1974).

B A primary hypothalamic or β cell defect without accompanying hyperphagia leads to overall increased β cell activity. The resulting hyperinsulinaemia produces an increase in liver and adipose lipogenesis and subsequent obesity.

Finally, an hypothesis was developed on assessing the metabolic status of obese individuals.

C With increasing duration of obesity secondary metabolic and hormonal alterations occur which lead to overstimulation of hepatic and adipose tissue. Insulin resistance may be apparent although the insulin levels continue to rise. The tissues may have reached the plateau phase of their insulin dose-response relationship and cannot respond to any further increases in plasma insulin.

Although the exact pathway for the development of obesity is not fully understood knowledge of the impaired metabolism of obese individuals may possible give clues as to its origin.

(ii) Impaired metabolism

Excess ingestion of calories, especially dietary carbohydrates, and physical inactivity promote deposition of body fat and lead to obesity. This increased food intake is thought to promote elevated levels of insulin and most obese individuals (including obese N.I.D.D.) show increased levels of plasma insulin which is related to the degree of obesity (Deschamps, et al, 1977).

Several workers (Abaira and Lawrence 1978; Stunkard and Blumenthal 1972) have shown that obese individuals have a normal response to an oral glucose challenge and glucose disappearance rate. However, under conditions of an intravenous glucose challenge obese individuals show an abnormal response and there is an increase in hepatic uptake of metabolic precursors for gluconeogenesis (Felig, et al, 1974). This indicates that peripheral insulin dependent tissues are showing a degree of insulin resistance and that hyperinsulinaemia does not affect the hepatic glucose metabolism (resistance?). The hyperinsulinaemia is thought to cause an increase in adipose, hepatic and muscular uptake of glucose which stimulates the production of fatty acids. Lipogenesis from various substrates including carbohydrate, and the activity of several lipogenic enzymes, were increased in the livers of obese animals (Assimacopoulos-Jeannet and Jeanrenaud 1976). The resulting elevation of circulating triglycerides and V.L.D.L. may inhibit glycolysis and glucose transport especially in muscle tissue thereby inducing peripheral insulin resistance and possibly, over a period of time, glucose intolerance.

In the case of obese N.I.D.D. the obese liver is thought to develop resistance to insulin so enhancing the hyperinsulinaemia. In an obese N.I.D.D. Sharkey (1968) showed that 75% of a 100g oral glucose load entered the peripheral circulation as compared to 50% in a normal weight diabetic. The resulting diabetics manifest hyperglycaemia and hyperinsulinaemia. These obese individuals also show elevated levels of free fatty acids after a glucose load which indicates a sluggish recovery of the metabolism.

Obese individuals after four months of weight loss (totalling 10.9 kg) showed a reduction in fasting levels of triglycerides, cholesterol and V.L.D.L. along with a decreased resistance to insulin and decreased hyperglycaemia in response to an oral glucose tolerance test (Olefsky, et al, 1974). It is also interesting to note that fasting glycerol and glucose levels before and after weight loss (total loss = 3kg) were higher in men than women (Hockaday, et al, 1975).

Another interesing aspect of obese individuals is their impaired thermogenesis which may be linked to the same cause as membrane insulin resistance. The sodium - potassium - ATPase (Na-K-ATPase) pump which is present in cell membranes to regulate intracellular sodium and potassium concentrations shows decreased activity in the hepatic tissue, muscle and erythrocytes of obese mice and men (De- Luise, et al, 1980). Obese individuals were shown to have a 22% reduction in the Na-K-ATPase activity of erythrocytes and this reduction was negatively correlated with body weight (De Luise, Blackburn and Flier 1980). A defect in thermogenesis will lead to the need for increased energy intake to maintain body temperature

which in turn will lead to obesity. Obese mice have been shown to need a greater proportion of ingested energy than pair-fed lean littermates and they have a decreased thermogenetic response to cold exposure (Trayhurn, et al, 1977; Chlouverakis 1970).

The sodium-potassium-ATPase mechanism has also been implicated as the possible defect in β cell release of insulin because the β cell membranes have been shown to be highly permeable to ions and the mechanism of accumulation is ATP dependent (Idahl, et al, 1978) - for further discussion see following section.

1.5 Glucose Metabolism in Diabetes Mellitus

(i) Pancreatic Abnormalities

One of the major pathological changes in diabetes is the altered endocrine content of the pancreas. Insulin requiring diabetics show a total absence of β cells yet their ratio of δ : α cells is the same as controls, whereas, noninsulin requiring diabetics have an increase in α cell mass and so the ratio of δ : α cells and β : α cells is altered (Rahier, Goebbels, Henquin 1983).

The maintenance of intracellular potassium concentration is important to allow β cell depolarisation and the release of insulin. The presence of extracellular glucose retards the slow efflux of potassium ions from the islet cells so increasing depolarisation and stimulating insulin release. Diabetic mice show persistent depolarisation of islet cells at low glucose concentration (3 $mmol.L^{-1}$) because their basal potassium permeability is decreased. This depolarisation appears to be

dependent on a glucose-sensitive K^+-electrodiffusion mechanism (Berglund, et al, 1980). Further evidence for the interaction of a glucose-sensitive potassium dependent pump comes from work by Thurston et al. (1977). In fed animals a 50 $U.kg^{-1}$ dose of insulin reduced plasma glucose by 50% but the potassium concentration was unaffected whereas in fasted animals a 50 $U.kg^{-1}$ dose of insulin decreased plasma glucose by 68% and potassium by 26%. Hence, potassium is important for glucose uptake at low glucose concentrations.

In opposition to this system cobalt ions inhibit the release of K^+-induced insulin from islet cells and the calcium uptake into islet cells. It has been suggested that cobalt and calcium may exhibit an antagonistic effect (Henquin, Lambert 1975). The implications of this antagonism comes clear when it is known that Ca^{2+} ions along with a high concentration of glucose (16.7 mM) increase islet cell communication so if Ca^{2+} uptake is inhibited the pancreas may not respond adequately to high levels of glucose (Kohen, et al, 1983).

(ii) Brain Metabolism

Of the insulin independent tissues erythrocytes show no difference from controls in glucose consumption either by the pentose phosphate pathway or glycolysis (Phillips and Menderschausen 1975), whereas the brain, which does not rely solely on glucose, can switch to ketones as its major source of metabolic fuel (Ruderman and Goodman 1980).

Glucose provides the main source of energy to the brain except in a state of starvation when blood glucose is decreased markedly and the brain utilises β-hydroxybutyrate, acetoacetate and in some cases lactate (Nemoto, et al, 1974) for 60% of its oxidative metabolism. If glucose is lacking the formation of neurotransmitters will also be affected because oxaloacetate is a precursor of glutamine and γ-aminobutyrate.

In diabetes the brain responds in a similar way to starvation and so there is a switch from glucose to ketone fuels in the presence of hyperketonemia. The increased metabolism of ketones produces high levels of acetoacetate and this together with diminished levels of neurotransmitters both lead to a reduction in central nervous system activity and induce diabetic coma. Also contributing to the ketoacidosis is the decreased release of oxygen from the erythrocytes because of reduced 2,3 diphosphoglycerate concentration (Ditzel and Standl 1975).

The main stimulus for altered brain metabolism in diabetes appears to be due to hyperketonemia and not hyperglycaemia because Kety, Polis, Nadler and Schmidt (1948) observed that the glucose concentration of confused and comatose diabetics was unchanged. Although the precise intracellular biochemical defect in diabetic ketoacidosis is not known all the above mentioned factors contribute and it is known that treatment of the metabolic disorders restores consciousness and reverses the ketoacidosis.

(iii)Peripheral Glucose Utilisation

Both insulin dependent and non-insulin dependent diabetics show hyperglycaemia following food intake and unless their condition is treated will show increasing levels of circulating fatty acids, amino acids and glucose. In the diabetics there is an increase in certain serum enzymes which are correlated with the degree of glycaemia. The lysosomal enzymes, acid phosphatase and amylase, are higher in diabetics and appear to be linked to the degradation of various cell constituents in the tissue catabolism of diabetics (Belfoire, et al, 1973).

Insulin dependent diabetics are hyperglycaemic because of a lack of insulin production by the pancreas leading to severely reduced uptake of glucose into cells. There is an intracellular increase in the production of glucose from carbohydrate stores and any excess is passed into the circulation so enhancing the hyperglycaemia. As soon as the concentration of circulating glucose reaches the renal threshold of approximately 10 mmoles.L^{-1}, glucose is excreted in the urine along with large volumes of water because of the hyperosmolarity of the urine.

Non-insulin dependent diabetics show a three-four fold increase in glucose turnover (Bowen and Moorehouse, 1975) in response to an increased level of glucose in their peripheral circulation. The increased insulin resistance observed in non-insulin dependent subjects (Ciaraldi, et al. 1982) especially where there is the additional problem of obesity further adds to the hyperglycaemia until the renal threshold is reached and glycosuria results.

(iv) Hepatic Metabolism

The main action of insulin in the hepatic tissue is induction of certain glycolytic enzymes and suppression of a number of gluconeogenic enzymes. Induction of glucokinase, phosphofructokinase and pyruvate kinase promotes the conversion of glucose, through a series of intermediary metabolites, to pyruvate. In diabetes, glucose utilisation in the liver is depressed to about one quarter of normal values (Steinner and Williams 1959) because the enzymes normally induced by insulin are low in activity and so there is an increase in the amount of glucose entering the peripheral circulation. However, the enzymes of the gluconeogenic pathway, i.e. pyruvate carboxylase, phosphoenolpyruvate kinase and glucose-6-phosphatase are increased in activity (no suppression from insulin) and so plasma glucose increases. Gahlaut and coworkers in 1974 investigated the serum pyruvate concentration of diabetics and controls. They observed that controls and insulin requiring diabetics had the same fasting pyruvate concentration but unlike the controls, the insulin requiring diabetics showed no increase in pyruvate after a glucose challenge. The noninsulin requiring diabetics had elevated fasting pyruvate compared to the controls and yet their response to the glucose load was poor. Hence, both types of diabetic patients had decreased glycolytic activity, as expected.

Observations of patients on total parenteral nutrition with hyperglycaemia led Robson and coworkers (1983) to believe that a high glucose load (>10g glucose per hour) would result in overloading of the citric acid cycle so increasing lipogenesis. Their

patients showed elevated levels of plasma cholesterol as observed in diabetes. They concluded that hyperglycaemia induced by diet may lead to glucose intolerance and also elevated cholesterol levels. Earlier Ginter and coworkers (1978) observed hypercholesterolaemia in diabetic patients associated with ascorbic acid deficiency. As ascorbic acid is an important feature of cholesterol catabolism any deficiency would inevitably lead to impaired cholesterol degredation. Ascorbic acid deficiency is often associated with deterioration of capillary integrity, oedema and impaired wound healing all of which are also observed commonly in diabetes.

Lipoprotein concentration in controls and diabetics shows a significant diurnal variation, however, these variations are more pronounced in the diabetics (Simpson, et al. 1980). The increased mobilisation of fatty acids in diabetics leads to an increase in the circulating concentration of acetyl CoA and acetoacetate, these two metabolites inhibit phosphofructokinase and there is a resulting decrease in glucose utilisation. The elevated levels of ketones will produce acidosis in the diabetic liver. It is known that ketoacidosis and hypoglycaemia are common in the non-obese group of diabetics whereas lactic acidosis and hyperosmolar comas are frequently seen in obese diabetics (Mirouze, Beraud 1979).

Diabetic subjects also show a disturbed protein metabolism which further complicates the hepatic disturbances already discussed. Diabetics often show hypoalbuminaemia which is not accounted for by gastointestinal losses (McPerson 1973). Albumin is extruded from the vessels of the eyes of diabetics resulting in diabetic

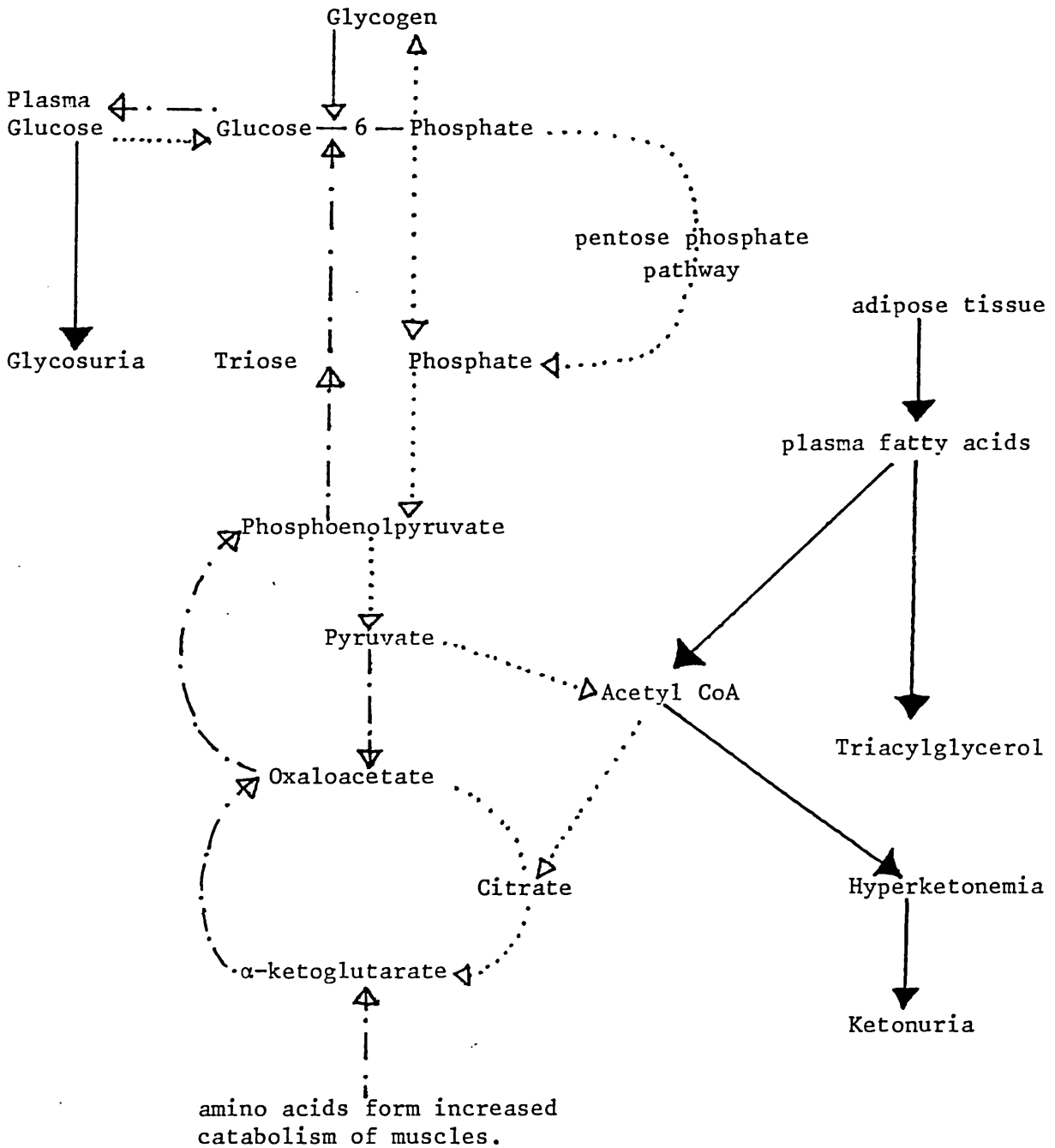
retinopathy and along with oedema of diabetic nephropathy, these two conditions being common pathological disturbances of diabetics. Differences in albumin production or turnover were not observed in diabetics, it was, however, suggested that extravascular pools of albumin were increased in diabetes resulting in a large pool of inaccessible albumin (McPherson 1973). Elevated levels of large plasma proteins i.e. haptoglobin, fibrinogen and caeruloplasmin have been recorded in diabetes and are also associated with diabetic complications (Jonsson and Wales 1976), β-lipoprotein and α_2-macroglobulins were positively correlated with the duration of the disease. As the majority of plasma proteins are synthesised in the liver these findings indicate the possibility of elevated levels of enzymes responsible for protein synthesis associated with the increased availability of precursor amino acids from muscular breakdown which occurs especially in insulin dependent diabetes.

The imbalance of carbohydrate, lipid and protein metabolism in the liver of diabetic individuals has been summarised in Fig. 1. There are other factors which affect the metabolism of diabetics (especially their response to a glucose challenge) i.e. hormones, diet and age, these will be discussed in a separate section.

(v) Hormonal responses to glucose

Twenty years ago it was demonstrated that endogen ous pituitary and adrenal cortical activity were responsible for insulin reversible inhibition of glucose uptake in the rat (Bornstein and Park 1953). This led investigators to believe that other factors as well as insulin were implicated in diabetes. Work by Phear (1962)

Fig. 1. Abnormal Metabolism in the liver during uncontrolled diabetes



Pathway Activity :-

Greater than normal

Markedly impaired

Increased gluconeogenesis

Normal

demonstrated that not only was the response of a diabetic to a glucose challenge different from a control individual but that differences occurred amongst the different types of diabetics.

Growth hormone has been suggested as a contributing factor to diabetes. Uncontrolled insulin dependent diabetics are known to have elevated levels of growth hormone (Vasquez, et al. 1973) most pronounced in association with fasting, whereas female non-insulin dependent diabetics were found to have increased growth hormone concentration in conjunction with elevated serum insulin (Florey, 1978). Sherwin and coworkers(1983) investigated the effect of a growth hormone infusion in a control subject. They observed no change in fasting glucose or somatomedins levels but there was an increase in the circulating fatty acids, ketones and insulin which indicates a rapidly developing resistance to insulin, whereas in lean non-insulin dependent diabetics on a somatostatin infusion the growth hormone response was suppressed and there was an initial increase in glucose and fatty acid levels (Christensen,et al. 1978). These data indicate that growth hormone produces an antagonistic effect of insulin and therefore may contribute to glucose intolerance, even when fasting glucose is unchanged.

Catecholamines may also be involved in glucose intolerance as an increase in their concentration appears to be related to the pathogenesis of diabetes (Efimov et al, 1973) and the reduced response of diabetics to metabolic stress. Insulin dependent diabetics maintained for sixty minutes on an infusion of noradrenalin showed no response in their concentration of circulating insulin whereas control subjects had an initial period (twenty

minutes) of insulin inhibition. Both groups exhibited elevated levels of circulating ketones and glucose although controls had a greater increase than diabetics (Schade and Eaton, 1978). As noradrenalin is known to increase in situations of stress or exercise the suppressed response observed in diabetes may lead to metabolic insufficiency resulting in ketoacidosis.

The response of the body to outside stimuli whether it be ingestion of food or a response to a situation will induce an internal hormonal response. In the case of glucose intolerance in diabetes the opposing action of an insulin antagonist is exaggerated and will in turn increase the metabolic output of major tissues to compensate. These hormonal changes overlay the fundamental tissue disturbances in diabetes and must be counteracted either by administering insulin or hypoglycaemic drugs.

1.6 Other Factors Affecting Glucose Tolerance

Various conditions of diet, life style and genetic susceptibility will affect normal glucose tolerance in man. Obesity and hyperglycaemia are known to be two important contributors to glucose intolerance (Kadowaki et al, 1984) although obesity is not the sole reason for the development of non-insulin dependent diabetes in glucose intolerant individuals (Stern et al, 1983). Kopf and coworkers (1973) demonstrated that insulin dependent diabetics had greater postprandial glucose than controls and this effect was not sex or weight but age related. Whereas in non-insulin dependent diabetics glucose concentrations were found to be sex related and were higher in women than men (Hockaday, et al, 1975). However, a greater number of women are obese and a reduction in their weight

improves their tolerance to glucose (Badran and Farid, 1977; Olefsky, et al, 1974). As glucose and insulin levels are known to correlate with meal size (Service et al, 1983) any weight loss by dieting will decrease circulating glucose and improve glucose tolerance especially the response to a glucose challenge. The weight loss program must include a reduction in the intake of fat as high dietary fat levels increase the risk of developing or aggravating existing diabetes (Portha, et al, 1982).

Work with complex carbohydrate diets containing high levels of cereal or guar, which is a storage polysaccharide of the bean Cyanopsis tetragonoloba (Gassull, et al, 1976), became popular as possible ways of alleviating hyperglycaemia. These products act as a source of unavailable carbohydrate and if added to the diet produce a decreased glucose and insulin response to a glucose challenge (Uram, et al, 1958; Kline, 1958; Goulder, 1979) possibly by alleviating the stress of hyperglycaemia on the pancreatic cells. However, these complex carbohydrates contain high levels of phytate which is known to impede uptake of essential trace elements which could prove detrimental in diabetes (Goulder and Alberti, 1978) and they are unpalatable in large quantities.

Smoking (Janzon et al, 1983) and exessive alcohol consumption (Berntrop et al, 1983) are known to impair the oral glucose response and if either is coupled to genetic susceptibility, may well lead to diabetes.

1.7 Hypoglycaemia

Hypoglycaemia is usually defined as a situation where blood glucose concentration has decreased to less than 2.2 $mmol.L^{-1}$ in an adult. Hypoglycaemia is often observed in badly controlled diabetics due to either an accidental insulin overdose or an increase in activity without the necessary boost of glucose. However, hypoglycaemia has many causes and is not only observed in diabetics. Hypoglycaemia may be due to:-

(a) Insulin excess

Excess circulating insulin will exist if there is an exogenous insulin overdose, islet cell carcinoma or with increased autonomic stimulation of the islet cells.

(b) Deficiency of an insulin antagonist

This often occurs due to adrenocortical insufficiency or hypopituitarism.

(c) Deficiency of available glucose

This may occur due to glycosuria, primary liver disease or as a consequence of glycogen storage disease.

(d) Functional hypoglycaemia

This may be due to an excessive production of insulin following transient hyperglycaemia (postgastrectromy syndrome) or hypersensitivity to a fall in blood sugar levels.

Whatever the cause hypoglycaemia is extremely dangerous because whilst in this condition permanent damage may be done to the heart or brain (Hardwick, 1962). The most vulnerable tissue is the brain and prolonged hypoglycaemia will lead to loss of consciousness. The brain uses glucose as its main metabolic fuel

although it can utilise ketones but, the aglycaemia must be reversed as quickly as possible by a glucose infusion especially where patients have lost consciousness. Administering oxygen has no effect as glucose is the fuel which would normally be oxidised, so anoxia may occur in certain areas of the brain. Although cardiac tissue contains stores of glycogen, if there is inadequate coronary circulation then the myocardium will also suffer anoxia and a heart attack occurs.

Hypoglycaemia must be avoided if at all possible and diabetics leading very active lives or undertaking excessive periods of driving must always be aware of the onset of the symptoms and alleviate them before any loss of concentration or consciousness occurs.

1.8 Therapy

Patients with Diabetes mellitus are able to live relatively normal lives with the appropriate treatment. Insulin dependent diabetics rely on sources of bovine or porcine insulin at the present time, although bacterial production of insulin is being developed with a hope of reducing the antigenicity and cross-reactivity of existing preparations. Non-insulin dependent diabetic treatment may vary depending on severity. Initially patients can often be controlled by dietary carbohydrate restriction and/or oral hypoglycaemic drugs. However, it sometimes becomes necessary to treat these individuals with insulin injections.

(a) Insulin Compounds

Insulin is produced commercially as an amphoteric protein forming soluble salts in weak acid solution. It must be administered either subcutaneously or intramuscularly by injection as it is inactivated by proteolytic enzymes in the gastrointestinal tract. Several types of insulin preparation have been developed by the pharmaceutical industry.

(i) Soluble insulin

This clear colourless liquid with a pH of 3 is rapidly absorbed and has a short duration of action.

(ii) Protamine zinc insulin

This colourless, turbid liquid consists of a suspension of insulin protamine and zinc chloride and has a pH of 6.9 - 7.4. The addition of protamine to insulin forms a complex which has a less intense but more prolonged action than soluble insulin because of its decreased solubility.

(iii)Globulin zinc insulin

This is a similar product to protamine zinc insulin although it has a pH of 3 and was developed as an alternative to protamine zinc insulin which often produces sensitivity reactions.

(iv) Lente Insulin

This is a slow acting insulin formulation prepared from crystals of insulin and zinc which have been neutralised by the addition of phosphate or citrate buffer. Their duration of action is increased to around 30 hours.

(b) Oral Hypoglycaemic Drugs

The drugs used in the treatment of noninusulin dependent diabetes can be divided into two groups

i) Sulphonylureas

ii) Biguanides

The sulphonylureas include Chlorpropamide, Glibenclamide and Tolbutamide and are administered to patients who still have functioning islet tissue. These drugs are readily absorbed from the gastrointestinal tract and bind to plasma proteins. They act initially by stimulating the islets to release insulin but are thought to have a long term effect on the liver and muscles. Whereas biguanides e.g. Metformin, Pherformin; exert their action on peripheral tissues by increasing glucose utilisation. In certain cases both types of drugs will be used together with their actions complementing each other.

I.2 Trace Elements and Diabetes Mellitus

Introduction

2.1 The multifactorial nature of Diabetes mellitus has been known for many years although an interest in the role that trace elements play in the disease has only developed fairly recently. It has been well documented by clinicians that diabetics produce large quantities of hyperosmolar urine especially when in a state of metabolic acidosis which leads to massive loss of electrophysiological salt solutions. The increased excretion of sodium, potassium and magnesium results in a reduction in the circulating levels of these trace elements (Heintz 1972, Kailakov 1979, Fijii et al. 1982). The effect of Diabetes on the trace elements is less well defined and the publication of data showing varying responses of the trace elements to glucose intolerance has led to a great deal of controversy.

2.2 Animal studies

Animal work on trace elements and Diabetes has been directed almost exclusively at insulin dependent diabetes as this condition can be easily induced in animals by drug therapy. Halim et al. (1977) worked with rabbits and initiated diabetes by administering dithizone which is known to selectively destroy pancreatic β cells. After 48 hours the animals had decreased circulating calcium and sodium along with elevated levels of zinc, iron and potassium. Treatment for ten days with insulin restored all elements, except magnesium and potassium, to their normal levels.

The resulting elevated potassium and depleted magnesium were unexplained, however, hyperkalaemia is a frequent occurrence in stable Diabetes mellitus (Preuss and Lombardo 1978). Very similar work by Failla (1983) using rats whose pancreatic tissue had been destroyed by streptozotocin also resulted in increased plasma zinc concentrations, probably as a result of islet cell release of zinc. However, this study concentrated on tissue abnormalities and it was observed that the hepatic tissue contained higher concentrations of zinc, copper and manganese than controls whereas the kidney had only elevated zinc and copper. These two tissues also demonstrated increased metallothionein turnover (mellallothionein being the inducible intracellular protein with high affinity for heavy metals) which may account for their accumulation of these three trace elements. Opposition to these data came from Gabovich and Mikhalyuk (1977) who observed depleted levels of copper and iron in alloxan diabetic rats.

An alternative approach to the problem was adopted by Mikhail and Awadallah (1977),who using alloxan and dithizone treated rats observed the protective effect of various trace elements on the pancreatic tissue of the animals. Of the four trace elements investigated only zinc chloride prevented the β cell destruction produced by the drugs; magnesium chloride, chromium chloride and cobalt chloride had no effect. Magnesium chloride and chromium chloride did decrease the hyperglycaemia produced by the drugs due to their peripheral hypoglycaemic action on tissue metabolism such as increased glucose utilisation. With the cobalt chloride, which was known to destroy α cells of the pancreas, there was no

stimulatory effect of gluconeogenesis. However, repeated subcutaneous injections of cobalt chloride are known to produce hypoglycaemia as a result of increased peripheral glucose uptake. This effect was observed by Dixit and Lazarow (1967) in isolated adipose tissue, where cobalt increased the uptake and oxidation of C-1 glucose in the tissue so increasing glucose utilisation. Continuation of this work by Awadallah and Hanna (1980) showed that pre-treatment of alloxan diabetic rats with chromium and cobalt increased the production of serum transaminases which is an indication of the hepatotoxic effect of alloxan. Disturbances in the liver metabolism will lead to leaking of trace elements into the circulation and possible depletion. Whereas dithizone has no toxic effect on the liver because dithizone treated rats showed only slight elevations of serum transaminases, so trace elements would be retained. These facts may explain why Gabovich and Mikhalyuk (1977) observed depleted trace elements in alloxan diabetic rats and Halim et al. (1977) observed elevated zinc and iron in the dithizone treated rabbits.

The investigation into the role of chromium in diabetes has been of interest since 1959 when Mertz and Schwarz demonstrated that chromium deficient rats developed glucose intolerance. Progress in trace element research has now made it possible to rear animals in a completely chromium deficient environment and these animals show severe carbohydrate intolerance. The degree of glucose intolerance observed under these conditions depends on the species of animal used. Guinea pigs were found to be more sensitive to a chromium deficient diet that other rodents (Preston et al. 1976).

In recent years the hypoglycaemic action of chromium has been disputed by Ghafghazi et al. (1979) because they demonstrated that in non-deficient animals acute administration of chromium (III) chloride resulted in hyperglycaemia and glucose intolerance, this hyperglycaemic action being reversible by adrenalectomy. They explained this effect as possible inhibition of insulin release by chromium because administration of chromium does not influence acute insulin hypoglycaemia. They also demonstrated hyperglycaemia and glycosuria in animals fed chromium deficient diets, indicating that chromium, both in excess and deficiency, acts as an hyperglycaemic agent.

Although diabetes-inducing drugs mimic the symptoms of Diabetes mellitus they may have effects on other tissues which are important in trace element metabolism such as the liver. The effects observed in animal studies therefore may not be a true reflection of the human metabolic disturbances. At this time there is no adequate animal model for the diabetic complications seen commonly in diabetics i.e. nephropathy and retinopathy which would indicate the disturbances in trace elements observed may not truly reflect the human condition.

2.3 Human studies

There has been much debate as to the involvement of trace elements in the pathology of human diabetes, not least of these was zinc. Zinc is known to be an important trace element in the body especially in relation to the glycolytic pathways, wound healing processes and as part of the insulin molecule. Any alteration in carbohydrate metabolism or an insulin deficiency would surely be

influenced, at least in part, by zinc concentration. A pattern emerges from the literature that diabetics, irrespective of type or duration, exhibit hyperzincuria and this is often greater in women than men (Mateo et al. 1978, Ishii et al. 1980). However circulating zinc levels do not appear to be clearly defined. Several groups observed no change in the fasting concentration of plasma or serum zinc levels between diabetics and controls (Prout et al. 1960, Chooi et al. 1976, Khattab et al. 1976, Svoboda and Palicka 1978, Lodhi and Khan 1979) and Pidduck (1970) found no differences in non-fasting zinc concentrations. In the cases where zinc concentrations were reported to be higher in the diabetics than controls, these patients also showed elevated levels of copper and caeruloplasmin (Mateo et al. 1978) which may indicate hepatic damage and one group observed a reduced response to a glucose challenge along with elevated zinc and copper levels (Fayek et al. 1984). Finally, several groups observed significantly reduced zinc concentrations in both insulin dependent and non-insulin dependent diabetics which appeared to be associated with patient's control and duration of the disease (Kumar and Rao 1974, Awadallah et al. 1978, Khandewal et al. 1981). The idea of a circulating deficiency of zinc would appear to be a logical progression of hyperzincuria, however, homeostatic mechanisms may overcome zinc losses because zinc is so metabolically important. A study of tissue zinc levels may give clues as to whether the distribution of zinc is affected although the animal studies discussed earlier indicated increased levels of zinc in the tissues. The main question to be answered would appear to be:

Are the levels of circulating zinc in diabetics different from matched controls?

Copper is also an important trace element in the body, it is needed for cellular oxidative metabolism, iron uptake and basement membrane integrity hence disturbances in copper metabolism will lead to decreased efficiency of the copper-dependent enzymes. It has been observed in rats that reduced levels of copper-dependent superoxide dismutase activity in the β cells resulted in islet cell destruction by free radicals leading to insulin dependent diabetes (Couch et al. 1981, Gandy et al. 1983). Reduced efficiency of lysyl oxidase may be responsible for disturbed basement membrane formation resulting in poor capillary transport and oedema often observed in diabetics. Low fasting copper levels were recorded by De Nicola et al. (1975) in aged diabetics and by Awadallah et al. (1978) in juvenile diabetic subjects.

Evidence of normal copper levels in human diabetic subjects was provided by Pidduck (1970) and Lodhi et al (1979). At the other end of the scale Mateo et al. (1978), Azevedo (1980) and Fayek (1984) observed elevated copper concentrations in both insulin dependent and noninsulin dependent subjects. However, Azevedo demonstrated that the copper levels decreased with advancing of the disease and at the same time as serum monoamine oxidase was increasing (responsible for catecholamine oxidation and inactivation). The question inevitably to be asked: are circulating copper concentrations affected in diabetes?

Apart from zinc and copper the other trace element for which a great deal of work has been carried out to establish its relation-

ship with human diabetes is chromium. It has been known for many years that tissue chromium decreases with advancing age (Schroeder et al. 1962) and that in many parts of the world individuals are subjected to chromium deficient diets. However, the majority of evidence for establishing the presence of a chromium-deficient state in human diabetics has come from supplementation studies, an improvement in glucose tolerance after supplementation indicating that a previous deficiency state existed. Elderly patients with glucose intolerance showed an improvement in glucose tolerance after addition of chromium-rich yeast to their diet (Offenbacher and Pi-sunyer 1980) whereas addition of chromium chloride as an inorganic salt only improved glucose tolerance in half of the patients (Levine, et al, 1968). Rabinowitz et al. (1983) demonstrated that although inorganic and organic chromium compounds increased hair and erythrocyte chromium content, the fasting glucose and lipid concentrations were unchanged. Only the ketosis-resistant subgroup demonstrated a postprandial insulin increment after supplementation. Other evidence supporting a possible chromium deficiency state came from patients relying solely on parental nutrition. They were found to need chromium dietary additions of at least $150 \mu g.day^{-1}$ to reduce the possibility of developing glucose intolerance (Jeejeebhoy et al. 1977, Freund et al. 1979).

Evidence opposing the concept of hyperglycaemia produced by chromium deficiency was put forward by Wise (1978), who observed that chromium chloride supplementation of diabetics enhanced their fasting hyperglycaemia. This effect was also observed by

Ghafghazi et al. (1979) with non chromium-deficient rats. One explanation may be that inorganic chromium opposes the effect of insulin whilst organic chromium in the glucose tolerance factor (GTF) enhances insulin action, an excess of either may have a detrimental effect on glucose tolerance. Ghafghazi et al. (1980) also demonstrated that chromium has an inhibitory effect on islet insulin production acting via an interference with intracellular calcium.

The Pima Indians from America provided an ideal population in which to study trace elemental abnormalities because the incidence of diabetes was almost 50% (Eatough et al. 1977). Investigations into their tissue chromium levels showed no significant differences from those of non diabetic Indians which suggests that depleted chromium stores were not implicated in diabetes. Unfortunately, the paper does not record the plasma and urine chromium concentrations which may have given clues as to whether chromium utilisation and excretion was the same as in non diabetics. Boyle et al. (1977) also looked into the problem of chromium depletion and its relationship with diabetes and atherosclerosis. They found that oriental nations with low serum cholesterol concentrations had relatively high tissue chromium and a low incidence of diabetes and atherosclerosis.

An alternative approach to the problem of whether or not a state of chromium deficiency associated with glucose intolerance exists in diabetes is to subject the patients to a glucose challenge and observe the chromium response. In individuals with normal glucose tolerance the relative chromium response peak was inversely

correlated with total insulin (Lui and Abernathy 1982) and this rapid increase in plasma chromium was associated with normal glucose utilisation. When glucose tolerance was impaired as in diabetes this rise in chromium was not observed (Glinsmann et al. 1966). Although the plasma response of diabetics appears to be suppressed as compared to the matched controls their excretion of chromium was increased (Gurson and Saner 1978). This increased chromium excretion was also observed in malnourished children with glucose intolerance who responded positively to chromium supplementation (Hopkins et al. 1968).

It was postulated that inorganic chromium obtained from the diet could be converted to a metabolically active form known as the glucose tolerance factor (GTF). The structure of this compound has not yet been elucidated but it is thought to contain a nicotinic acid complex (Mertz 1974) and a biologically active fraction with GTF-activity has been isolated from yeast and pork kidney (Haylock et al. 1983). Tracer studies using Cr - 51(III) and Cr - 51(VI) have shown that trivalent chromium binds to plasma transferrin, is located in the nuclear fraction of liver and spleen cells (especially associated with a 70K protein) and is excreted via the urine (Okado 1984). Cr-51(VI) enters the erythocytes where it binds firmly (Holland 1979) and its main excretory pathway is in the bile (Edel et al. 1984). Hence, it would appear that Cr(III) enters the glucose tolerance factor.

The above data suggests that there are still questions to be answered regarding the involvement of chromium or GTF in diabetes:

a. Do diabetic individuals exhibit low circulatory chromium concentration?

b. Do all diabetics have a poor chromium response to a glucose challenge? If so, is it related to the plasma glucose or insulin concentration?

c. If chromium is related to the hyperglycaemia observed in diabetes, will the additional problem of obesity accentuate the chromium disturbances?

The amount of available data on other trace elements and their association with diabetes is sadly lacking although a certain amount of interest has been shown in iron and manganese. Iron was suspected of being involved in the pathogenesis of diabetes because individuals with genetically inherited hemochromatosis often develop diabetes as a secondary complication. Chemically diabetic rats show increased serum iron concentration and excretion which are returned to normal by insulin treatment (Halim et al. 1977, Failla 1983). However, human diabetic subjects demonstrated normal circulatory iron levels and enhanced urinary excretion (Culebras et al. 1977, Awadallah et al. 1978 1 and 2, Lodhi et al 1979) as compared to controls. These results may indicate an association between iron metabolism and the pathogenesis of diabetes.

The evidence of the possible importance for the role of manganese in diabetes came from work with manganese deficient rats who had decreased pancreatic insulin content and release, glucagon was unaffected (Baly et al. 1984). Manganese had previously been observed to be necessary for islet cell release of insulin

(Mikhail et al. 1977) and animal studies revealed that hepatic tissue of diabetic rats had increased activity of manganese-responsive enzymes possibly up to 50% (Wagle 1966, Failla 1983). The stimulation of the enzymes of gluconeogenesis may not be due to a primary manganese disturbance in the liver but as a consequence of reduced insulin output. There has been one reported case of an insulin dependent diabetic who showed an hypoglycaemic response to oral ingestion of lucerne (high manganese content) and inorganic manganese chloride where previously large doses of insulin were needed to control his diabetes (Rubenstein et al. 1962). Manganese would, therefore, appear to be implicated as an important trace element for insulin production by the pancreas. However, human individuals with diabetes were found to have normal levels of serum manganese (Sullivan et al. 1979).

Research into the possible control mechanism of pancreatic islet cells, using isolated preparations, has revealed interesting information as to the role of rubidium and cobalt. The alkali metals rubidium and caesium are known to be readily interchangeable with potassium in tissue metabolism. As uncontrolled diabetics show hypokalaemia associated with glucosuria then presumably the cellular contribution of rubidium and caesium will increase. Insulin release by islet cells is dependent on the availability of adenosine triphosphate (ATP), zinc, manganese, chromium and cobalt (Mikhail et al. 1977, Idahl et al. 1978). The presence of extracellular glucose reduces the rubidium and potassium efflux from islet cells to allow cellular depolarisation and consequently insulin release (Berglund et al. 1980). This

response in diabetic animals was severely reduced and is thought to be associated with the reduction in cellular thermogenesis observed in diabetic and obese individuals (De-Luise et al. 1980). Data supporting this idea of rubidium compensating for potassium in transmembrane ATPase pumps comes from Lodhi (1979) who observed a reduction in the rubidium excretion of diabetics, whereas whole blood concentration was unchanged. The positive influence of cobalt on glucose uptake and utilisation in adipose tissue was demonstrated by Dixit and Lazarow (1967) and this, along with insulin release from islet cells, confirms the idea that cobalt, manganese, zinc and chromium all interact to facilitate insulin release and peripheral action. Obese individuals exhibiting hyperinsulinaemia have reduced levels of cobalt in their blood (Shurygin et al. 1974) and diabetics have increased urine cobalt concentration (Ishii et al. 1980).

One element scantily covered in the literature in relation to Diabetes mellitus was selenium. Fasting serum selenium concentrations were not significantly different from controls (Sullivan et al. 1979, Behne and Diel 1972), although after an oral glucose load diabetics exhibited a decrease in selenium along with zinc. The urinary excretion of selenium was seen to be deficient in diabetics and this deficiency has also been observed in children suffering from Kwashiokor often accompanied by diabetes (Sandstead et al. 1970). Selenium is also known to play a role in reducing the toxic effects of heavy metals such as mercury (Hill 1975) so that if diabetes was due, in part, to heavy metal exposure there would be a decrease in selenium excretion to counteract this

effect. Evidence for a possible role of mercury in diabetes came from personal observations of the author who noted an increasing incidence of diabetes in men working in a mercury related industry.

Finally, a few research groups have looked into the problems of diabetic complications especially with the increased plasma viscosity and erythrocyte aggregation normally associated with diabetic retinopathy. An increased incidence of retinopathy was found in individuals with high plasma concentrations of large plasma proteins especially α_2-macroglobulin and β-lipoproteins (Jonsson and Wales 1976, Muller et al. 1975). Cheta et al. (1981) demonstrated that IgA and Transferrin were highest in insulin dependent diabetics and whilst IgA increased with duration of the disease the circulating Transferrin levels fell. This was an important observation as chromium is known to be transported attached to Transferrin and so this may account for the increased excretion of chromium by diabetics.

2.4 Areas Requiring Further Study

It would appear correct at this stage to conclude that the roles of copper, zinc and chromium in diabetes need further characterisation whilst the interaction of the other elements needs more experimental evidence to support a possible involvement in this disease. The experiments undertaken in this research project will hopefully clarify some of the points discussed above by following the events listed below:

a. Observe fasting concentrations of trace elements in diabetics and non-diabetics.

b. Observe the diurnal variation in trace elements of diabetics and non-diabetics.

c. Observe the effects of hyper and hypoglycaemia on a control individual on the circulating trace element concentration.

d. Observe the effect of a glucose load on the circulating trace element concentrations of lean and obese diabetics and controls.

e. Observe the effect of weight control in reducing hyperglycaemia in noninsulin dependent diabetics and the subsequent trace element fluctuations to maintain euglycemia.

f. Measure the trace element concentration of head and pubic hair from lean and obese subjects.

g. Observe the effect of the trace element content of certain plasma proteins during a glucose load indicating the possible interation of a chromium binding protein.

II

CHAPTER II

NEUTRON ACTIVATION ANALYSIS

1.1 INTRODUCTION

Activation analysis is a technique of elemental analysis that is dependent on the properties of the nucleus. The process involves the excitation of a nucleus by inelastic collisions with nuclear particles to produce an activated nucleus, a charged particle and/or a photon. In neutron activation analysis the radionuclides (activated nuclei) are known to emit β particles and electromagnetic radiation (γ). Some nuclei may go through several stages of β and γ emission before they achieve their ground state. These characteristic radiations produced by individual elements allows identification of the nuclides of interest within the radioactive sample.

1.2 Advantages of neutron activation analysis

This technique has the following features;

a) Good sensitivity

b) High level accuracy

c) Precision

d) Specificity

e) Multielemental

f) Non-destructive (physically)

1.3 Disadvantages of neutron activation analysis

a) Hazards of handling radioactive materials

b) Destructive (chemically)

c) Expense

d) No speciation

e) In certain cases the analysis can be time consuming

1.4 Possible Neutron Sources for Use in Neutron Activation Analysis

(a) Radioactive Sources.

(i) Cf-252 is a nuclide which undergoes spontaneous fission with a half life of 2.65 years. A 10 mg Cf-252 source will produce a thermal neutron flux of $10^{12} n.m^{-2}.s^{-1}$.

(ii) The (α,n) sources consist of an alpha-emitter e.g. Am-241 and a Be target which has a low (α,n) threshold. A 7 Ci Am-Be source (23 mm internal diameter, 31 mm high) will produce a neutron flux of $3.1 \times 10^{9} n.m^{-2}.s^{-1}$.

(iii) The (γ,n) sources are not routinely used because they need a high degree of shielding. The most common arrangement of this type is a Sb/Be source.

(b) Accelerators

There are a wide variety of neutron producing reactions induced by charged particles. Some of the most important ones are shown below:

(i) $^{3}H(d,n)^{4}He$

(ii) $^{3}H(p,n)^{3}He$

(iii) $^{9}Be(\alpha,n)^{12}C$

(iv)) $^{9}Be(\gamma,n)^{8}Be$

The choice of reaction used in generating neutrons is entirely dependent on the source of the accelerator. For example a low energy deuteron accelerator can be used to produce 14 MeV neutrons from the $^{3}H(d,n)^{4}He$ reaction.

(c) Nuclear Reactor

A nuclear reactor can be used to supply a high, stable, isotopic flux of neutrons. The neutrons are produced from the fission of either natural uranium or enriched uranium. Natural uranium is composed of $6 \times 10^{-3}\%$ U^{234}, 0.71% U^{235} and 99.3% U^{238} whereas enriched uranium can contain anything from a few percent up to ninety-five percent U^{235}. The core of the CONSORT reactor at Silwood Park is enriched with 80% U^{235}.

The enrichment of the uranium by U^{235} increases the rate of fission within the core because U^{235} has more favourable capture and fission cross-sections for thermal neutrons than U^{238}. The resulting neutron flux within the reactor will be in the range $10^{16} - 10^{18}$ $n.m^{-2}.s^{-1}$.

The neutron activation analysis carried out as part of this project made use solely of the neutron producing CONSORT reactor at Silwood Park.

1.5 The Neutron Cycle in a Thermal Neutron Reactor

A nuclear reactor can be considered as a system in which a controlled chain reaction takes place. Propagation of the chain reaction requires the recycling of the neutrons produced during fission, after they have been thermalised by scattering within the reactor core. The resulting neutrons are in thermal equilibrium with the atoms of the moderator and have a probable velocity of 2200 $m.s^{-1}$ at 20°C. See figure 1.5.1.

Figure 1.5.1

A Diagram to Show the Progress of Neutrons in the Reactor Core.

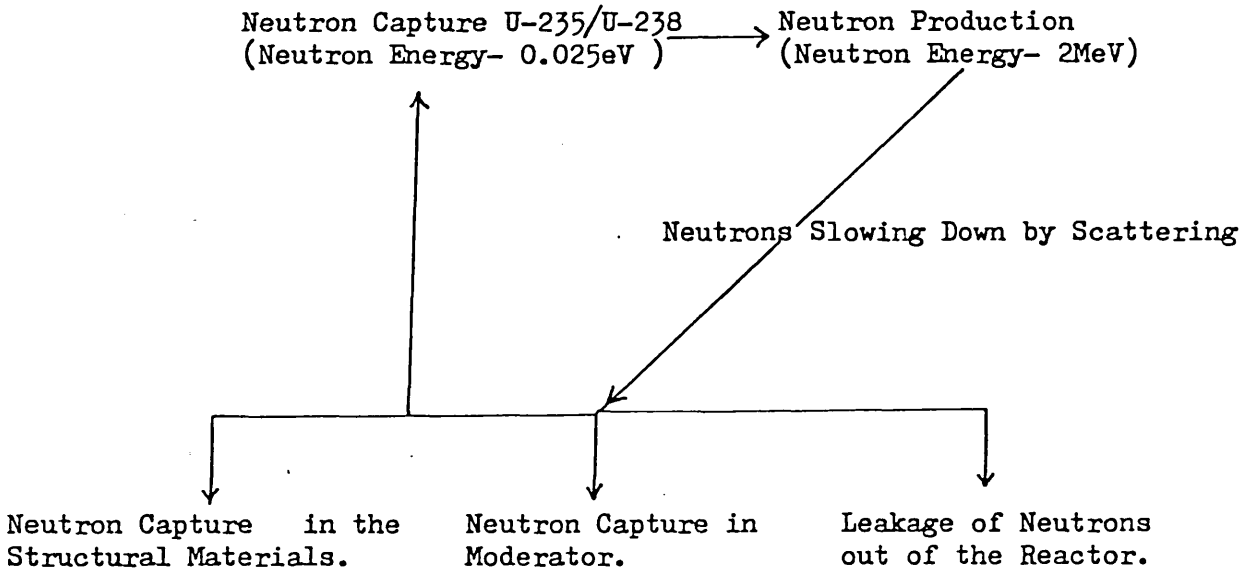
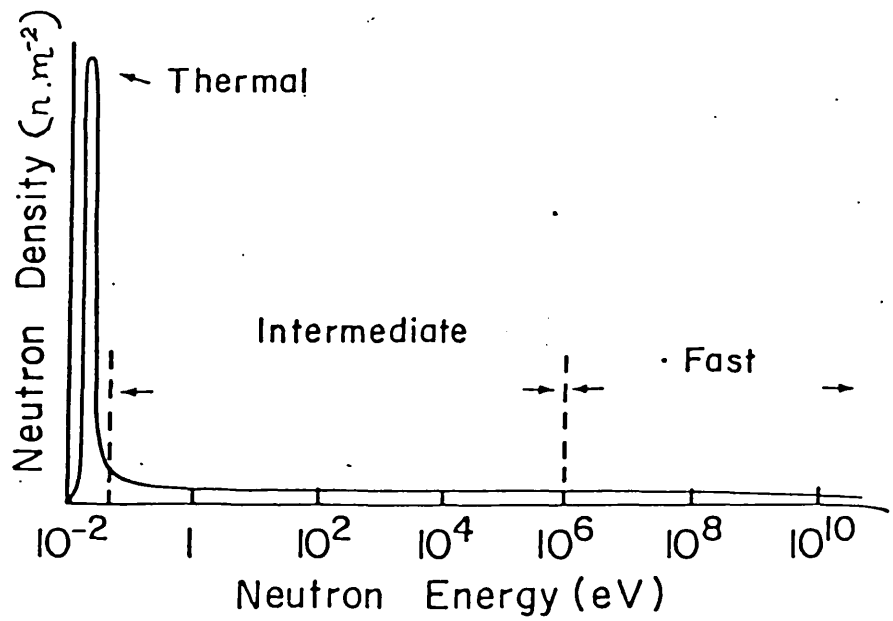


Figure 1.5.2

Generalised Distribution of Neutron Energies in a Reactor.



In a well moderated nuclear fission reactor a constant fission rate can be established which results in a stable neutron flux providing ideal conditions for neutron activation analysis. There will, however, be a range of neutrons of varying energy which may lead to nuclear interferences and this should always be taken into consideration when designating the ideal irradiation conditions for a particular sample - see figure 1.5.2.

1.6 Reactor Facilities Available at Silwood Park

The CONSORT reactor at Silwood Park is a swimming pool type reactor. The reactor core is suspended in a tank of de-ionised water at a depth of seven metres and is one metre in diameter. The water circulating within the tank acts as a coolant, by passing through a series of air cooled pipes, and as a moderator for the twenty-four fuel elements. Each fuel element is composed of enriched uranium in a high purity aluminium sheath. The full list of reactor specifications are shown below:

Max. Thermal Operating Power	:	100 kW
Mean Water Temperature (above core)	:	$44^{o}C$
Max. Temperature Rise (inlet/outlet)	:	$10^{o}C$
Coolant flow	:	9000 $L.hr^{-1}$

Reactor Core

Operating Mass of U^{235}	:	3265g
Number of Fuel Elements	:	24
Moderator, Coolant, Reflector	:	Light Water
Excess Reactivity	:	1%

Control Rods

	Material	Reactivity Worth
Safety Rod	Cd in Stainless Steel	2.2%
Coarse Rods (2)	Cd in Stainless Steel	1.4%, 2.2%
Fine Rod	Stainless Steel	0.5%

Within and around the reactor core there are facilities available for the irradiation of samples. Selection of the facility to be used is dependent on the irradiation time and whether a thermal, epithermal or fast flux of neutrons is required.

For long irradiations i.e. >8 hours, samples can be placed in the vertical core tubes (eight in total) surrounding the core. One of these tubes is clad in cadmium and therefore provides the conditions of an epithermal neutron flux. The in-core irradiation system (ICIS) is available for fast transfer of samples into and out of the core and provides the position of highest thermal flux within the reactor ($0.24 \times 10^{17} n.m^{-2}.s^{-1}$). The ICIS facility can be linked directly to the radiochemistry laboratories via a tube system containing compressed nitrogen. For very fast irradiation and transfer of samples into the detection position (transfer within 0.5 seconds) the cyclic activation system (CAS) on the 0^{o} face of the reactor can be used. This system connects with the core via one of two possible irradiation tubes; an aluminium tube for irradiation with the total reactor neutron spectrum or a cadmium tube for irradiation in an epithermal neutron spectrum. Both the ICIS and CAS systems are linked to multichannel analysers for prompt collection of sample spectra.

Other facilities available include a fast neutron spectrum assembly and a thermal column with horizontal and vertical branches.

The neutron flux in each of the above positions within the reactor is shown in table 1.6(a).

Each of the eight vertical thermal columns surrounding the reactor core can hold six sample tubes. Each of these six positions is numbered with position one being at the bottom of the core and six at the top, hence there is a flux difference observed between each of these sample positions. Table 1.6(b) shows the neutron flux at each position within the core tubes.

Knowledge of the neutron flux density is an important factor in neutron activation analysis, hence, the flux at each position within the Imperial College reactor is well documented.

1.7 Important Nuclear Reactions in Neutron Activation Analysis

The (n,γ) reaction is an important one for Neutron Activation Analysis since it produces the same radioactive nuclide as the inactive one, e.g.

$$^{23}_{11}Na + ^{1}_{0}n \rightarrow {}^{24}_{11}Na + \gamma\,(\text{prompt})$$

$$^{24}_{11}Na \xrightarrow{-\beta} {}^{24}_{12}Mg + \gamma\,(\text{delayed})$$

The above equation is a simplified monomolecular reaction of the process involved in neutron activation analysis. In any one sample only a small proportion of all the sodium nuclei will go through the above sequence of events and the eventual production of stable magnesium atoms will be dependent on a number of parameters which are to be discussed below.

Table 1.6 (a)

Facility	Thermal Flux ($n.m^{-2}.s^{-1}$)	Fast Flux ($n.m^{-2}.s^{-1}$)
Vertical Core Tube (8 in total)	0.14×10^{17} (max.)	0.03×10^{17} (max)
I.C.I.S.	0.24×10^{17}	0.10×10^{17}
C.A.S.	0.13×10^{17}	0.05×10^{17}
Fast Neutron Assembly	-	1.20×10^{12}
Thermal Column (including horizontal branching)	3.2×10^{12} (max.)	2.5×10^{10} (max.)

Table 1.6 (b)

Core Tube Position	Neutron Flux $\times 10^{16}$ ($n.m^{-2}.s^{-1}$)							
	Number of Core Tubes							
	1	2	3	4	5	6	7	8(epith)
1	0.60	0.80	0.69	0.64	0.70	0.79	0.84	0.56
2	0.82	1.09	0.95	0.88	0.95	1.08	1.14	0.77
3	0.96	1.27	1.10	1.02	1.11	1.25	1.33	0.89
4	0.96	1.27	1.10	1.02	1.11	1.25	1.33	0.89
5	0.82	1.09	0.95	0.88	0.95	1.08	1.14	0.77
6	0.60	0.80	0.69	0.64	0.70	0.79	0.84	0.56

1.7.1 Half life

In neutron activation analysis the measurement of decay radiation is usually made within a specified time interval after the end of irradiation. During the period before counting takes place the number of active nuclei will decrease according to the following equation:

$$\frac{dN}{dt} = -\lambda N \qquad (1.1)$$

where

λ = decay constant

N = total number of radioactive atoms.

The above equation (1.1) can be integrated to give,

$$N = N_o e^{-\lambda t} \qquad (1.2)$$

where

N_o = number of radioactive atoms at time zero

N = number of radioactive atoms at the present time

The half life of a radionuclide can be defined as the amount of time required for an initial number of radioactive atoms (N_o) to be reduced by half that value. Hence,

$$N = \frac{1}{2} N_o \qquad (1.3)$$

Combining this expression with equation (1.2);

$$\frac{1}{2} N_o = N_o . e^{-\lambda t_{\frac{1}{2}}} \qquad (1.4)$$

$$\ln 2 = \lambda . t_{\frac{1}{2}} \qquad (1.5)$$

$$t_{\frac{1}{2}} = \frac{0.693}{\lambda} \qquad (1.6)$$

The half life of a nuclide can be calculated from equation (1.6). As a decay constant is specific to each individual nuclide, nuclides can be characterised by measuring their half lives.

1.7.2 Saturation Activity

When a sample is exposed to a neutron source, the number of radioactive nuclei accumulating in the sample is dependent on the following equation:

$$\frac{dN^*}{dt} = \sigma_{act} \cdot \phi \cdot N - \lambda N^* \qquad (1.7)$$

where

σact = activation cross section (barn) (1 barn = $10^{-28} m^2$)

ϕ = neutron flux ($n.m^{-2}.s^{-1}$)

N = number of target nuclei

λN^* = decay rate of radionuclides in the sample

$$N^* = \frac{\sigma_{act} \cdot \phi \cdot N \cdot (1 - e^{-\lambda t})}{\lambda} \qquad (1.8)$$

This equation can be expressed as the number of disintegrations per unit time.

$$\lambda N^* = \sigma_{act} \cdot \phi \cdot N \cdot (1 - e^{-\lambda t}) \qquad (1.9)$$

If the sample were irradiated for an infinite period of time i.e. $t = \infty$ then

$$(1 - e^{-\lambda t}) \rightarrow 1$$

Hence,

$$\text{Saturation Activity} = A_{sat} = \sigma_{act} \cdot \phi . N \qquad (1.10)$$

1.7.3 Comparator Method

After an irradiation time of t_i and a cooling period of t_d, the remaining radioactive nuclei in the target sample are as follows:

$$\sigma_{act} . \phi . N(1 - e^{-\lambda t_i})e^{-\lambda t_d} \qquad (1.11)$$

A number of the nuclei decay during the counting period, t_c, so the equation can be modified to give

$$\lambda N^* = \frac{\sigma_{act}}{\lambda} . \phi . N \, (1 - e^{-\lambda t_i}) . e^{-\lambda t_d} \, (1 - e^{-\lambda t_c}) \qquad (1.12)$$

Hence, the specific activity induced in any parent nuclide can be calculated from the formula

$$S = \sigma_{act} . \phi . N . (1-e^{-\lambda t_i}) . e^{-\lambda t_d} \, (1-e^{-\lambda t_c}) . I_\gamma . \varepsilon_\gamma \qquad (1.13)$$

where

$I\gamma$ = intensity of γ-rays emitted

$\varepsilon\gamma$ = efficiency of the detector

N = sample mass (g)

If samples and standards are irradiated under identical experimental conditions then equation (1.13) can be simplified to

$$\frac{\text{specific activity of unknown}}{\text{specific activity of standard}} = \frac{\text{conc. of element in unknown}}{\text{conc. of element in standard}}$$

The use of comparator standards in neutron activation analysis is very important because it takes into account any changes in neutron flux to which the samples are subjected. Determination of a large number of elements within a sample using a multi-element comparator is only possible with the use of a high resolution Ge(Li) detector, the process involved will be discussed in a later section.

1.7.4 Interfering Nuclear Reactions

In instrumental neutron activation analysis there are two sources of interference:

1. Spectral Interference
2. Nuclear Reaction Interferences

Spectral interferences will be discussed in a later section on gamma-ray spectrometry (see section 1.8.4).

The production of a specific radionuclide from trace elements in the sample, other than the parent nuclei, will result in nuclear interferences. For example:

$^{50}Cr(n,\gamma)^{51}Cr \quad \sigma_{th} = 16 \times 10^{-4}$ barns

$^{54}Fe(n,\alpha)^{51}Cr \quad \sigma_{f} = 0.74 \times 10^{-7}$ barns

If samples are irradiated in a position in the reactor where the ratio of thermal neutron density to fast neutron density is 4.67 (core tubes five and six, positions three and four - see Table 1.6(b), the probability of the $^{54}Fe(n,\alpha)^{51}Cr$ reaction taking place is very small. Hence the problem of interference with measurements of Cr-51 is negligible. However, this was verified by an experiment using iron wire which gave no significant peak at 320 keV.

1.8 Gamma Spectrometry

1.8.1 Interaction of Gamma Radiation with Matter

Radionuclides formed during the activation process emit gamma radiation of characteristic energy and the interaction of this gamma radiation with matter leads to ionisation in the target material. The type of gamma interaction with the target is dependent on the energy of the emission and the physical properties of the target. In gamma radiation detection there are three possible ways a photon can interact:

1. photoelectric effect
2. Compton effect
3. pair production

Photoelectric effect

In this type of collision all the energy of the incident gamma is absorbed by the atoms of the target. With these atoms in an excited state there is emission of an electron (photoelectron) and consequently a rearrangement of the remaining orbital electrons resulting in the production of an X-ray (which is characteristic of the absorbing material).

Compton effect

A collision occurring between a gamma photon and an outer orbital electron, where there is partial loss of energy, resulting in the scattering of a lower photon is known as the Compton effect. The scattered photons have continuous energy because their energy content is dependent on the angle of scattering;

$$\text{Energy of scattered photon} = \frac{mc^2}{(1 - \cos \theta)}$$

where

θ = the angle of scattering between 0^{o} and 180^{o}.

This type of interaction is important with gamma energies of 0.5 - 5 MeV.

Pair production

A gamma photon with energy > 1.02 MeV can interact with the electrostatic field of a nucleus to produce an electron-positron pair which is annihilated and two photons of energy 0.51 MeV are emitted. These photons can then interact by photoelectric absorption or Compton interaction.

1.8.2 Types of Detector

The two types of detector most commonly used in neutron activation analysis are:

1. Scintillation Detectors e.g. NaI(Tl)
2. Semiconductor Detectors e.g. Ge(Li)

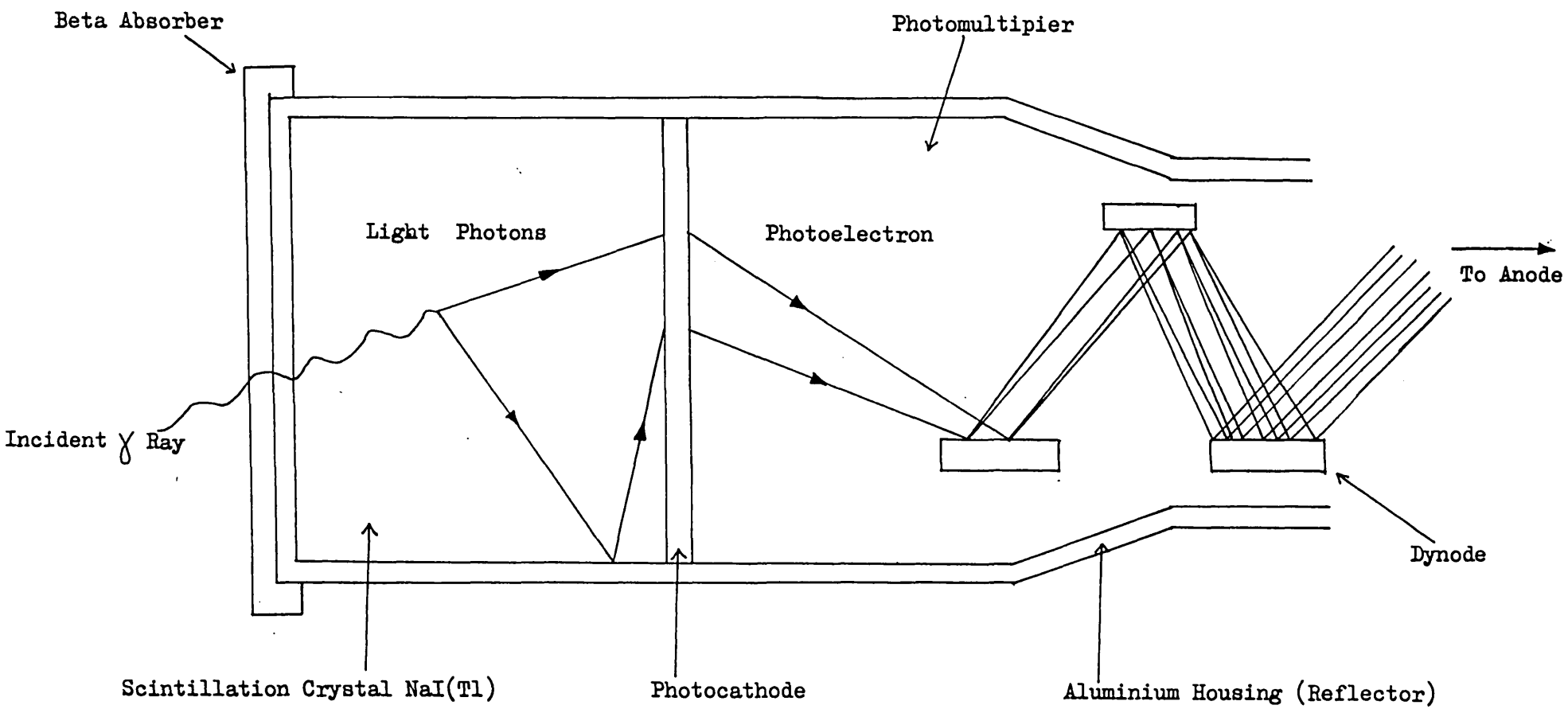
1. Scintillation Detectors

Scintillation detectors have developed for use in neutron activation analysis where only a small number of gamma-rays are to be measured. These types of detectors produce low resolution photopeaks with a high degree of efficiency.

The process involved in detection of gamma rays by an inorganic crystal relies on the ability of that crystal to demonstrate luminescence. As can be seen from the figure 1.8.2. incident gamma rays penetrate the aluminium housing of the detector and interact with the ions of the crystal. Scattering occurs within the crystal and a fraction of energy will be deposited in the ions of the crystal with each interaction. Consequently one or

Figure 1.8.2

A Schematic Diagram of a NaI(Tl) Scintillation Detector Optically Coupled to a Photomultipier Tube.



more high energy electrons will be produced and in the case of luminescent materials a certain proportion of these electrons will lose excitation energy by emission of light. Light interacting with the photocathode produces photoelectrons which are intercepted at the first dynode. These photoelectrons are then accelerated to the next dynode along with secondary electrons produced at the dynode surface. These steps are then repeated several times and eventually electrons emitted by the final dynode are collected at the anode. The resulting effect is that the quantity of charge i.e. number of electrons, arriving at the anode will be proportional to the energy lost by the interacting gamma ray. For this process to be efficient the luminescent material must possess the following qualities:

1. High density to allow for maximum absorption of gamma rays
2. Produce a high yield of photoelectrons
3. Fast fluorescence decay time
4. Linear response

The most commonly used detector of this type is a NaI(Tl) detector which has a crystal density of $3.67 g.cm^{-3}$, a fluorescence decay time of 2.5×10^{-7} sec and requires only 300 eV to release one photoelectron. See figure 1.8.2.

2. Semiconductor Detectors

As neutron activation developed to become a multielement technique it became necessary to produce a detector which could handle the increased number and variety of gamma-rays produced by radioactive samples. This resulted in the development of high resolution gamma spectrometers which are usually coupled to

a multichannel analyser system with the capability of manipulation of large quantities of data.

Semiconductor materials have various properties which make them favourable for use in this type of detector. In a semiconductor a large percentage of the electrons are fixed, however, a small addition of an impurity can render the material conductive i.e. it will have electrons which are free to move under the influence of an applied potential difference.

Elements belonging to group IV in the periodic table e.g. germanium, have four outer orbital electrons which are all involved in bonding and so conduction cannot occur. However, the addition of a small amount of a group V element e.g. phosphorus, to a Ge crystal will result in four of its outer electrons being involved in bonding and the fifth electron will be free to allow conduction within the lattice. This type of semiconductor is known as n-type because it has a number of conduction electrons. If impurities of a group III element e.g. gallium, are introduced into the Ge crystal only three electrons are available for bonding. The missing electron will leave a 'positive hole' in the lattice structure, this type of semiconductor is known as p-type. The p-type of materials are important in the conduction process because an electron from an adjacent atom can enter a 'positive hole', leaving another hole from whence it came. The overall effect produced is one of electrons and 'positive holes' travelling in opposition within the lattice.

The combination of an n-type and a p-type region within a germanium crystal at a temperature of absolute zero produces a

depletion region which is devoid of electrical charge. If a voltage is applied across the material, with the +ve to the n-type region, the internal electrical field of the crystal is enhanced and the depletion region increases to about 2 mm. This junction now formed can be used as a radiation detector because any ionisation resulting in the production of electrons will set up an electric current. The absorption of incident gamma-rays produces electron hole pairs and collection of the charge gives rise to an output signal which is proportional to the energy of the gamma. The amount of energy needed to produce an output signal in germanium is 2.9 eV whereas the same output in a NaI(Tl) detector requires 300 eV. Consequently the conversion of gamma energy into electrical energy is improved in a Ge detector and this in turn results in the higher resolution of semiconductor detectors.

In order to increase the efficiency of this type of detector it is necessary to increase the width of the depletion region i.e. producing a larger area for gamma absorption. This is achieved by drifting lithium ions into the n-type region of the crystal where there will be a resulting charge annihilation and a thicker depletion region is produced. This type of semiconductor detector is known as a p - i - n junction detector and the most widely used of these is a Ge(Li) detector.

These high resolution Ge(Li) detectors are operated at the temperature of liquid nitrogen ($-196^{o}C$) which stops the lithium drifting through the whole of the lattice and overcomes a certain amount of detector noise. For efficient detection of gamma radiation the detector should possess the following qualities:

1. Minimum of noise interference

2. Absence of impurities in the crystal which may lead to loss of signal - 'trapping centres'

3. High charge carrier mobility to allow for efficient charge collection

4. Low energy requirement for ion pair production to optimise resolution

A Ge(Li) detector fulfils all these criteria and is routinely used in the laboratories at ICRC for multielemental analysis of gamma spectra. The detector used in this project for instrumental neutron activation analysis of biological materials has the following characteristics;

Crystal Dimensions:-

Diameter = 39×10^{-3}m

Length = 39.5×10^{-3}m

Drift Depth = 16×10^{-3}m

Drift Configuration = Coaxial, open one end

Nominal Active Volume = 42 cm^3

Resolution

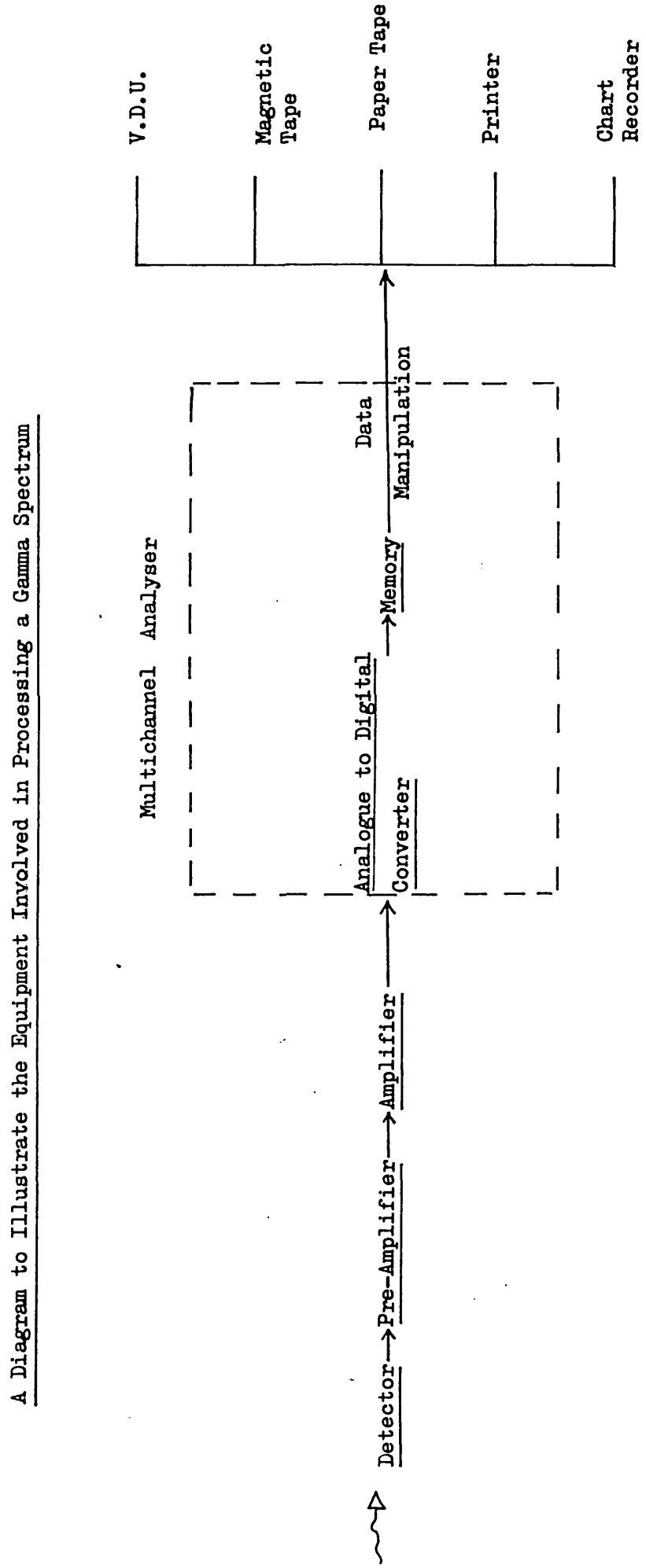
1.81 keV(FWHM) at 1.33 MeV

Efficiency

8.1% with respect to a NaI detector

Figure 1.8.3 shows the chain of events from interaction of a gamma-ray in the detector through to an account of the number and energy of the gamma energies. Each step along the chain will be discussed below.

Figure 1.8.3



A Diagram to Illustrate the Equipment Involved in Processing a Gamma Spectrum

Pre-amplifier

The pre-amplifier receives a charge sensitive input from the detector and transmits this signal (without distortion) to the amplifier.

Amplifier

The amplifier, amplifies the signals to bring them in the energy range of 0 - 10 volts whilst maintaining the linearity of the pulses. The peaks are also shaped and the discriminator rejects pulses with amplitude less than a pre-determined level i.e. cutting out ones produced by electronic noise.

Multichannel Analyser

The multichannel analyser can be divided into two regions:

i Analogue to Digital Converter (ADC)

The ADC is the module that converts peak height into its digital equivalent (channel number). The signals from the amplifier of 0 - 10 volts are divided into 4096 equal channels or memory locations. During the analysis time of pulse height to channel number the system is busy and cannot receive anymore signals from the amplifier, this is known as the dead time of the analyser. An automatic correction is made within the analyser to correct for the dead time.

ii Memory

Information on peak height in a digital mode is stored in the memory. It is possible to manipulate this information to provide an output of gamma energy versus peak area/activity. In order to be able to do this the spectrometer must be calibrated so that the resulting spectra can be interpreted.

Calibration of a spectrophotometer is performed using 1 μCi

sources of known radioactive species. The sources used each week to calibrate the Ge(Li) detectors at Imperial College Reactor Centre (I.C.R.C.) are Co-60 (1173 keV, 1332 keV), Cs-137 (662 keV) and Ba-133 (356 keV, 81 keV). The channel numbers are then set to represent 1 keV per channel so that in the final output channel number is equivalent to gamma energy. The other important detector parameter is energy resolution. This is a measure of how many channels are used to discriminate between peaks lying close together in the spectrum. For semiconductor detectors the resolution is usually expressed as the full width half maximum of the 1332 keV peak. For a coaxial Ge(Li) detector a resolution of 1.8 keV at 1.332 keV (Co-60) is attained routinely at ICRC.

Output Data

Manipulation of the spectra produced by each sample to give an unknown sample report is carried out at ICRC by an ND6600 multi-channel analyser (Nuclear Data Inc.) with the appropriate software packages. The spectra can either be viewed on a visual display unit or a paper copy can be obtained for data collection.

1.8.4 Spectral Interferences in Instrumental Neutron Activation Analysis

In the biological application of neutron activation analysis there are two categories of spectral interferences:

a Matrix Interference

b Compton Edge Interference

a Matrix Interferences

Biological samples contain high concentrations of the elements sodium, chlorine and phosphorus. To be able to measure long lived isotopes e.g. Zn-65 these elements pose no problems because their half lives are

Na-24 $t_{\frac{1}{2}}$ = 15 hrs

Cl-38 $t_{\frac{1}{2}}$ = 35 mins

P-32 $t_{\frac{1}{2}}$ = 14 days

The half life of Zn-65 is 245 days so all the matrix activity will have disappeared before the quantity of zinc has reduced by half and hence zinc can easily be measured.

However, in the case of Cu-66 ($t_{\frac{1}{2}}$ 5.1 mins) the activity of the matrix will be so high that when this sample has been irradiated the copper peak will be completely swamped by the Na, Cl and P. The only alternative is to carry out a radiochemical separation to remove the matrix components.

b Compton Edge Interference

The photopeak of the high energy gammas will have a Compton edge due to scattering in the detector crystal. This gives rise to a large tail on the low energy edge of the peak. Consequently if the photopeak of an element of interest is at a slightly lower energy than one of the major photopeaks and in much lower concentration within the sample, the smaller peak may be obliterated by the Compton edge of the larger peak, e.g. Rb-86 has a peak at 1076 keV which falls within the region of the Compton edge of the Fe-59 at 1099 keV. In order that the Rb-86 peak can be measured the technique not only requires a high resolution gamma detector but the manipulation of the irradiation and decay times to optimise for the measurement of the 1076 keV peak.

1.8.5 Applications of Neutron Activation Analysis in Medical Research

In Vitro (i) Detection of Normal trace element levels in man and comparison with levels of the same elements in various disease states.

(ii) Metabolic balance studies using stable tracers.

(iii) Production of radioactive tracers for all types of medical research.

(iv) Forensic science to detect possible toxicity.

In Vivo Non-destructive activation analysis using low doses of radiation to measure macroelements in the whole body. The elements that can be determined using a thermal neutron flux are Br, Ca, Cl, I, K and Na. Fast neutron fluxes have been used to measure F, O, N and P.

II Sample Selection, Collection and Preparation

2.1 Sample Selection

The selection of suitable patients and control individuals in clinical trace element research is of fundamental importance to ensure correct and adequate sampling with the minimum amount of discomfort to the individuals involved. Candidates must be adequately screened prior to the trials and fully informed as to the nature of the investigation. In order that these conditions be fulfilled there must be close collaboration between the analyst and the medical staff and all investigators must be aware of the factors which can affect trace element levels in clinical samples i.e.

1) Age
2) Sex
3) Nutritional Status
4) Posture
5) Pregnancy
6) Lactation
7) Environmental and Occupational Exposure
8) Disease States

All patients and controls were matched as closely as possible in all the experimental groups.

2.2 Sample Collection

Sample collection took place in a hospital environment and medical staff were made aware of the problems of possible contamination. For the purposes of these investigations the following clinical samples were collected:

Whole blood

Erythrocytes

Plasma

Serum

Urine

Hair - head and pubic

Whole blood was collected by venipuncture whilst patients were resting (Jurgensen, Behne 1977) using Becton Dickinson vacutainers (10 mls) and 21 G TW 1.1/2 needles. The vacutainers were internally coated with lithium heparin to inhibit clotting. Heparin functions as a cofactor in the formation of a complex between the coagulation factors in the blood and antithrombin III to prevent activation of the clotting mechanism. The blood samples were centrifuged at 2500g for twenty minutes in a Beckman centrifuge to separate erythrocytes and plasma.

For the collection of serum silicone-coated Becton Dickinson vacutainers were used and the sample was left to stand at room temperature for twenty minutes to allow adequate clotting before the serum was removed. Processing of all samples took place within two hours of collection and all the remaining sample was stored at 80^{o}C for future reference (Stoeppler, Durbeck, Nurnberg 1982).

Incorrect sampling or insufficient care when handling human blood samples can lead to haemolysis (rupture of the erythrocytes) which renders the samples useless for trace element analysis. The photographs overleaf indicate examples of haemolysed samples.

Collection of urine samples was carried out by the patients themselves. In the first part of the study urine was collected in

Figure 2.2.(a)

An Illustration of Correctly Sampled Human Plasma

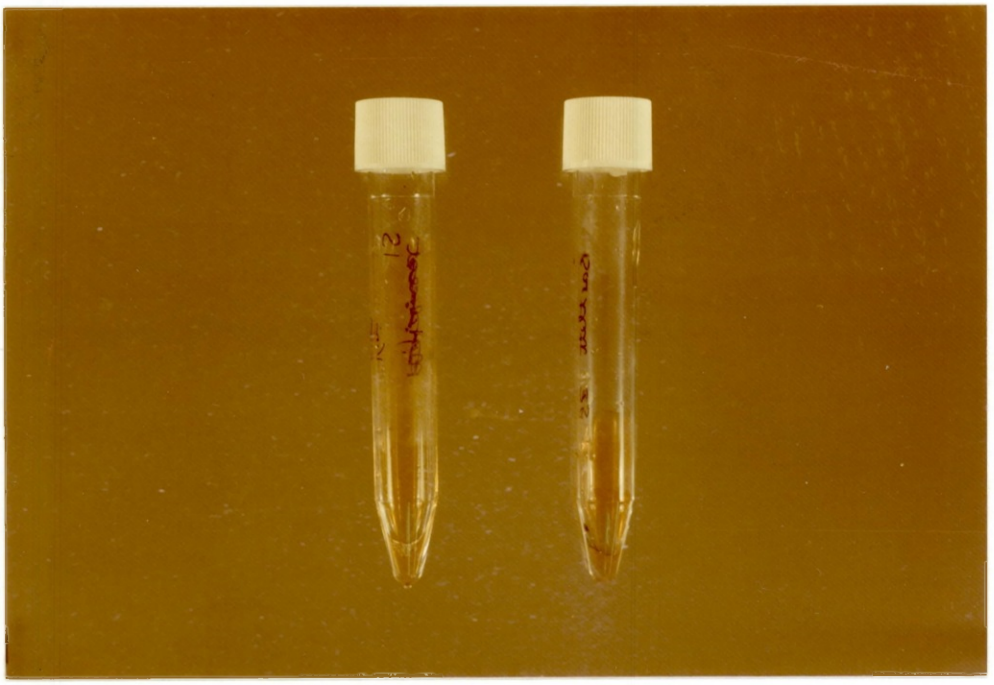
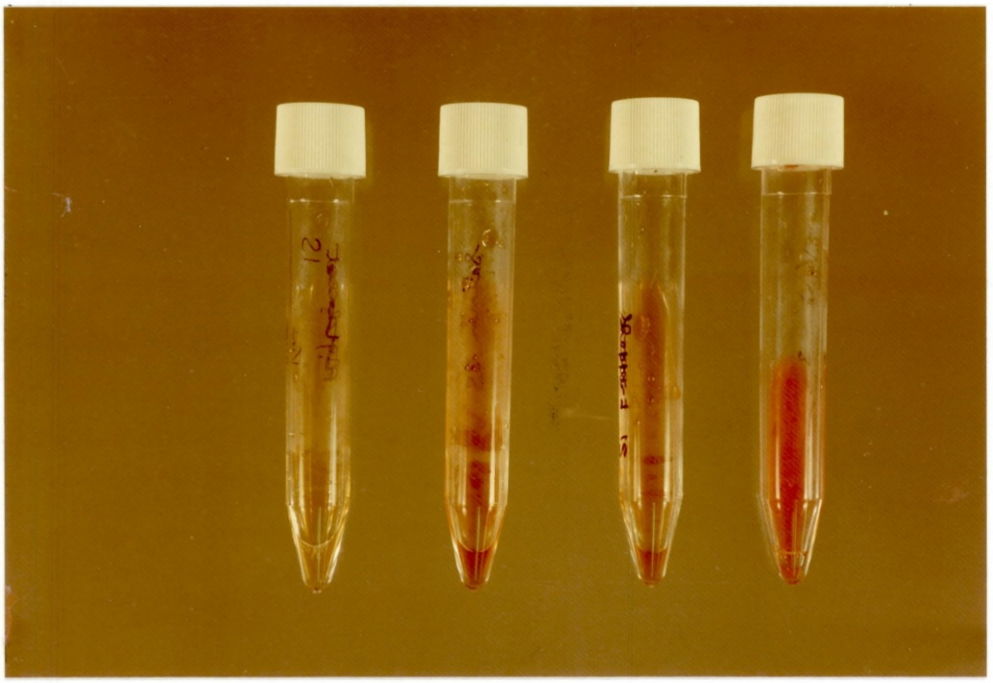


Figure 2.2.(b)

Several Examples of Haemolysed Human Plasma.



fifty millilitre polyethylene bottles (acid cleaned) at each stage of the experiment. In the second study 24 hour urine samples were collected in five litre polyethylene containers throughout the period of the experiment. Records were kept of the volume of urine produced by these individuals and also the pH, creatinine concentration, glucose and ketone concentrations.

Hair samples of approximately 10 cm length were cut from one area at the nape of the neck and the groin of the patients, and then the hair was heat sealed into plastic bags for transport to the laboratory.

Transportation of the samples from the hospital to the laboratory was by motorcycle and proved to be very efficient. The hospitals taking part in these clinical experiments were Radcliffe Infirmary (Oxford), West Middlesex Hospital (Isleworth) and Addenbrooke's Hospital (Cambridge).

2.3 Experimental Protocol

All investigations took into account the factors listed in Section 2.1. The investigations can be divided into several sections:

I Investigation into the diurnal variation of trace element concentrations in biological fluids of control and diabetic subjects.

Subjects were divided into three groups: Controls, Maturity onset diabetics (M.O.D.) and Insulin requiring diabetics (I.R.D.). All three groups contained patients of both sexes.

Urine and blood samples (10 mls) were collected after an overnight fast, at mid-day (pre-lunch) and again at approximately 18.00 hours (post dinner). All subjects were given an identical evening meal : soup, chicken salad with mashed potatoes, fruit salad/yoghurt and tea/coffee.

II Investigation into the effects of hyper and hypo-glycaemia on the levels of circulating trace elements

Both sets of experiments were carried out on control subjects after an overnight fast.

(a) Hyperglycaemia Experiment.

Intravenous glucose (20g per square metre of body surface) was given at time zero when the first blood sample was taken. Whole blood and plasma samples were then collected at ten minute intervals for one and a half hours. Plasma was analysed for glucose as well as trace element concentration. Glucose analysis was carried out by the glucose oxidase method at the John Radcliffe Infirmary and urine creatinine was measured on an Instrumentation Laboratory Microcentrifugal Analyser (Multi Stat III).

(b) Hypoglycaemia Experiments

A series of experiments were carried out on the same control subject and each time the subject was given one unit of Actrapid (soluble insulin preparation) intramuscularly at time zero after an overnight fast. The subject was then put on an insulin infusion ($3U.hour^{-1}$) for the following two hours and blood was collected every thirty minutes. In each case the experimental details differed slightly:

(i) In the first experiment the subject was established in a hypoglycaemic state and after fifty minutes was given 10g of glucose orally.

(ii) In the second experiment, after ninety minutes, the subject was given 20 mls of 50% dextrose solution intravenously.

(iii) In the final experiment 10g of Guar (high fibre plant extract - glucose free) were given orally after fifty minutes.

All plasma samples collected were analysed for glucose and insulin as well as trace elements.

III An investigation to observe trace element fluctuations in obese maturity onset diabetics in response to an oral glucose tolerance challenge

The subjects in this trial were separated into two groups:

(1) Female obese maturity onset diabetics (M.O.D.)

(2) Female obese non-diabetics

As an indication of obesity, individuals were included in these groups if their weight was greater than the maximum acceptable weight for their height - see table 2.3(a). All the diabetics were matched as closely as possible to the controls.

Patients were admitted into hospital and put on a 1000 calories per day diet to ensure each person received equal quantities of carbohydrate, protein and fat. Twenty-four hour urine collections were made pre and post the glucose tolerance test, all diabetic drugs were withdrawn 24 hours before the trial. Patients underwent an overnight fast and at time zero were given 75g of glucose in 500 mls of water orally. Blood was collected every thirty minutes for three hours, using the individual collection regimes as shown below:

Zero hours	50 mls whole blood were centrifuged to give erythrocytes and plasma for trace element analysis.
	+ 5 mls plasma for glucose analysis.
	+ 5 mls plasma for insulin analysis.

Table 2.3 (a) Guidelines for Body Weight*

Height (m)	Men - Weight (kg)			Women - Weight (kg)		
	Mean	Acceptable Range		Mean	Acceptable Range	
1.45				46.0	42	53
1.48				46.5	42	54
1.50				47.0	43	55
1.52				48.5	44	57
1.54				49.5	44	58
1.56				50.4	45	58
1.58	55.8	51	64	51.3	46	59
1.60	57.6	52	65	52.6	48	61
1.62	58.6	53	66	54.0	49	62
1.64	59.6	54	67	55.4	50	64
1.66	60.6	55	69	56.8	51	65
1.68	61.7	56	71	58.1	52	66
1.70	63.5	58	73	60.0	53	67
1.72	65.0	59	74	61.3	55	69
1.74	66.5	60	75	62.6	56	70
1.76	68.0	62	77	64.0	58	72
1.78	69.4	64	79	65.3	59	74
1.80	71.0	65	80			

*Adapted from the recommendations of the Fogarty Centre Conference on Obesity, 1973. From G.A. Bray: Obesity in America, NIH Publication No. 79. Washington, D.C., 1979, p. 359.

30 minutes	20 mls whole blood for trace element analysis.
	+ 5 mls plasma for glucose analysis.
	+ 5 mls plasma for insulin analysis.
60 minutes	as for 30 minutes.
90 minutes	as for 30 minutes.
120 minutes	as for zero time.
150 minutes	as for 30 minutes.
180 minutes	as for 30 minutes.

Blood glucose measurements were carried out at the West Middlesex Hospital and all other biochemical tests including urine creatinine were carried out in Addenbrooke's Hospital laboratory. The 24 hour urine samples were tested using Multistix (Miles Laboratories Limited) for pH, glucose and ketones; blood volume was also recorded.

In addition to the blood and urine samples each patient supplied a sample of pubic and head hair (cut from the nape of the neck) at the beginning of the investigation.

A certain number of the patients remained in hospital after their glucose tolerance test to go through a period of supervised weight loss. Each was given 330 calories per day of a liquid dietary supplement (Cambridge diet) see table 2.3(b) for one month. The starting weight and final weight of each individual was recorded. After a period of one month the glucose tolerance test was repeated, as above.

Table 2.3 (b)

FORMULATION OF THE CAMBRIDGE DIET*		
Calories		330
Protein		33g
Carbohydrate		44g
Fat		3g
Vitamins:	A	1×10^{-3}g
	B6	2×10^{-3}g
	B12	3×10^{-6}g
	C	60×10^{-3}g
	D	7.5×10^{-6}g
	E	10×10^{-3}g
	K	67×10^{-6}g
Elements:	Ca	0.80g
	Cl	1.80g
	Cr	60×10^{-6}g
	Cu	3×10^{-3}g
	I	0.15×10^{-3}g
	Fe	15×10^{-3}g
	Mg	0.35g
	Mn	4×10^{-3}g
	Mo	0.15×10^{-3}g
	P	0.80g
	K	2.01g
	Se	60×10^{-6}g
	Na	1.5g
	Zn	15×10^{-3}g
Folic acid		0.4×10^{-3}g
Pantothenic acid		7×10^{-3}g

* One daily allowance

IV An investigation to observe trace element fluctuations in lean maturity onset diabetics in response to an oral glucose tolerance challenge

The subjects of this trial were divided into two groups:

(1) Male lean maturity onset diabetics

(2) Male lean non-diabetics

Patients were matched as closely as possible and were all in the acceptable range of weight/height ratio according to table 2.3(a). Patients taking part in this trial were not hospitalised but were treated as Out-Patients at Addenbrooke's Hospital. Each individual was asked to cease taking their diabetic drugs for 24 hours before the trial and arrive at the hospital having fasted overnight. Twenty-four hour urine was collected during the trial and blood samples were taken as previously described in section III every 30 minutes for 2 hours after the oral administration of 75g of glucose in 500 mls of water.

All sample analysis, apart from the trace element analysis, was carried out at Addenbrooke's Hospital.

2.4 Preparation of Apparatus for Trace Element Analysis

The analysis of biological samples by INAA requires that the samples be exposed to the minimum amount of contamination either from the atmosphere, apparatus, reagents or containers used during irradiation. The procedure described below for sample preparation includes all information available as to possible sources of contamination or errors in the analysis.

The collection and transport of blood samples in vacutainers has been suggested as a possible area for contamination of the samples from the rubber stoppers. Versieck, Barbier, Cornelius and Hoste

(1982) carried out a study to investigate this problem and found that contamination of the sample by copper and zinc was negligible whereas manganese contamination, after two hours contact with the stopper, was in the region of 11-24%. In order to clarify this point two different types of vacutainer stoppers were analysed by INAA - table 2.4(a). The red stopper was from a silicone coated tube and the green stopper was from a lithium heparin coated tube. The stoppers were found to contain large quantities of trace elements especially zinc and iron.

It was possible that the blood samples were being contaminated by these elements due to contact with the vacutainer lid during transport. To investigate this samples of a pooled blood were stored for periods of three and six months in lithium heparin vacutainers with half the samples stored upright i.e. no contact with the stopper and the other half stored upside down to allow contact. The results of this investigation are recorded in table 2.4(b). After a period of three months the following elements had leached from the stoppers into the samples - Cs, Cr, Sb and Zn. After six months Cs, Cr, Co, Fe, Rb, Sb, Se and Zn levels were higher than in the initial pooled blood sample, when the sample had been in contact with the stopper. The concentration of mercury in the samples was reduced by 78% after three months storage and by 93% after six months irrespective of whether the sample had contact with the lid. Losses of mercury from stored samples were not uncommon and this is one of the main reasons why the samples must be processed as rapidly as possible after collection.

Table 2.4 (a) Analysis of Stoppers From Becton Dickinson Vacutainers by I.N.A.A.

Element	Mean Elemental Concentration (μg. g^{-1})	
	Red Stopper	Green Stopper
Cs	0.14	0.19
Cr	2.01	2.59
Co	0.64	1.16
Fe	1639	1478
Hg	0.07	0.06
Rb	6.62	8.37
Sb	0.09	0.11
Se	0.27	0.86
Zn	1892	6491

Table 2.4 (b) Long Term Study to Investigate the Possible Contamination of Blood Samples by Becton Dickinson Lithium Heparin Vacutainers

Element	Mean Elemental Concentration (μg. ml^{-1})				
	Initial Sample	3 mths Storage at -80°C		6 mths Storage at-80°C	
		Lid Contact	No lid Contact	Lid Contact	No lid Contact
Cs	0.008	0.010	0.009	0.010	0.005
Cr	0.004	0.006	0.005	0.092	0.032
Co	0.003	0.003	0.008	0.007	0.009
Fe	411.5	408.9	421.3	522.9	380.3
Hg	0.19	0.042	0.040	0.013	0.016
Rb	0.93	0.93	0.94	1.53	1.26
Sb	0.003	0.008	0.005	0.014	0.015
Se	0.12	0.12	0.13	0.14	0.14
Zn	6.97	7.84	6.96	7.31	7.85

The samples analysed in all the experiments were left in the vacutainers for no longer than a few hours and so it can be assumed that contamination of the sample would be negligible. However, if the sample were stored prior to analysis the degree of contamination would be quite considerable.

All apparatus used in the preparation of samples (including pipette tips and irradiation capsules) was soaked overnight in concentrated nitric acid (to remove surface contamination) then rinsed in de-ionised water and oven dried. The acid and water used in the cleaning process was analysed and found to contain very low levels of the trace elements of interest - table 2.4(c). Acids that are to be used in sample digestion can be purified by methods such as sub-boiling distillation or ion-exchange chromatography (Mitchell 1982) (Moody, Beary 1982), to contain even lower concentrations of trace metals. However, in this study the acid and water were used purely for washing purposes and so further purification was not necessary. After washing all apparatus was stored in clean plastic bags.

2.5 Sample Preparation

Samples were opened on arrival in a laminar flow cabinet in the Clean Room to avoid contamination from atmospheric dust. Table 2.5(a) shows the concentration of trace elements in laboratory and external air (Hamilton, et al, 1971) which contribute to this problem.

All liquid samples were dispensed into small polythene capsules by 'reverse' pipetting (Medina, et al, 1977) using a 'Finipet' pipette to avoid errors in sample volume. All samples were then frozen overnight at -80^{o}C and then freeze dried at -40^{o}C under

Table 2.4 (c) Trace Element Concentration of Conc. Nitric Acid and De-ionised water from I.C.R.C. Laboratories

Element	Mean Elemental Concentration x 10^{-4} ($\mu g.ml^{-1}$)	
	De-ionised water	Nitric acid
Cs	< 1.96	< 1.80
Cr	< 0.96	< 1.04
Co	< 1.74	< 5.60
Fe	<1400	< 900
Hg	< 5.60	< 1.50
Rb	< 1.89	< 2.01
Sb	< 12.20	< 9.80
Se	< 3.80	< 1.70
Zn	< 8.40	< 18.0

Table 2.5 (a) A Comparison of the Trace Element Concentration in Laboratory and External Air.

Element	Mean Concentration in Laboratory Air ($\mu g.g^{-1}$)	Mean Concentration in External Air ($\mu g.g^{-1}$) ± S.D.
Cs	< 1×10^{-2}	1.3 ± 0.5
Cr	< 6×10^{-3}	39.2 ± 12.2
Co	< 0.10	9.0 ± 3.7
Fe	< 6×10^{-3}	3230 ± 650
Rb	< 1×10^{-2}	22.6 ± 8.3
Sb	< 3×10^{-2}	14.8 ± 5.2
Se	< 2×10^{-2}	0.6 ± 0.2
Zn	< 2×10^{-2}	1640 ± 510

vacuum for one day. Freeze drying was the method of choice for drying the samples in order to avoid losses by volatisation which can occur when dry ashing. Samples were stored in a dessicator until required.

In order that all the samples should be of equivalent geometry each one was made into a small pellet (diameter 10 mm, thickness - 4 mm). These pellets were made by compressing a weighed amount of the freeze dried sample between two stainless steel dies with a pressure of $\sim$3 tons applied.

The contamination from the pelleting equipment was established as negligible. The resulting pellets were placed in small size one polyethylene capsules ready for irradiation. For long term irradiation i.e. > one day, the samples must be packed inside a large polyethylene capsule (height 750 mm) along with chemical standards and a pellet of a reference material e.g. Bovine liver. All these samples were irradiated in the same position in the reactor core at a known flux.

Polyethylene capsules were used because they are more resistant to radiation than other polymers, can be easily moulded into shape (Heydorn and Damsgaard 1982) and once acid washed they provide no contamination to the sample. They do, however, contain trace elements within their structure (Kucera and Soukal 1983) and so after irradiation samples were transferred to non-active containers before counting.

Hair samples were prepared for irradiation by washing in deionised water in an ultrasonic bath for one hour (water changed twice) to remove surface dirt and grease (Othman, Spyrou 1978) (Holzbecher and Ryan 1981). To remove the water from the surface of the

samples the hair was freeze dried under vacuum at -40°C for approximately one hour. These samples of dry hair were weighed then coiled and packed into polyethylene capsules for irradiation. The preparation of dry, solid biological samples prior to irradiation was necessary because when irradiating solutions radiolysis can occur giving rise to gases which result in a build up of pressure within the capsules. Blood can be considered as an organic system and so the mechanism of radiation decomposition is very complex. A variety of products would be produced including gases such as H_2, CO and CO_2 along with small fragments of the irradiated material. To avoid problems of possible explosion and subsequent contamination from liquid samples the reactor personnel encourage the use of dry samples.

2.6 Preparation of Chemical Standards

The simultaneous irradiation of standards containing known amounts of the elements of interest allows for quantitative determination of trace element levels in the unknown samples. Chemical standards were prepared from a stock solution - see table 2.6(a) - of metal ions in dilute (0.01M) HNO_3 acid. The solution was freeze dried onto 5.5 cm Whatman filter papers and pelleted in the same manner as all other samples. Filter paper was used as a carrier medium because it contained low levels of trace elements - see table 2.6(b). Each new batch of standards was checked before use and the reproducibility of the standards was continually monitored.

Table 2.6 (a) Composition of Chemical Standard for I.N.A.A. of Biological Samples

Element	Elemental Concentration ($\mu g.ml^{-1}$)	Reproducibility (%)
Cs	0.05	11.9
Cr	0.10	11.7
Co	0.04	7.9
Fe	500	7.2
Hg	0.10	12.9
Rb	5.00	9.4
Sb	0.01	8.1
Se	0.10	16.0
Zn	10.0	5.5
Additional elements*		
Al	0.50	
Br	4.00	
Ca	100	
Cu	1.00	
I	0.10	
Mg	50.0	
Mn	0.10	
Ni	0.20	
K	2000	
Na	2000	
F	0.50	

*Elements added to the stock solution to match the matrix of the unknown samples, although not actually measured in the unknowns.

Table 2.6 (b) Trace Element Concentration of 5.5cm diameter Whatman Filter Paper Discs

Element	Mean Elemental Concentration ($\mu g.\ g^{-1}$)
Cs	< 0.03
Cr	< 0.004
Co	< 0.001
Fe	< 1.14
Hg	< 0.005
Rb	< 0.03
Sb	< 0.008
Se	< 0.02
Zn	< 0.09

III Optimisation of Parameters for the Analysis of Long Lived Nuclides in Biological Materials

3.1 Elements of Interest

The trace elements of interest in this study which have long lived nuclides are listed below:

Antimony

Caesium

Chromium

Cobalt

Iron

Mercury

Rubidium

Selenium

Zinc

In order to establish the ideal conditions necessary to measure the above trace elements in biological material, the data in table 3.1(a) along with the results of exploratory experiments must be considered.

A series of experiments were carried out to establish the irradiation time (t_i), decay period (t_d) and counting time (t_c) most suitable for the necessary multielement analysis. All samples were irradiated in a thermal neutron flux = 1.11×10^{16} $n.m^{-2}.s^{-1}$ and consisted of pellets of whole blood. For each set of experimental conditions a detection limit was calculated.

3.2 Limit of Detection of a Photopeak

The specific activity per gram of element (A_o) observed as a specific photopeak can be expressed by the following equation,

Table 3.1 (a)

Radionuclide	Half Life ($t_{\frac{1}{2}}$)	Major -energies (keV)	Thermal Neutron Cross Section (barns)	Degree of Difficulty of I.N.A.A.*
Cs-134	2.05y	795.8, 604.7	140	difficult
Cr-51	27.8d	320	16	difficult
Co-60	5.3y	1173.1, 1332.4	2	possible
Fe-59	45.1d	1098.6, 1291.5	1.2	possible
Hg-203	46.9d	279.1	4.8	possible
Rb-86	18.7d	1076.6	0.40	possible
Sb-124	60.9d	602.6, 1690.7	7	?
Se-75	121d	264.6, 136	58	possible
Zn-65	245d	1115.4	0.81	possible

*taken from Nondestructive Activation Analysis editor S. Amiel.

$$A_o = \frac{w.\ a.\ N_A \cdot \phi \cdot \sigma \cdot (1 - e^{-0.693 t_i / T})}{A} \qquad (3.1)$$

where

w = mass of the element (g)

a = fractional isotopic abundance of the target

N_A = Avogadro's number (6.022×10^{23} atoms.g-atom^{-1})

ϕ = thermal neutron flux (n.m^{-2}.s^{-1})

σ = cross section of the target (barns)

t_i = irradiation time (s)

T = half life of the radionuclide produced

A_o = counts.s^{-1}.g^{-1}

A = atomic mass of the element

However, if no peak is discernible in the pulse-height spectrum (digital) the multichannel analyser will calculate a limit of detection by comparison with the relevant signal in the spectrum of the standard. The limit of detection is usually expressed in terms of mass, w, of the element of interest under given irradiation and detection conditions:

$$w = L_D(c).\gamma \qquad (3.2)$$

where

$L_D(c)$ = count rate (counts.s^{-1})

γ = calibration constant (g.s.counts^{-1})

γ is related to A_o by the following expression,

$$A_o = \frac{1}{\gamma} \qquad (3.3)$$

Consequently each of the variables in equation (3.1) will affect the detection limit.

The detection limit can also be defined as "the true net signal which may be expected to lead to detection". Currie (1968) defined a set of 'working' expressions for calculating the detection limit for radiation measurements. The limit of detection is taken as $2.33\sqrt{\text{background}}$ where background is a measure of the possible error in the background reading and 2.33 represents the 99% confidence level of a Poisson distribution (the photopeak is assumed to show the characteristics of a Poisson distribution). In other words when showing a result as a detection limit there is a 99% chance that the signal measured is a photopeak.

$$L_D = 2.33\sqrt{\text{Background}} \qquad (3.4)$$

3.3 Exploratory Experiments

(a) t_i = 3d

t_d = 20d*

t_c = 5000s

sample size = 100 mg

*decay time of 20 days was chosen to allow for a reduction in the activity of the matrix components i.e. Na-24, P-32.

Table 3.3(a)

Element	Detection Limit ($g.ml^{-1}$)
Cr-51	0.014
Co-60	0.004
Fe-59	3.60
Hg-203	0.008
Rb-86	0.24
Se-75	0.092
Zn-65	0.160

(b) t_i = 3d

t_d = 20d

t_c = 5000s

sample size = 200 mg

Table 3.3(b)

Element	Detection Limit ($g.ml^{-1}$)
Cr-51	0.010
Co-60	0.002
Fe-59	3.30
Hg-203	0.006
Rb-86	0.020
Se-75	0.03
Zn-65	0.116

(c) t_i = 65 hrs

t_d = 30d

t_c = 7200s

sample size = 200 mg

Table 3.3(c)

Element	Detection Limit ($g.ml^{-1}$)
Cs-134	0.002
Cr-51	0.001
Co-60	0.0007
Fe-59	2.21
Hg-203	0.006
Rb-86	0.013
Sb-124	0.002
Se-75	0.01
Zn-65	0.089

Several conclusions were drawn from these data

(i) It was necessary to irradiate the samples for at least 60 hours to ensure adequate activation of these long lived nuclides.

(ii) Decay period must be sufficiently long for a reduction in the Na-24 and P-32 matrix interference.

(iii) Counting time must be increased beyond 5000s to alleviate the problem of having poor counting statistics due to the use of a low efficiency Ge(Li) detector.

(iv) The detection limits obtained were by purely instrumental activation analysis and so use of time consuming radiochemistry did not appear to be justified.

Once the irradiation conditions for a 200 mg pellet of blood were established this method was then applied to various sample types - table 3.3(d) - for calculation of the detection limits. Table 3.3(e) shows the expected normal range of trace elements in the biological fluids analysed in the following experiments.

Conclusions

The optimum parameters for the instrumental detection of long lived nuclides in several biological samples are:

t_i = 65 hrs

t_d = 30d

t_c = 7200s

Neutron flux = $1.11 \times 10^{16} n.m^{-2}.s^{-1}$

Sample Size

whole blood : one millilitre (0.2g)

erythrocytes : two millilitres (0.4g)

plasma/serum : two millilitres (0.2g)

urine : eight millilitres

hair : 0.1g dry weight

Table 3.3 (d) Limits of Detection for Various Biological Media.

Element	Detection Limit ($\mu g.ml^{-1}$)						
	Erythrocytes (1ml)	Erythrocytes (2ml)	Plasma (1ml)	Plasma (2ml)	Urine (6mls)	Urine (8mls)	Hair (0.1g)
Cs	0.002	0.0009	0.001	0.0007	0.0014	0.0013	0.0015
Cr	0.0025	0.0019	0.0016	0.0015	0.0016	0.0014	0.0017
Co	0.0019	0.0017	0.0011	0.0006	0.00098	0.00107	0.0009
Fe	6.77	4.19	0.92	0.58	0.94	0.86	1.68
Hg	0.0076	0.0017	0.0042	0.0021	0.0021	0.0019	0.005
Rb	0.37	0.31	0.083	0.074	0.112	0.106	0.069
Sb	0.0007	0.0003	0.0008	0.00013	0.00088	0.00081	0.0001
Se	0.020	0.0149	0.034	0.018	0.036	0.024	0.016
Zn	0.126	0.059	0.051	0.039	0.045	0.042	0.072

Table 3.3 (e) Table showing the Normal Range Values of Individual Trace Elements in Biological Samples.

Element	Range of Elemental Concentration ($\mu g.ml^{-1}$)				
	Whole blood	Erythrocytes	Plasma	Urine (24hr)	Hair
Cs	0.003-0.0047	?	.0007-.0013	9-50	?
Cr	.0065-0.107	0.019-0.066	0.14-1.67	0.6-150	0.13-3.65
Co	.0003-0.099	.00016-.086	.0007-.012	0.73-235	0.20-1.05
Cu	0.64-1.28	0.75-1.31	0.61-1.41	11-325	11-34
Fe	301-530	985-1140	0.71-1.27	131-1200	5-44.7
Hg	<.005-0.02	.007-0.05	.002-.011	1-17	1.25-7.60
Rb	1.17-5.98	4.2-5.3	.083-1.14	1100-2400	-
Sb	.0012-.0041	0.0046	.0005-.005	1.53-3.50	0.09-3.00
Se	0.057-0.32	0.07-0.24	< .03-0.59	20.4-187	0.64-2.53
Zn	4.80-9.30	7.60-16.1	0.79-1.70	42-1250	99-450

The main limiting factors in all the experiments carried out were the actual amounts of available sample and flux deviation during reactor operations.

3.4 Quality Control

Extreme importance is put on quality control to monitor any major changes in the conditions of the analysis. The necessity for adequate reference materials has led to the production of many types of synthetic materials i.e. polycrylamide gels containing trace elements (Masumoto and Suzuki; 1980) and a phenol-formaldehyde resin (Mosulishvili, et al, 1984), and powders made from pooled biological samples of known composition i.e. National Bureau of Standards (NBS) Bovine Liver or International Atomic Energy Authority (IAEA) animal muscle. Any reference material in order to be useful in quality control must meet the following requirements, (Brits and De Wet; 1979).

(i) Trace element concentrations well characterised and checked by several techniques.

(ii) Homogeneity.

(iii) Preferably have same matrix constituents as unknown sample.

(iv) It should not be hygroscopic nor decompose on irradiation.

(v) Preferably be a powder allowing preparation of samples of equivalent geometry.

The material of choice in this study was N.B.S. (ref 1577) Bovine Liver. This reference material contains the nearest overall trace element concentration profile to those of the samples and the matrix is not dissimilar - see table 3.4(a).

Table 3.4 (a) Table to Illustrate the Similarity between the concentration of trace elements in NBS Bovine Liver and whole blood

Element	Mean Elemental Concentration (p.p.m.)	
	Whole blood* ($SD\bar{x}$)	NBS Bovine Liver
Cs	0.0065 ± .004	0.013
Cr	0.057 ± .005	0.20
Co	0.049 ± .004	0.18
Fe	415.5 ± 114.5	270 ± 20
Hg	0.012 ± .007	0.016± .002
Rb	5.25 ± .09	18.30± 1.0
Sb	0.011 ± .006	0.010
Se	0.171 ± .13	1.1 ± 0.1
Zn	7.0 ± 2.25	130 ± 10

*calculated from ten readings obtained under the ideal irradiation conditions

Table 3.5 (a) Comparison of Experimental and Certified Trace Element Concentrations of Bovine Liver Reference Material.

Element	Mean Elemental Concentration ($\mu g.g^{-1}$)	
	Expected Values ($SD\bar{x}$)	Calculated Values* ($SD\bar{x}$)
Cs	0.013	0.022 ± .005
Cr	0.20	0.22 ± .027
Co	0.18	0.21 ± .034
Fe	270 ± 20	284.5 ± 31.4
Hg	0.016 ± 0.002	.017 ± .005
Rb	18.3 ± 1	18.2 ± 2.9
Sb	0.016	0.018 ± .004
Se	1.1 ± 0.10	1.18 ± .23
Zn	130 ± 10	129.9 ± 21.7

*Mean sample weight was 0.258g.

The analysis of NBS Bovine Liver under well defined conditions can be used to estimate the precision and accuracy of the technique. However, it must be stressed that these reference materials should not be used as the standard for each analysis.

3.5 Analytical Accuracy and Precision

The accuracy of the technique is defined as "the closeness of agreement between the true value and the mean result which would be obtained by applying the experimental procedure a large number of times". Table 3.5(a) shows a comparison between the expected values (NBS certified values) and the mean calculated values for the repeated analysis of bovine liver samples under the following experimental conditions:

t_i = 65 hrs

t_d = 30d

t_c = 7200s

The reproducibility of the technique or precision is 'a measure of the spread of a number of measurements carried out on the same sample'. Precision can be expressed as the percentage standard deviation from the mean, see table 3.5(b).

Table 3.5 (b) A Table Showing the Reproducibility of the Analysis of Bovine Liver Reference Material

Element	Mean Concentration ($\mu g.g^{-1}$) (n = 10)	% standard deviation
Cs	0.20	7.09
Cr	0.21	3.84
Co	0.19	5.06
Fe	281.3	3.44
Hg	0.18	9.18
Rb	18.7	4.97
Sb	0.012	6.94
Se	1.09	6.06
Zn	131.7	5.22

IV Cumulative Neutron Activation Analysis of Biological Materials for Measuring Short-Lived Nuclide

4.1 Introduction

Over the last 20 years there has been growing evidence that ultra-trace elements (< 1 $ng.g^{-1}$) such as vanadium (Mountain et al. 1956, Curran 1960, Schroeder et al. 1963) may be involved in the trace element metabolism of diseases with disturbances in cholesterol biosynthesis. Short-lived nuclides such as these are impossible to measure by INAA and so a method of radiochemical separation (post-irradiation) must be employed (Cornelius, et al, 1981, Simonoff, et al, 1984).

The difficulty of measuring these nuclides is due to the matrix elements contributing to the high background activity. Presumably, if a way were found to reduce this component of the spectrum then the possibility of INAA would exist.The use of an epithermal irradiation is not sufficient as it also depresses the flux. In the case of vanadium, with a $t_{\frac{1}{2}}$ = 3.75 mins, optimisation of the signal to background ratio at the vanadium photopeak (1434 keV) would be necessary. Two possibilities exist which may achieve the necessary detection limit,

(i) Cyclic activation analysis requiring repeated short irradiation of one sample or

(ii) Cumulative activation analysis involving the short irradiation of several equal portions of a sample and summing of the recorded spectra.

The idea of cumulative activation analysis was first introduced by Guinn (1980) and has already been successfully used to measure short lived isotopes in geological materials (Parry 1982). This technique has several advantages over cyclic activation analysis:

(i) Each of the samples irradiated are of equal mass and are irradiated for the same period of time, hence analyser dead time and pulse pile-up are the same each time. Corrections for these two parameters are made only on the final spectrum, whereas in cyclic activation analysis (C.A.A.) corrections for dead time and pulse pile-up must be made after each cycle.

(ii) In C.A.A. the build up of activity within the sample after each irradiation eventually limits the number of cycles. This is not evident in cumulative activation analysis (Cum.A.A.) because each sample is irradiated only once.

(iii) Cum.A.A. can lead to a more accurate representation of the sample content because several samples are analysed, this is an especially useful technique for analysing inhomogeneous samples.

4.2 Theory of Cumulative Activation Analysis

A signal (S) will have a relative standard deviation measured above a background (B). Where,

$$\sigma . S_{rel} = \frac{(S + 2B)^{\frac{1}{2}}}{S} \qquad (4.1)$$

In a cumulative spectrum for n replicates

$$\sigma . n . S_{rel} = \frac{n^{\frac{1}{2}} . (S + 2B)^{\frac{1}{2}}}{n . S} = \frac{\sigma . S_{rel}}{n^{\frac{1}{2}}} \qquad (4.2)$$

Hence, the precision of the measurement was improved by $n^{\frac{1}{2}}$. Using this to predict the improvement in the detection limit (L_D) where,

$$L_D = 2.33\ B^{\frac{1}{2}} \quad \text{(Currie 1968)} \qquad (4.3)$$

The detection limit of the total spectrum is improved by a factor $n^{\frac{1}{2}}$.

$$L_D = \frac{2.33\, n^{\frac{1}{2}}\, B^{\frac{1}{2}}}{n} = \frac{L_D}{n^{\frac{1}{2}}} \qquad (4.4)$$

4.3 Method

Although the cyclic activation system (neutron flux = 0.13 x $10^{17} n.m^{-2}.s^{-1}$) would have provided the ideal conditions for carrying out short irradiations and rapid transfer to a detector, the capsules used in this system were found to contain on average 4.2 $ng.g^{-1}$ vanadium. As this set up does not allow for the transfer of irradiated samples out of the capsule before counting and the vanadium content of the capsules was found to be inconsistent, the detection of nanogram levels of vanadium was impossible. The alternative system, in-core irradiation system, I.C.I.S. (neutron flux = 0.24 x $10^{17} n.m^{-2}.s^{-1}$) does allow for samples to be transferred into non-active polyethylene capsules by the use of a rabbit system which connects ICIS with the radiochemical laboratory. The minimum amount of time to transport a sample from the reactor core to the laboratory, transfer the sample into another capsule and place on the detector was 75 seconds.

Transfer of the sample into another capsule with ease necessitated the use of a carrier onto which the sample could be placed prior to irradiation. To establish the vanadium content of several carrier materials each was irradiated in ICIS for 225 seconds ($t_{\frac{1}{2}}$ V-52) the results are shown in Table 4.3(a). Alkathene is the raw

Table 4.3 (a) Vanadium Content of Various Materials used in Cumulative Activation Analysis

Sample	Mean Vanadium Concentration ($ng.g^{-1}$) ($SD\bar{x}$)
C.A.S. capsule	4.2 ± 0.45
Alkathene Polymer	< 0.029
Size One Capsule	0.57 ± 0.011
1.3cm Whatman Filter Paper disc	< 0.057
2.0cm Whatman Filter Paper disc	< 0.146

Table 4.3 (b) Concentration of Trace Elements in CAS capsules used at ICRC

Element	Mean Concentration ($ng.g^{-1}$)
V	4.2
Cu	0.16
Mg	11.10
Mn	48.90
Ti	24.40

Table 4.3 (c) Analysis of Various Aliquots of Biological Materials by Cumulative Activation Analysis

Individual Sample Aliquot (µℓ)	Analyser Dead Time (%)	Limit of Detection ($ng.ml^{-1}$)	
		Erythrocytes	Plasma
200	33	1.60	1.42
100	14	3.56	2.44
50	9	5.70	7.63
10	3	48.0	81.10

material supplied by Imperial Chemical Industries (polymer 21) which is moulded into capsules by Precision Machining Engineers (Harrow) for use at ICRC. As well as 4.2 $ng.g^{-1}$ vanadium the C.A.S. capsules also contained other trace elements see Table 4.3(b), which made them unsuitable containers for trace element analysis. The 1.3 cm diameter Whatman filter papers contained <0.057 $ng.g^{-1}$ vanadium and therefore were used as carriers for the blood samples.

The following conditions for irradiation and detection were used with various aliquots of sample to establish the maximum amount of sample that could be irradiated.

t_i = 225s

t_d = 75s

t_c = 180s

Replicates = 7

Neutron flux = $0.24 \times 10^{17} n.m^{-2}.s^{-1}$

The results are recorded in Table 4.3(c).

4.4 Discussion

Sample aliquots of 200, 10, 50 and 10 µl of erythrocytes and plasma were analysed and the limits of detection calculated in each case . Seven replicates were chosen because the total amount of available sample was only 1.5 mls and to maximise the analysis it could be divided easily into seven aliquots of 200 µl, samples larger than 200 µl resulted in very high dead times.

The cumulative activation of 7 x 200 µl of red blood cells and plasma produced detection limits within the expected normal range for vanadium, however, the dead time and pulse pile-up were unacceptably high. The 7 x 100 µl aliquots had an analyser dead

time of 14% but the calculated limits of detection were not sufficiently low to allow the vanadium concentration of the sample to be measured.

In order to verify the results determined by this method a set of samples were also analysed by a method of pre-irradiation radiochemical separation by another researcher at ICRC. Hydrated antimony pentoxide columns were used to remove the matrix interference of the Na-24 nuclide so that the vanadium peak could be observed in the spectrum. The results are documented in Table 4.4(a).

Assistance with verification of the results was also requested from the group working at the Institute for Nuclear Sciences, Gent, Belgium who had worked on the same problem previously (Cornelius, et al, 1981). They were unable to assist by analysing any of my samples as they no longer had the use of the necessary irradiation facility. However, they did suggest the analysis of IAEA Bowen's Kale which has a certified value for vanadium - see Table 4.4(b). the results obtained by Cum. A.A. of Bowen's Kale fall just within the lower limits of the acceptable values and the analytical precision is 5.84%. The results in Table 4.4(a) indicate that the technique is capable of measuring vanadium with a fair degree of accuracy above 5 $ng.g^{-1}$ and so the possibility of developing this technique for routine use was not totally unacceptable.

Table 4.4 (a) Comparison of the Results obtained for the detection of Vanadium by two different analytical methods

Sample	Vanadium Concentration ($ng.ml^{-1}$) ($SD\bar{x}$)	
	H.A.P. Separation	Cumulative A.A.
0.01g aqueous V standard	9.80	11.00 ± 0.199
Foetal blood	5.40	$\leqslant$ 5.15
Maternal blood	4.20	$\leqslant$ 3.62
Amniotic fluid + 0.01µg V spike	10.80	14.00 ± 0.21

Table 4.4 (b) Reproducibility of cumulative activation analysis of Bowen's Kale[1]

Sample	Mean Vanadium Concentration ($ng.g^{-1}$) ($SD\bar{x}$)
Bowen's Kale (mass = 7 x 0.05g)	297.7 ± 17.4

[1]Certified value for vanadium is 351 $ng.g^{-1}$ ± 36

4.5 Final Comments

As the technique developed it became apparent that with improvement of detection limits by irradiation in a higher thermal neutron flux and/or increasing the number of individual samples analysed to produce the cumulative spectrum, this method could become a useful analytical technique. It has the following advantages over the more traditional radiochemical separation methods:

i) Sample quantity was small ($\simeq$700 µl)

ii) Sample preparation was minimal

iii) Possibility of contamination was reduced due to direct incorporation of liquid sample onto the filter paper carrier in a clean room

iv) No expensive column materials were required

For the purposes of this research project the work with Cum.A.A. progressed no further, but this initial investigation could be used as a starting point for further work in a future project.

V The Determination of Short-lived Isotopes in Human Hair

5.1 Introduction

Hair can be considered as a horny, fibrous derivative of the skin which, on reaching the surface, is no longer considered to be part of the body's metabolic activities. Sampling is relatively easy and the samples require no special storage conditions, however, there is a great deal of controversy surrounding the analysis of hair as a possible indicator of body content.

The rate of growth of human hair is different for various regions of the body i.e. hair on the crown of the scalp grows at approximately 0.35 $mm.day^{-1}$ whereas pubic hair grows at 0.30 $mm.day^{-1}$, and also differs in its structure. Head hair consists of a long shaft (100 - 1000 mm) which has a tapered tip and a small root whereas pubic hair is more coarse and irregular, only 10 - 60 mm in length and has many twists in its structure. The regional growth rate of hair can be affected by many parameters:

i) Nutrition

Severely malnourished individuals show symptoms of hair brittleness, loss of natural curl and frequently alopecia (loss of hair).

ii) Age

The density of hair decreases with increasing age.

iii) Hormones

An increase in circulating androgens e.g. testosterone, in males at puberty is responsible for the growth of facial and body hair.

iv) Genetic Disorders

Kinky-hair syndrome (Menkes, et al, 1962) is associated with an X-linked recessive inheritance and is characterised by hair fragility.

v) Disease States

Alopecia may occur during acute illnesses such as typhoid, tuberculosis and scarlet fever or as a result of therapy (chemotherapy, radiotherapy) for diseases such as cancer.

Environmental exposure of hair to sweat, dust, cosmetics (including dye and perming lotion) and toxic fumes can also affect the internal and structural features of the hair. Pubic hair is not necessarily a better medium to work with than head hair because contamination from clothing and cosmetics will take place.

Both the factors affecting growth and the environmental contamination are possible areas leading to erroneous results in hair analysis. In order to minimise these all samples should be collected from matched individuals (excluding persons with genetic disorders or severe malnutrition) with adequate sampling and washing procedures carried out to remove surface contaminants.

One area where hair analysis has been used successfully for many years is forensic science. The uptake of toxic elements, e.g. arsenic or mercury, which are not normally present in large quantities in the human body can be easily monitored by I.N.A.A. or atomic absorption.

A number of papers have been published where the trace element concentration has been used to identify certain disease states (Katz and Samitz 1974) (Ryan, et al, 1978), (Ward and Minski, 1982). In this study head hair and pubic hair were analysed to observe whether a state of trace element deficiency or excess in the patients blood samples would be reflected in the hair samples. The samples were analysed for the following elements by the method

already described in Section III, caesium, chromium, cobalt, iron, mercury, rubidium, antimony, selenium and zinc. In addition the washed hair samples were analysed for the following short-lived nuclides:

Nuclide	$t_{\frac{1}{2}}$	Major Energies (keV)
Br-80	4.5 hrs	617.0, 665.7
Cu-66	5.1 min	1039
I-128	25.4 min	442.7
Mg-27	9.5 min	844.0, 1014.1
Mn-56	2.6 hrs	846.9, 1810.7
V-52	3.8 min	1434.4

5.2 Method

Samples of head and pubic hair (approximately 0.1g) were analysed in the cyclic activation system available at ICRC. This system allows samples to be irradiated for a short period and be rapidly transferred to the counting position on the Ge(Li) detector, the whole apparatus is mounted on the top of the reactor so that the transfer time from the core is approximately 0.5 seconds. Samples were irradiated using small, polyethylene capsules which function as aerodynamic carriers for efficient transport within the system - see figure 5.2(i). The samples could not be transferred into clean capsules before counting so it was important to use ones with low trace element content to avoid the need for a correction factor for individual elements. The capsules were analysed to determine their elemental content - Table 5.2(a) - the levels recorded were sufficiently low that there was no appreciable interference in the analysis of hair.

Figure 5.2.(i)

A Diagram to Illustrate the Dimensions of a CAS Capsule.

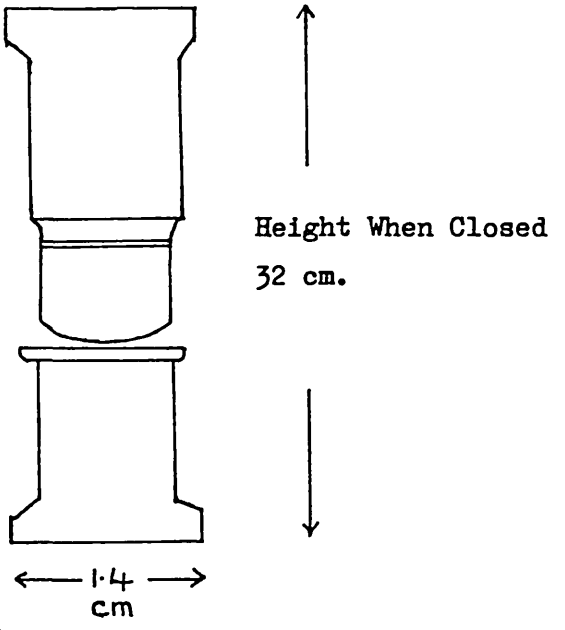


Table 5.2 (a) A Comparison between the Trace Element Content of a CAS Capsule and the 'normal' levels found in human hair*

Element	Mean Capsule Content (µg.caps)	Range of hair element Concentration ($\mu g.g^{-1}$)
Br	< 0.08	0.65 - 53.3
Cu	< 0.67	11.0 - 34.0
I	< 0.07	0.1 - 15.1
Mg	< 3.67	19 - 163
Mn	< 0.05	0.25 - 5.7
V	< 0.004	0.005 - 0.53

* Values taken from:- The Elemental Composition of human tissues and body fluids by G.V. Iyengar, W.E. Kollmer and H.J.M. Bowen.

Irradiation parameters were optimised for the two shortest lived nuclides i.e. Cu-66 and V-52, and an irradiation time of 300 seconds was selected. In order that as little activity as poss ible was lost during the transfer of samples to the detector the decay period was set to zero. Operation of the CAS system in mode B allowed one sample to be counted whilst the next sample was being irradiated, as long as the counting time was set one second shorter than the irradiation time to allow ejection of the counted sample from the Ge(Li) detector. The system can be operated in one of four modes depending on the desired irradiation scheme - figure 5.2(ii).

Irradiation Conditions:

$$t_i = 300s$$

$$t_d = 0s$$

$$t_c = 299s$$

$$\text{mode} = B$$

$$\text{neutron flux} = 0.13 \times 10^{17} (n.m^{-2}.s^{-1})$$

Chemical standards were prepared for direct comparison with the unknown samples. An aqueous solution of the elements of interest was dispensed into the capsule and then the capsule was freeze dried to contain the solution within it. Table 5.2(b) shows the composition of the standards used in these experiments. The levels were chosen to be representative of a hair sample.

Quality control was carried out using Bowen's Kale reference material to verify the accuracy and precision of the experimental procedure - Table 5.2(c).

Table 5.2 (b) Trace Element Composition of Chemical Standards for cyclic activation analysis of human hair samples

Element	Elemental Concentration (µg)
Br	22.0
Cu	19.0
I	3.50
Mg	70.0
Mn	2.00
V	0.20

Figure 5.2 (ii) Diagram to show the different ways a sample can progress within the C.A.S. system

Mode	No. of samples within system at any one time	Position of sample			Comments
		Core	Waiting	Counting	
A	3	1	1	1	$t_d > ti, tc$
B	2	1	-	1	$t_d = 0$
C	1	1	(or 1)	(or 1)	
D	1	1	(or 1)	(or 1)	ti, t_d repeated several times

Table 5.2 (c) Accuracy and Precision of Analytical Method

Element	Mean Elemental Concentration ($\mu g.g^{-1}$)		
	Certified Value*	Mean Calculated Value	Reproducibility (%)
Br	25.62 ± 1.5	27.3	9.20
Cu	4.89 ± 0.75	5.01	14.90
I	0.13 ± 0.08	0.14	11.50
Mg	1590 ± 93.3	1657.5	5.43
Mn	14.9 ± 1.45	13.9	10.79
V	0.35 ± 0.04	0.39	7.80

* Bowen's Kale

5.3 Comments

Once the method was established for analysis of the hair samples it was carried out routinely on all samples received. After a period of two or three weeks these samples were unloaded from the CAS capsules and prepared for irradiation in the main core tubes. The main problem encountered in this analysis was the inadequate sample size. In general subjects were not willing to give more than a few centimetres of their hair so in certain cases the sample was insufficient for the analysis.

II.6

Atomic Absorption Spectrometry

6.1 Introduction

Flame atomic absorption spectrometry (F.A.A.S.) is an analytical technique widely used to measure metallic elements in liquid samples. Direct nebulisation of a sample into an air-acetylene flame provides the thermal energy for vaporisation and atomisation of the metallic ions present. The atomic species then present in the flame can be excited to a higher energy state if exposed to light energy of a wavelength equivalent to the energy required to raise the electron to a higher energy level. The ability of neutral atoms to absorb light energy from an atomic spectral line source (hollow cathode lamp), so reducing the light intensity of the beam measured on the far side of the flame burner, has been utilised to develop the technique of F.A.A.S. The amount of energy absorbed by the atoms of the sample can be calculated from the equation shown below:

$$I_{\lambda o} = I_{o\lambda o} \exp(1 - k_o \cdot \ell) \qquad (6.1)$$

where

$I_{\lambda o}$ = intensity of the emerging light

$I_{o\lambda o}$ = intensity of the incident radiation from a source of wavelength λ_o

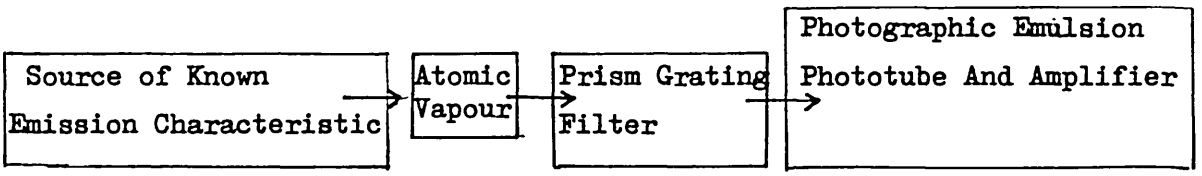
k_o = Peak absorption coefficient

ℓ = thickness of the atomic vapour (cm)

Figure 6.1.a illustrates the main components of a system for detecting the amount of energy absorbed by the atoms.

Figure 6.1.a

Block Diagram to show the set up of a F.A.A.S.



This technique obeys Beer's law i.e. absorbance of the sample is directly proportional to the concentration. With the use of standards of known concentration the method can be used to detect the concentration of metallic ions in unknown samples. The method can be hampered by the presence of interference from non-specific absorption by molecular species or scatter within the flame. In order to overcome this it is necessary to use a form of 'background' correction. Deuterium-arc background correction involves the use of a continuum light source in the UV - visible region of the spectrum. Subtraction of the absorbance of the deuterium-arc source from the absorbance value obtained by the hollow cathode lamp at the wavelength of the absorption line gives the net absorbance of the atomic species in the sample. The use of a double beam instrument, where the incident beam splits so that one (reference) beam does not pass through the flame, increases the stability of the signal from the source lamp by automatically compensating for changes in background absorption.

6.2 Method

The range of concentration of copper in whole blood, erythrocytes and plasma samples is 0.5 - 2.5 $\mu g.ml^{-1}$, in comparison with many of the other elements measured in this study the levels of copper were well within the capabilities of the F.A.A.S. system. The half life of Cu-66 is 5.1 minutes, which is somewhat shorter than the half lives of the interfering matrix component nuclides, and so unless a method of radiochemical separation were to be used the analysis of copper by N.A.A. would not be possible. Hence, the method of choice was F.A.A.S. of which there were many accounts in the literature (Sprague and Slavin 1965) (Dawson, et al, 1968) (Parker, et al, 1967).

6.3 Instrumentation

Spectrophotometer	:	Perkin Elmer 2380 Atomic Absorption Spectrophotometer (double beam)
Gas	:	Content = air-acetylene flow
Lamp	:	Hollow Cathode Cu lamp current = 10 mA λ = 324.7 nm
Slit Width	:	0.7 nm
Signal Mode	:	Concentration
Background Corrector	:	Deuterium Arc
Sampling Conditions	:	6 aliquots per second
Burner	:	High Solids type

6.4 Sample Preparation

Samples of whole blood, erythrocytes and plasma were prepared by dilution 1:1 with 0.01% Triton-X 100 as used by Thompson 1980 to analyse plasma for chromium. Dilution with 0.01% Triton-X 100 rather than water was used because the presence of the Triton

decreased the incidence of blockages in the nebuliser due to its surfactant properties. Aqueous standards were prepared in 5% Triton-A 100 to increase the viscosity so they matched with the viscosity of the unknown samples. Standards in the range 0.5 - 5.0 $\mu g.ml^{-1}$ of copper were used routinely.

6.5 Analytical Method

The nebuliser system of the F.A.A.S. requires a minimum of one millilitre of sample for one reading so this technique consumed a large quantity of each sample. Experiments were carried out to assess the reliability of the results and the limit of detection, all the results are recorded below.

6.6 Results

Table 6.6(a) and graph 6.6(b) show the comparison of aqueous copper samples with standard additions. In certain cases a method of standard addition was thought to be desirable to allow for matrix differences, however, in these experiments standard additions did not appear to be necessary.

Tables 6.6(c)(d) show the intra- and inter-assay reproducibility of the analytical method which are all within an acceptable range.

The use of Flame Atomic Absorption Spectrometry in the determination of copper in biological fluids has the following advantages:-

(1) Straightforward sample and standard preparation

(2) It exhibits no interference in an air-acetylene flame, i.e. virtually independent of size and shape of the flame and the lamp current (Platte, Marcy 1965)

(3) Less time consuming than radiochemical separations

(4) Analysis time is short (can analyse on average forty - fifty samples per hour).

Table 6.6 (a) Comparison of Aqueous Copper Standards and Standard Additions

Sample	No. of Replicates	Mean Absorbance ($SD\bar{x}$)
Aqueous Standards		
10ppm Cu	10	0.17 ± .04
5ppm Cu	10	0.08 ± .07
2ppm Cu	10	0.04 ± .04
1ppm Cu	10	0.01 ± .02
Blood. 1:1 with 0.01%	10	0.005± .002
Triton-X100		
5ppm spike Cu	10	0.096± .05
2.5ppm spike Cu	10	0.05 ± .03
1ppm spike Cu	10	0.022± .01

Detection limit = 0.4 $\mu g.ml^{-1}$

Graph 6.6(b) Calibration Curve for Copper Determinations by F.A.A.S.

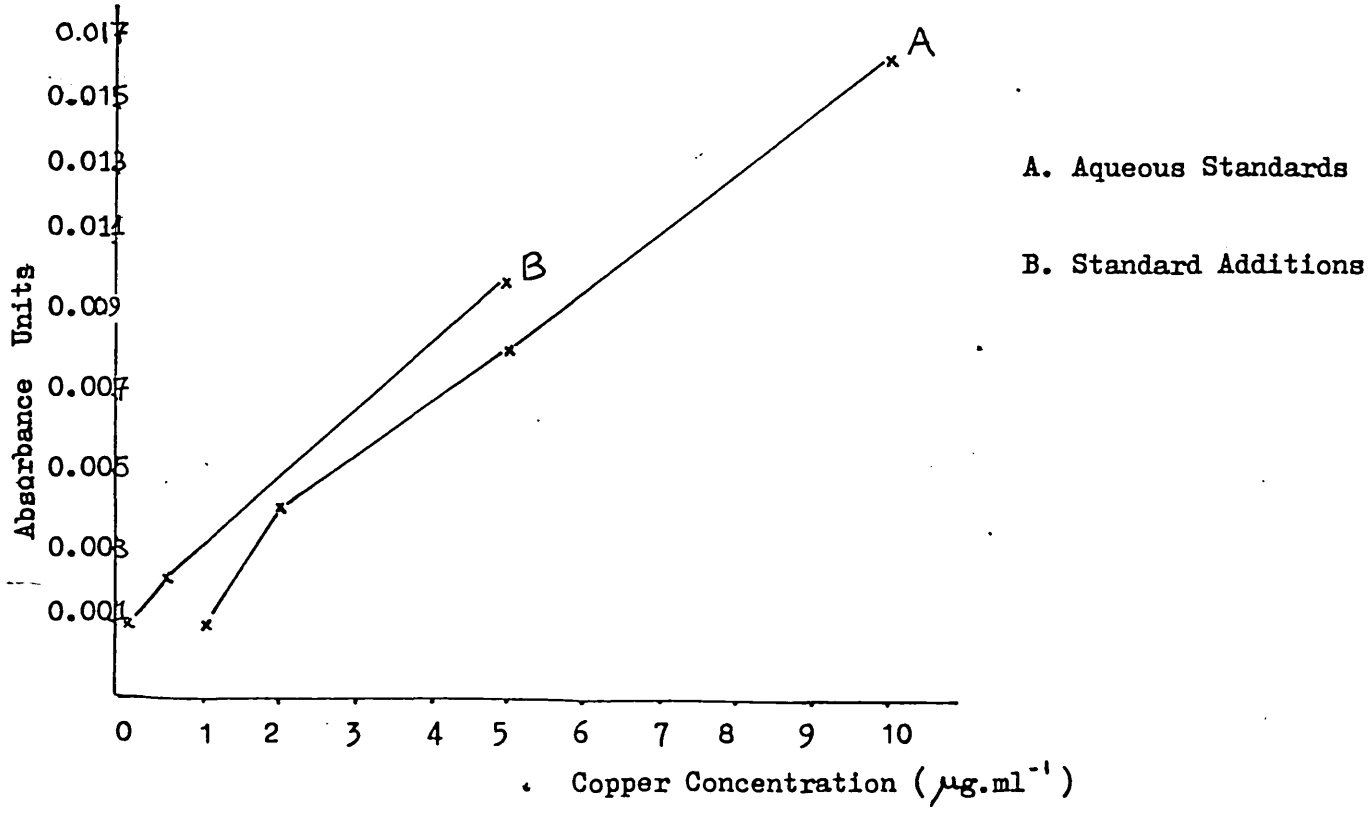


Table 6.6 (c) Intra-assay Reproducibility of Copper Determinations

Sample	No. of Replicates	Mean Absorbance ($SD_{\bar{x}}$)	Reproducibility (%)
5ppm aq. Cu standard	6	0.095 ± .005	5.3
2ppm aq. Cu standard	6	0.040 ± .0002	0.5
1ppm aq. Cu standard	6	0.020 ± .0002	1.0
0.5ppm aq.Cu standard	6	0.010 ± .0002	2.0
Blood 1:1, 0.01 % Triton-X100	6	0.006 ± .0002	3.3
Plasma 1:1, 0.01% Triton-X100	6	0.008 ± .0003	3.8

Table 6.6 (d) Inter-assay Reproducibility of Blood Copper Determinations

Sample	No. of Replicates	Mean Absorbance ($SD_{\bar{x}}$)	Reproducibility (%)
5ppm aq. Cu standard	12	0.09 ± .005	5.6
2ppm aq. Cu standard	12	0.04 ± .0004	1.0
1ppm aq. Cu standard	12	0.02 ± .0002	1.0
0.5ppm aq.Cu standard	12	0.01 ± .0003	3.0
Blood 1:1, 0.01% Triton-X100	12	0.006± .0003	5.0
Plasma 1:1, 0.01% Triton-X100	12	0.007± .0004	5.7

Analysis of copper in all the blood samples was carried out by this method on the sample volume that remained after trace element analysis by I.N.A.A.

II.7 Plasma Protein Separation by Ultrafiltration

7.1 Introduction

Information on the beneficial and detrimental effects of trace elements has been growing steadily over the last fifteen to twenty years and as yet the exact sites of their interactions with body components are not known. However, it is known that trace elements are present in plasma in three forms:

(1) Bound to albumin - 'free' for transport and exchange

(2) Bound to low molecular weight substances - e.g. amino acids, metabolites

(3) Incorporated into metalloproteins

Albumin acts as the main carrier for metabolically active trace elements whereas the metalloproteins contain elements that are tightly bound and therefore do not readily exchange (Van den Hamer, Houtman 1980). Non-specific measurement of the total trace element concentration in plasma gives little information as to the availability of the elements to the body. In a limited number of cases information is available on the carrier protein for a number of essential elements e.g. copper is transported attached to caeruloplasmin, iron binds to transferrin and cobalt forms part of the vitamin B-12 molecule. In recent years work has been carried out in an attempt to localise trace element interactions within the body and to be able to carry out such experiments it was necessary to have both accurate separation techniques and analytical evaluation of the trace element concentrations (Malvano, et al, 1967; Woittiez, et al, 1982).

There are two possible ways of analysing the available samples:

I Protein separation of plasma followed by trace element analysis of each fraction.

II Isolation of trace elements, followed by protein analysis.

Method II requires knowledge of the ionic form of the trace element of interest so that the appropriate chemical separation procedure can be set up. At the present time speciation of individual trace elements is largely unknown so this approach proves to be unsatisfactory. Speciation is a topic outside the scope of this work.

Method I allows separation of proteins from the original plasma sample followed by analysis of the total trace element concentration of each protein fraction. Protein separation can be achieved by utilising any one of the following properties:

A. Solubility — i.e. precipitation of protein with trichloroacetic acid to remove all protein-bound elements (Vogt, et al. 1980).

B. Molecular Weight — 1) Chromatography - (Evans, Fritze 1969; Chilvers, et al. 1984;
2) Ultrafiltration - (Behne, et al. 1978).

C. Biological Activity i.e. enzyme determination.

The method of choice in these investigations was ultrafiltration. A system of ultrafiltration has been used by several groups to separate biological materials (Blatt, et al, 1965; Blatt, et al, 1967; Hancher, Ryon 1973) and has been successful with proteins up to 100,000 Daltons (molecular weight).

Ultrafiltration has the advantages of speed, minimum contamination of the sample (no buffer additions necessary or contact with column materials) and cost over many of the other available techniques and avoids any denaturation or ionic changes within the sample.

7.2 Theory

Ultrafiltration can be defined as:-

'the separation of colloidal liquors from solids through semi-permeable media'.

In the Model-3 Amicon Ultrafiltration cell (see figure 7.2.1) the semipermeable medium was an anisotrophic (operates only in one direction) diffusive membrane composed of a thin layer of a homogeneous polymer (0.1 - 10μ) on top of a microporous sponge structure (20 - 10 mm), the polymer layer being in contact with the sample inside the cell. The solvent and solutes are transported through the membrane by a process of molecular diffusion under the influence of an applied pressure. For dilute solutions at low pressure the solvent activity hydrostatic pressure. The flux rate through the membrane will decrease as the concentration of the retained solutes increases according to the following equation (Ng et al,1976).

$$J = k.\ln \frac{C_w - C_u}{C_b - C_u} \qquad (7.1)$$

where

J = ultrafiltrate flux

k = mass transfer coefficient for protein between the bulk solution and the membrance surface

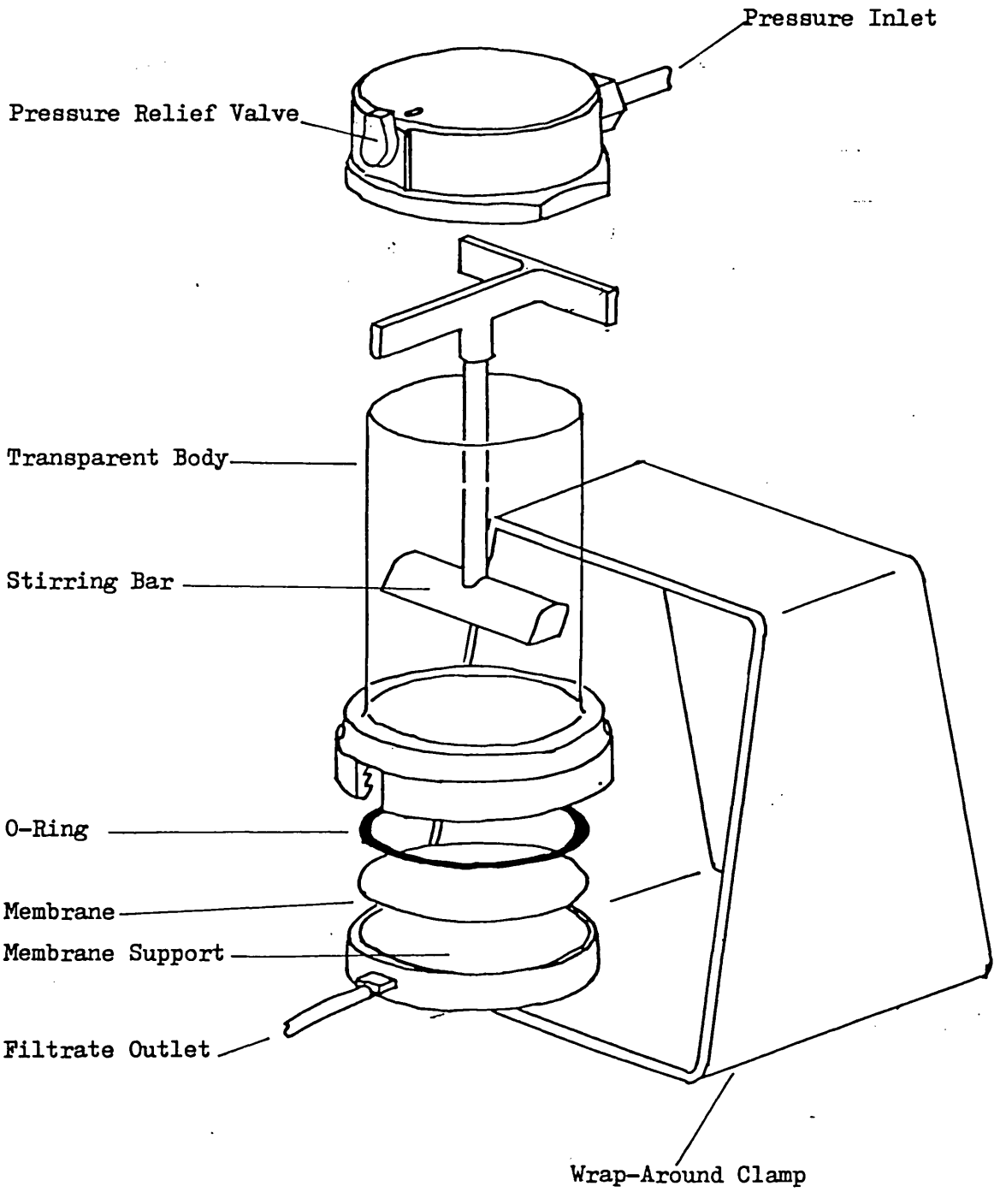
C_w = concentration of solute at the wall of the cell (g.100 ml^{-1})

C_u = concentration of solute in the ultrafiltrate (g.100 ml^{-1})

C_b = concentration of solute in original solution (g.100 ml^{-1})

Figure 7.2.1

A Schematic Diagram of an Amicon Model - 3 Ultrafiltration Cell



In conditions of high membrane rejection, $C_w >> C_u$ hence,

$$J = k.\ln \frac{C_w}{R.C_b} \qquad (7.2)$$

where

R = rejection coefficient = $1 - (C_u/C_b)$

To prevent a decrease in flux rate due to the build up of retained solute and adequate separation of the desired ultrafiltrate, the bulk solution must be continuously agitated by the magnetic stirrer and diluted with four times its own volume of de-ionised water (Amicon recommendation) to allow 99% passage of the solute through the membrane.

If stirring is inadequate with biological samples a colloidal, gel layer builds up at the membrane surface (concentration polarisation) and the membrane can no longer function correctly as a molecular sieve. The design of the Amicon diffusive membranes with a polymer layer of small pore size has the advantage that membrane fouling does not readily occur. Once the molecules have passed through the initial layer, the microporous understructure does not impede them and hence there is no build up of back pressure within the membrane that will impede further filtration. These small pores also allow for a more discrete molecular weight cut-off than will be seen with a purely cellulose (microporous) membrane.

The removal of solute by repeated dilution steps, as carried out in this study, is known as 'discontinuous diafiltration', and is governed by the following equation:-

$$\frac{C}{C_o} = \frac{V_F}{V_o} \qquad (7.3)$$

where

C_o = Initial microsolute concentration (g.100 ml^{-1})

C = Concentration of solute after concentration and dilution steps (g.100 ml^{-1})

V_F = Sample volume after the concentration step (ml)

V_o = Initial sample volume (ml)

7.3 Instrumentation

Figure 7.2.1 shows the components of an Amicon Model-3 ultrafiltration cell. Below are the specifications of this equipment:

Material Components

Top Cap and Base	:	reinforced nylon
Stirrer	:	reinforced nylon coated with teflon
Transparent Cylinder	:	polycarbonate
Clamp	:	stainless steel
Membranes	:	inert, synthetic polymer

Model 3 Cell

Weight	:	0.25 kg
Height	:	11 cm
Max. Process Volume	:	3 ml
Min. Process Volume	:	0.075 ml

Membranes

Diameter	:	25 mm
Surface Area	:	1.4 cm^2
Types	:	Ym30, XM100A, XM300
Storage	:	10% ethanol. Refrigerated

Operating Conditions

Sample Volume	:	3 ml
Stirrer Speed	:	1000 r.p.m.
Temperature	:	$20^{o}C$
N_2 Pressure	:	10 p.s.i.

7.4 Method

Plasma was collected from patients taking part in glucose tolerance experiments and stored at $-40^{o}C$ for a maximum of one week before undergoing separation.

Membranes were rinsed thoroughly in de-ionised water to remove all traces of ethanol before placing in the cell. The cell was assembled and the sample (3 ml) entered into the vessel via the detachable pressure release valve. The stirrer was switched on and the pressure valve replaced allowing the N_2 pressure to be applied (10 p.s.i.) to the cell, regulation being via the cylinder valve. The whole apparatus was assembled in a laminar flow cabinet and the ultrafiltrate was collected directly into a Sterilin, conical bottom test tube. The sample volume was allowed to decrease to one millilitre before the cell was depressurised and two millilitres of de-ionised water introduced, this process was repeated four times (established by preliminary experiments to be adequate for separation). The whole process took two to three hours.

The resulting ultrafiltrate was analysed for trace element content by neutron activation analysis as discussed in Section III and the remaining solution in the filtration cell was discarded.

Thorough cleaning of the cell and membrane after separation was of prime importance to reduce the possibility of contamination. All

equipment having contact with the sample was washed repeatedly in dilute nitric acid and water. The membranes were rinsed in 0.1M NaOH to remove any trace of protein deposit, rinsed repeatedly in de-ionised water and stored at -4°C in 10% ethanol.

One of the problems with pre-irradiation separation of samples can be contamination from the apparatus used. The degree of contamination with this method was minimal because all the cell structure that came into contact with the sample was made of inert material and the plasma was removed from the patient and entered the filtration cell with the minimum time delay. However, the cell was checked for contamination by flushing through both water and a plasma sample of known chemical composition and no effect was observed.

7.5 Comments

The ultrafiltrate produced by this method was dependent on the sieving properties of the individual membrane types. The cut-off range will not be as discrete a molecular weight as the manufacturers suggest so to verify the content of the ultrafiltrate it was necessary to analyse it for protein content. Verification of the protein content of the dialysate can be achieved by either qualitative or quantitative methods. Qualitative determination by a method of gel electrophoresis and quantitative assessment of the membrane cut-off regions by protein determination using samples of known molecular weight, were both used.

7.6 Qualitative Analysis of Ultrafiltrates by Gel Electrophoresis

(a) Introduction

Plasma proteins are large molecules containing numerous $-NH_3^+$ and $-COO^-$ groups within their structure and therefore unless the

protein is at its isoelectric point the molecules will have an overall net charge. If exposed to an electrical field in a carrier medium (gel) the proteins will move to the pole of opposite charge. The velocity of this movement being dependent on the net molecular charge and the strength of the electrical field. Migration of the proteins will also be hindered by the size and shape of the molecules of the supporting gel. Hence,

$$\text{Specific Mobility} = \frac{S/t}{F} \quad cm^2.V^{-1}.s^{-1} \qquad (7.4)$$

where

S = distance (cm)

t = duration of migration (secs)

F = field strength (volts.cm^{-1})

The specific mobility of a protein under defined conditions of pH and gel composition can be used for identification and this technique is known as gel electrophoresis.

Electrophoretic mobility is dependent on

(a) pH

(b) composition of sample

(c) ionic strength of the buffer

The technique employed was used routinely in Biochemistry Department of Medical Research Council Unit (Mill Hill, N. London). The author was allowed the use of their facilities.

(b) Method

Table 7.6.(a) shows the composition of the stacking and running gels used in the experiments.

Two glass plates were held together with bulldog clips and sealed with agar to avoid leakage of the gel, and a small space was left

Table 7.6 (a) Composition of Stacking and Running Electrophoresis Gels

Reagents	Quantity of each Reagent	
	Running Gel	Stacking Gel
30% Acrylamide	10ml	1.67ml
2.5% Bisacrylamide	3.25ml	0.65ml
Water	5.24ml	6.43ml
1M Tris pH 8.8	11.25ml	(pH 6.8) 1.25ml
20% sodium dodecyl sulphate	0.15ml	0.05ml
TEMED*	20µℓ	10µℓ
10% Ammonium persulphate	0.2ml	0.1ml

*TEMED N, N, N', N' - tetramethylethylene diamine

Table 7.6 (b)

Composition of Gel Running Buffer	
$30.3 g.L^{-1}$	Tris base
$144.2 g.L^{-1}$	Glycine
$10 g.L^{-1}$	Serva sodium dodecyl sulphate

for entry of the running and stacking gels. The running gel was poured between the plates and overlaid with butanol which has been saturated with Tris pH 8.8/S.D.S. The plates were left until the phases had separated and the running gel completely set before washing away the butanol. The stacking gel was poured onto the running gel just prior to use and at the same time a 'comb' was inserted which allowed for discrete application of each sample. The samples were suspended in 25 l of the sample buffer - table 7.6.(c) - and then loaded onto the gel. The gel used was 15 cm x 15 cm x 2 mm.

The bulldog clips were removed from the plates and the top and bottom reservoirs were filled with gel running buffer - table 7.6.(b). The whole apparatus was placed in a perspex tank and run at 45 volts for approximately fifteen hours (until the dye front had just run off the gel). Finally the gel plate was removed and underwent a process of staining for one hour followed by de-staining for one hour at 60°C and 50 r.p.m. in a shaking incubator; photographs were taken of the gel.

Standards of known molecular weight were applied to the gel at the same time as the unknown samples and migration of these standard proteins enabled the calibration of the gel.

The composition and pH of the gels and buffer solutions were chosen for optimum mobility of plasma proteins. At pH 8.8 most of the proteins within the sample were negatively charged and would move towards the anode, additional negative charge comes from the sodium dodecyl sulphate which binds to the protein. This excess of negative charge along with the molecular sieving allowed for adequate separation of the proteins towards the anode.

Table 7.6 (c)

Composition of Gel Sample Buffer	
0.3ml	20% sodium dodecyl sulphate
0.3ml	Glycerol
0.15ml	0.2% Bromophenol blue
0.3ml	1M Dithiothreitol
0.24ml	1M Tris pH 6.8
1.71ml	Water

Table 7.6 (d) Table to illustrate the minimum molecular weight species found in the sample

Sample	Molecular Weight Range (Daltons)
Original Plasma	25,000 - >300,000
Ultrafiltrate using YM30	27,500 - 53,000
Ultrafiltrate using XM100A	< 57,000
Ultrafiltrate using XM300	28,500 -270,000

(c) Results

The photograph clearly shows the separation into bands of each sample. On the left hand side can be seen the bands corresponding to the protein standards. The tracks from right to left represent:

1. standard proteins
2. 1/10 dilution of plasma
3. XM100A membrane ultrafiltrate
4. XM300 membrane ulttrafiltrate
5. Ym30 membrane ultrafiltrate

The distance migrated can be plotted against the logarithm of molecular weight for each of the calibration proteins - Graph 7.6.a. Table 7.6(d) shows the range of molecular weight species present in the ultrafiltrate from each of the Amicon membranes. The XM100A membrane did not appear to be as efficient as the other two membranes because very few bands appeared on the gel. This experiment was repeated and produced the same results, so the separations using these membranes (XM100A) were disregarded.

7.7 Quantitative Analysis of Ultrafiltrate by Direct Protein Determination

(a) Introduction

The membanes used in this study included YM30, XM100A and XM300 which had a molecular weight cut-off of 30,000; 100,000 and 300,000 Daltons respectively. In order to ensure either complete retention of larger molecules or complete filtration of smaller molecules than indicated by the manufacturers cut-off limit the cell was tested with known molecular weight protein solutions. Each sample of protein solution was put through exactly the same

Figure 7.6

Photograph of an Electrophoresis Plate Used for Analysing Plasma Proteins.

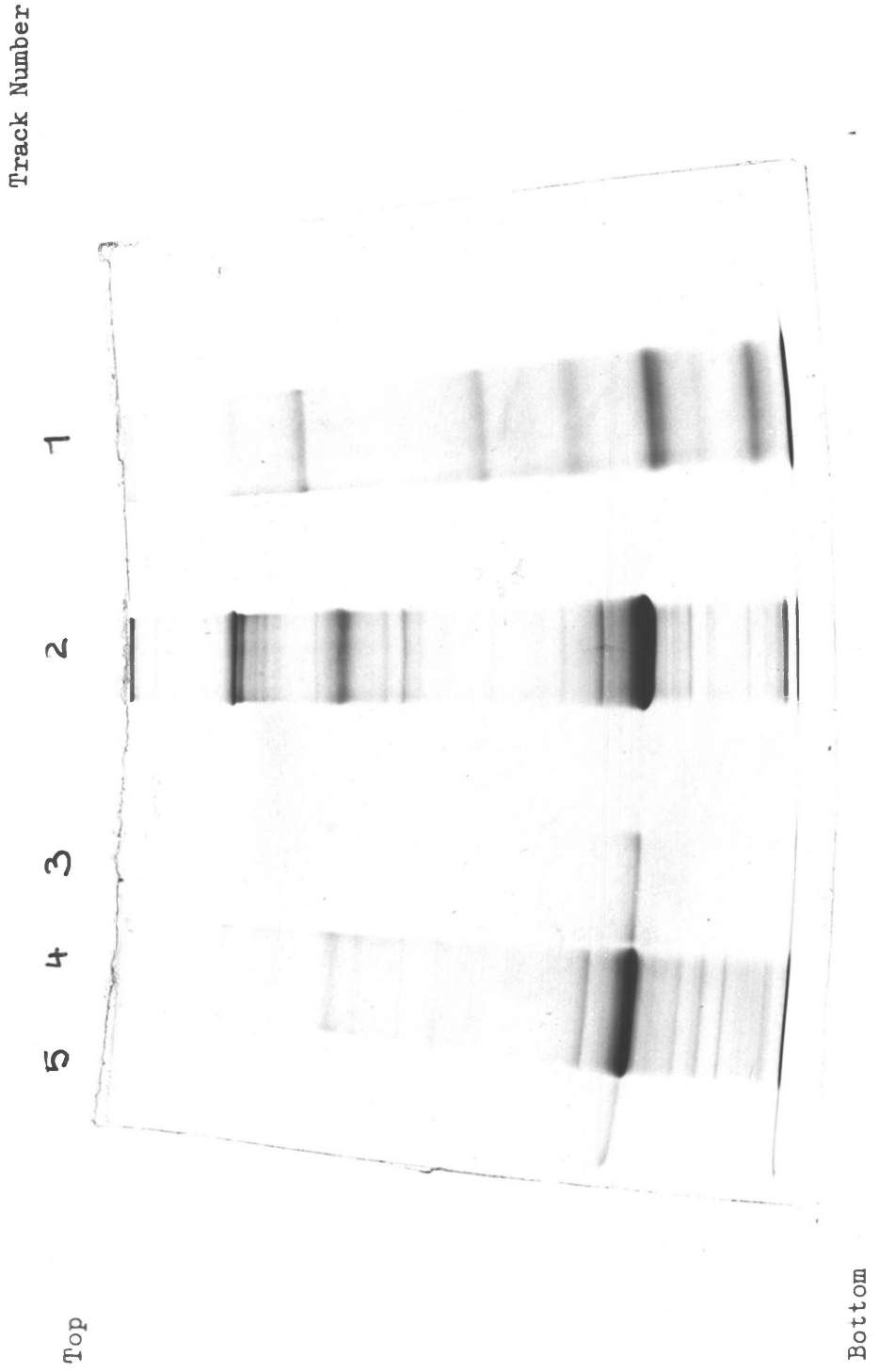
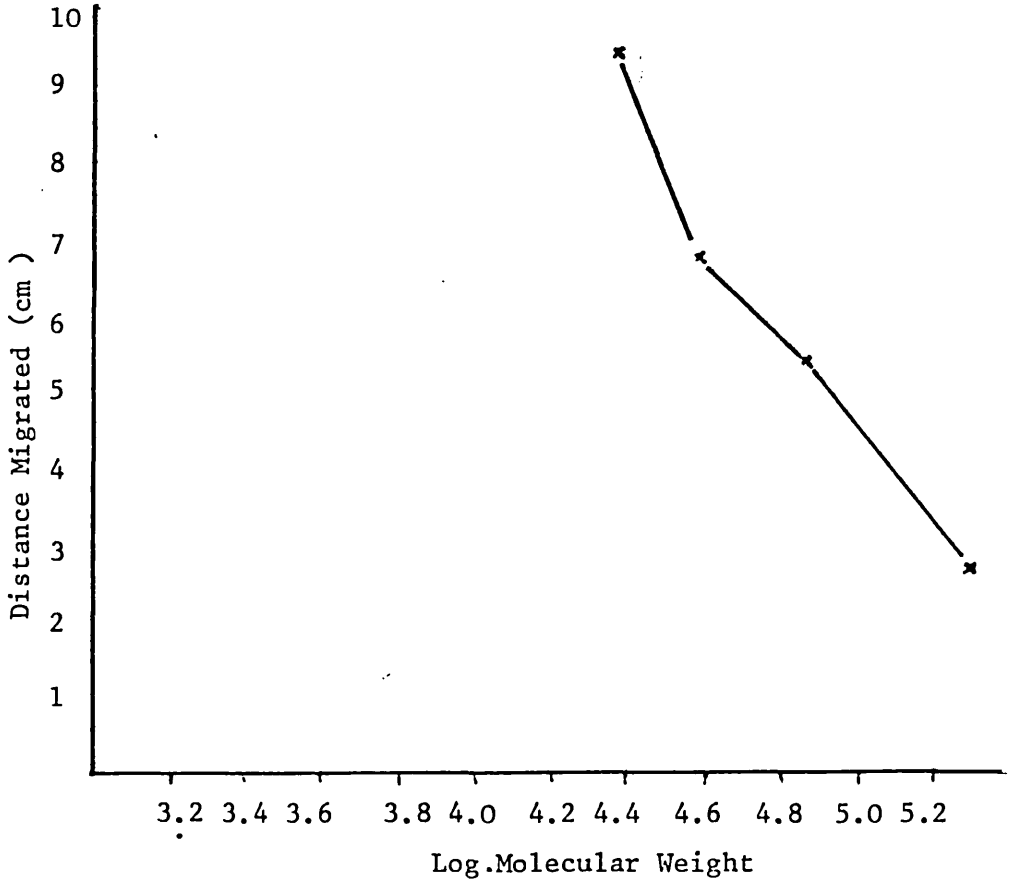


Figure 7.6.a

Calibration Curve for the Electrophoresis Gel, using Proteins of Known Molecular Weight



Molecular Weight of CalibrationProteins:

200,000 D
97,400 D
68,000 D
25,700 D

procedure as the plasma samples obtained experimentally. However, instead of pooling the ultrafiltrate before analysis all fractions were analysed separately for protein content.

The protein solutions containing 30 $mg.ml^{-1}$ were from Boehringer Mannheim and were:

Chymotrypsinogen A	25,000 D
Hen Albumin	45,000 D
Bovine Albumin	68,000 D
Aldolase	158,000 D
Catalase	240,000 D
Ferritin	450,000 D

(1 Dalton = 1.67×10^{-24}g i.e. mass of a hydrogen atom)

Each lyophilised sample was made up to one millilitre with de-ionised water before use.

(b) Method

The protein solutions were ultrafiltered in a model-3 Amicon stirred cell according to the following scheme:

Membrane used	Protein Ultrafiltered
YM30	Chymotrypsinogen A
	Hen Albumin
XM100A	Bovine Albumin
	Aldolase
XM300	Catalase
	Ferritin

Each solution was analysed separately and washed through the system four times. The protein concentration of each stage was measured (including the water and buffer used to prime the system) using the Biuret method and the system was calibrated by analysis of quality control serum having a concentration of 60 $mg.ml^{-1}$.

The biuret reaction used in the assay involves a reaction between the nitrogen atoms of peptide bonds and copper (Cu^{2+}) ions in an alkaline medium to produce an intense, stable purple colour. All soluble proteins give the biuret reaction (Weicheselbaum 1946).

0.1 ml of each sample was diluted with 5 mls of biuret reagent which contains:

0.1M	NaOH
16 m $mol.L^{-1}$	K-Na-tart rate
15 m $mol.L^{-1}$	Potassium iodide
6 m $mol.L^{-1}$	Cupric Sulphate

The samples were then incubated for thirty minutes at 25°C and their absorbance at 546 nm was measured. The U.V. spectrophotometer was calibrated using a reagent blank containing only NaOH and K-Na-tartrate and a standard protein solution (60 $mg.ml^{-1}$).

(c) Results

Tables 7.7(a)-(f) show the results of the six experiments carried out. In each case the results show that the proteins of slightly larger molecular weight than the membrane pore size were retained 100% and the molecules of molecular weight smaller than the membrane pore size were completely filtered. However, as each of these proteins was observed in isolation the same phenomenon may

Calibration of YM30 membrane

Table 7.7 (a)

Chymotrypsinogen A (mw = 25,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0
6g.$100ml^{-1}$ Standard	0.28	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml Chymotrypsinogen A	0.16	3.4
1ml H_2O to wash cell	0.02	0.4
1ml H_2O to wash cell	0.01	0.2
1ml H_2O to wash cell	0.01	0.2
1ml H_2O to wash cell	0.00	0
Extraction from within cell	0.00	0
Washings from membrane	0.00	0

Table 7.7 (b)

Hen albumin (mw = 45,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0
6g.$100ml^{-1}$ Standard	0.29	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml her albumin	0.00	0
1ml HO to wash cell	0.00	0
1ml HO to wash cell	0.00	0
1ml HO to wash cell	0.00	0
1ml HO to wash cell	0.00	0
Extraction from within cell	0.13	2.7
Washings from membrane	0.01	0.21

Calibration of XM 100A membrane

Table 7.7 (c)

Bovine albumin (mw = 68,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0.00
$6g.100ml^{-1}$ Standard	0.29	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml bovine albumin	0.11	2.9
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
Extraction from within cell	0.00	0
Washings from membrane	0.04	0.9

Table 7.7 (d)

Aldolase (mw = 158,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0
$6g.100ml^{-1}$ Standard	0.30	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml Aldolase	0.01	0.06
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
Extraction from within cell	0.16	3.20
Membrane washings	0.01	0.06

Calibration of XM300 membranes

Table 7.7 (e)

Catalase (mw = 240,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0
6g.$100ml^{-1}$ Standard Catalase	0.30	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml Catalase	0.22	4.40
1ml H_2O to wash cell	0.01	0.20
1ml H_2O to wash cell	0.01	0.20
1ml H_2O to wash cell	0.00	0
1ml H_2O to wash cell	0.00	0
Extraction from within cell	0.01	0.20
Washings from membrane	0.00	0.0

Table 7.7 (f)

Ferritin (mw = 450,000)

Sample	Absorbance	Concentration ($g.100ml^{-1}$)
Reagent Blank	0.00	0
6g.$100ml^{-1}$ Standard	0.29	6
H_2O pre-protein	0.00	0
buffer pre-protein	0.00	0
1ml Ferritin		0
1ml H_2O to wash	0.01	0.21
1ml H_2O to wash	0.00	0
1ml H_2O to wash	0.00	0
1ml H_2O to wash	0.00	0
Extraction from within cell	0.17	3.52
Washings from membrane	0.01	0.21

not be true when analysing a complex protein mixture. Without using a more sophisticated method of protein determination this was the only way the experiment could be carried out.

7.8 Final Conclusions

The gel electrophoresis results give the best insight into the actual ultrafiltrates produced. The results show that the YM30, XM100A and XM300 Amicon diffusive membranes show cut-off values of 53,000, 57,000 and 300,000 respectively. The second set of experiments with known protein solutions indicated that washing through the cell with four times the sample volume of solvent ensured maximum separation of the desired microsolute.

The method of ultrafiltration for plasma protein isolation, described in the above section, was applied routinely to samples collected at time zero, time 90 minutes and time 120 minutes in a selection of samples from patients undergoing an oral glucose tolerance test.

CHAPTER III

III EXPERIMENTAL RESULTS

The introduction indicated that copper, zinc and chromium play a major role in Diabetes mellitus and that certain other trace elements may play a minor role. The following experiments were undertaken to clarify some of the points discussed in the introduction :

3.1 Observe the fasting concentrations of trace elements in Diabetics and Non-Diabetics.

3.2 Observe the diurnal variation in the trace elements of Diabetics and Non-Diabetics.

3.3 Observe the effects of hyper and hypoglycaemia on a Control individual and his circulating trace element concentrations.

3.4 Observe the effect of a glucose load on the circulating and excreted trace element concentrations of lean and obese Diabetics and Controls.

3.5 Observe the effect of weight control in reducing hyperglycaemia in non-insulin dependent Diabetics and the subsequent trace element fluctuations to maintain euglycaemia.

3.6 Measure the trace element concentration of head and pubic hair from lean and obese subjects.

3.7 Observe the effect of trace element content of certain plasma proteins during a glucose load indicating the possible interaction of a chromium binding protein.

3.8 Measure the trace element concentration of the most commonly used Diabetic drugs.

Section 3.1

Table 1 Individuals Participating in Initial Study

Subject Group	Mean Age (years) ($SD\bar{x}$)	Mean Weight (kg) ($SD\bar{x}$)	Mean Height (m) ($SD\bar{x}$)	Duration of Disease (years) ($SD\bar{x}$)
Controls n = 11	44.8 ± 5.5	75.5 ± 6.70	1.67 ± 0.10	-
Insulin Requiring Diabetics n = 10	57.6 ± 5.9	77.8 ± 7.37	1.66 ± 0.03	18.3 ± 4.5
Maturity Onset Diabetics n = 11	70.6 ± 4.3	68.9 ± 5.20	1.60 ± 0.07	21.7 ± 6.2

Diurnal Trace Element Profile of Control Subjects

Table 2 (a) : Fasting

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.69 (.039)	0.0047 (.0006)	5.21 (.35)	0.12 (.015)	428.5 (16.99)	2.20 (.11)	0.017 (.002)	0.0024 (.0003)	0.006 (.001)	0.008 (.001)
Plasma	1.10 (.11)	0.014 (.002)	2.53 (.34)	0.24 (.04)	2.12 (.46)	0.51 (.08)	0.026 (.005)	0.0078 (.0007)	0.031 (.007)	0.028 (.009)
Serum	0.99 (.10)	0.006 (.002)	1.09 (.11)	0.14 (.009)	1.32 (.09)	0.37 (.06)	0.018 (.002)	0.0021 (.0002)	0.013 (.003)	0.008 (.002)
Urine*	0.003 (.0005)	0.0002 0.00004)	0.04 (.004)	0.003 (.0005)	0.07 (.006)	0.09 (.007)	0.0002 (.00005)	0.0009 (.00005)	0.0002 (.00005)	0.0004 (.00003)

Table 2 (b) : Midday

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.87 (.087)	0.005 (.0006)	5.36 (.62)	0.14 (.011)	444.5 (14.57)	2.01 (.14)	0.0035 (.017)	0.0034 (.0003)	0.008 (.002)	0.009 (.002)
Plasma	1.25 (.12)	0.017 (.004)	2.58 (.38)	0.15 (.04)	1.75 (.25)	0.49 (.06)	0.018 (.002)	0.0061 (.0006)	0.004 (.0006)	0.002 (.0003)
Serum	1.14 (.09)	0.009 (.002)	1.27 (.12)	0.14 (.02)	1.55 (.43)	0.50 (.09)	0.017 (.002)	0.0049 (.0009)	0.015 (.002)	0.008 (.001)
Urine*	0.003 (.0003)	0.0002 (.00005)	0.04 (.005)	0.002 (.0001)	0.045 (.006)	0.22 (.03)	0.0002 (.00002)	0.0006 (.00009)	0.0002 (.00002)	0.0004 (.00005)

Table 2 (c) : post dinner

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.75 (.07)	0.004 (.0006)	4.95 (.39)	0.13 (.11)	429.0 (17.90)	2.26 (.10)	0.014 (.002)	0.0032 (.0003)	0.007 (.001)	0.009 (.002)
Plasma	0.97 (.06)	0.016 (.003)	1.61 (.14)	0.21 (.03)	1.46 (.30)	0.67 (.06)	0.017 (.003)	0.007 (.001)	0.002 (.0004)	0.002 (.0003)
Serum	1.11 (.08)	0.007 (.001)	1.24 (.13)	0.15 (.01)	1.36 (.19)	0.81 (.06)	0.007 (.002)	0.0042 (.0006)	0.011 (.002)	0.007 (.001)
Urine*	0.004 (.0008)	0.0002 (.00002)	0.03 (.006)	0.003 (.001)	0.07 (.01)	0.18 (.02)	0.0002 (.00002)	0.008 (.0001)	0.0002 (.00002)	0.0005 (.00005)

* all urine samples corrected for Creatinine

Table 3 (a) : fasting

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 10									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.63 (.07)	0.004 (.0007)	6.44 (.31)	0.17 (.01)	434.9 (13.2)	2.40 (.14)	0.012 (.002)	0.002 (.0005)	0.005 (.001)	0.006 (.002)
Plasma	1.16 (.07)	0.009 (.001)	1.62 (.08)	0.20 (.02)	2.96 (.40)	0.60 (.11)	0.027 (.04)	0.008 (.001)	0.017 (.002)	0.007 (.001)
Serum	1.34 (.09)	0.007 (.001)	1.87 (.12)	0.18 (.02)	2.19 (.21)	0.54 (.07)	0.012 (.001)	0.006 (.001)	0.013 (.002)	0.008 (.001)
Urine*	0.004 (.002)	0.0006 (.0003)	0.10 (.03)	0.002 (.0008)	0.11 (.005)	0.06 (.02)	0.0002 (0)	0.002 (.0001)	0.0001 (.00002)	0.0004 (.00005)

Table 3 (b) : midday

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 10									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.74 (.07)	0.004 (.0003)	6.81 (.38)	0.14 (.012)	445.5 (12.5)	2.20 (.18)	0.017 (.002)	0.003 (.0005)	0.005 (.0012)	0.008 (.001)
Plasma	1.10 (.08)	0.009 (.001)	0.82 (.09)	0.15 (.03)	2.99 (.80)	0.39 (.08)	0.018 (.002)	0.005 (.001)	0.015 (.002)	0.007 (.001)
Serum	1.26 (.12)	0.007 (.001)	1.81 (.15)	0.24 (.02)	2.14 (.31)	0.42 (.05)	0.019 (.001)	0.0029 (.0006)	0.007 (.001)	0.005 (.0008)
Urine*	N.D.	0.0004 (.0002)	0.15 (.04)	0.004 (.001)	0.30 (.006)	0.13 (.04)	0.0005 (.0001)	0.0014 (.0002)	0.0005 (.0003)	0.0007 (.0001)

Table 3 (c) : post dinner

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 10									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.66 (.06)	0.004 (.0007)	7.25 (.70)	0.17 (.013)	445.5 (12.6)	2.37 (.15)	0.017 (.002)	0.003 (.0005)	0.005 (.0009)	0.007 (.001)
Plasma	1.08 (.07)	0.006 (.001)	1.35 (.07)	0.22 (.013)	2.68 (.60)	0.53 (.07)	0.027 (.006)	0.004 (.001)	0.005 (.0007)	0.004 (.0005)
Serum	1.27 (.07)	0.006 (.0007)	1.35 (.09)	0.23 (.014)	2.97 (.42)	0.40 (.028)	0.021 (.001)	0.004 (.001)	0.009 (.001)	0.006 (.001)
Urine*	N.D.	0.0002 (.00008)	0.038 (.002)	0.004 (.0008)	0.08 (.048)	0.079 (.009)	0.0003 (0)	0.0008 (.0003)	0.0002 (.00006)	0.0003 (.0001)

* all urine samples corrected for creatinine
N.D. Not determined

Diurnal Trace Element Profile of Maturity Onset Diabetic Subjects

Table 4 (a) : fasting

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.57 (.04)	0.0019 (.0001)	6.67 (.39)	0.17 (.009)	472.2 (22.5)	2.48 (.11)	0.037 (.005)	0.003 (.0003)	0.002 (.0006)	0.005 (.0007)
Plasma	1.19 (.09)	0.002 (.0001)	1.65 (.15)	0.19 (.02)	1.80 (.07)	0.26 (.03)	0.023 (.002)	0.002 (.0002)	0.002 (.0006)	0.002 (.0005)
Serum	1.03 (.06)	0.003 (.001)	1.22 (.11)	0.17 (.02)	2.02 (.11)	0.26 (.04)	0.023 (.002)	0.002 (.001)	0.0019 (.002)	0.002 (.0002)
Urine*	N.D.	0.00007 (.00001)	0.092 (.010)	0.0036 (.0007)	0.102 (.011)	0.17 (.011)	0.0003 (.0001)	0.0012 (.0007)	0.00008 (.00005)	0.0006 (.0001)

Table 4 (b) : midday

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.59 (.07)	0.003 (.0007)	6.27 (.25)	0.16 (.01)	451.7 (24.3)	2.44 (.21)	0.043 (.06)	0.002 (.0002)	0.0028 (.0003)	0.005 (.0002)
Plasma	0.89 (.06)	0.0028 (.0006)	1.14 (.08)	0.14 (.012)	1.80 (.10)	0.22 (.02)	0.031 (.0012)	0.0010 (.0003)	0.003 (.0004)	0.002 (.0001)
Serum	N.D.	0.0037 (.0006)	0.93 (.51)	0.15 (.007)	1.83 (.40)	0.29 (.03)	0.035 (.001)	0.0014 (.0002)	0.0034 (.0007)	0.003 (.0007)
Urine*	N.D.	0.00013 (.00006)	0.092 (.012)	0.0023 (.0003)	0.039 (.007)	0.15 (.012)	0.0007 (.0003)	0.0007 (.0001)	0.00004 (.00001)	0.0005 (.00007)

Table 4 (c) : post dinner

Sample	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 11									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Whole blood	0.49 (.07)	0.002 (.0002)	6.28 (.30)	0.19 (.01)	495.0 (21.4)	2.31 (.08)	0.06 (.006)	0.009 (.0002)	0.003 (.0001)	0.005 (.0007)
Plasma	1.01 (.07)	0.002 (.0006)	1.22 (.12)	0.15 (.004)	1.62 (.05)	0.19 (.01)	0.039 (.001)	0.011 (.002)	0.004 (.0005)	0.002 (.0005)
Serum	1.08 (.10)	0.002 (.0001)	0.91 (.12)	0.17 (.012)	1.47 (.07)	0.19 (.011)	0.043 (.005)	0.005 (.0006)	0.0039 (.002)	0.0023 (.0004)
Urine*	N.D.	0.0002 (.0001)	0.10 (.02)	0.005 (.002)	0.11 (.03)	0.32 (.09)	0.001 (.0003)	0.0008 (.0002)	0.0004 (.0001)	0.0009 (.0002)

* all urine samples corrected for creatinine
N.D. Not Determined

SECTION 3.1

3.1 OBSERVE FASTING CONCENTRATIONS OF TRACE ELEMENTS IN DIABETICS AND NON-DIABETICS

The following hypotheses were tested using a Student t-test where the variances were assumed unknown and not necessarily equal (Handbook of Tables for Probability and Statistics edited by W.H. Beyer, p.25-27).

NULL HYPOTHESIS 3.1(i)

Controls and Insulin requiring Diabetics show no fasting differences in their concentration of circulating and excreted trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWERED IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Whole blood	Cs , Hg	Rb, Se, Zn
Plasma	Co, Cs, Sb, Se, Zn	Fe, Rb
Serum	Hg	Cr, Cu, Fe, Rb, Se, Zn
Urine	Rb, Sb	Co, Cr, Fe, Zn

All the other trace elements show no differences between the two groups.

* indicates that the values were rejected at the t 0.99 probability level.

NULL HYPOTHESIS 3.1(ii)

Controls and Maturity onset Diabetics show no fasting differences in their concentration of circulating and excreted trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWERED IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Whole blood	Co, Cs, Cu, Sb	Cr, Fe, Hg, Rb, Se, Zn
Plasma	Cr, Cs, Rb, Sb, Se, Zn	-
Serum	Co, Cs, Rb, Sb	Fe, Hg, Se, Zn
Urine	Sb	Cs, Hg, Rb, Zn

All the other trace elements show no differences between the two groups.

- indicates that their were no trace elements which fell into this category.

SECTION 3.2

3.2 OBSERVE THE DIURNAL VARIATION IN CIRCULATING TRACE ELEMENTS OF DIABETICS AND CONTROLS

NULL HYPOTHESIS 3.2(i)

Control subjects show no diurnal variation in their circulating trace element concentrations.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWERED at the end of the day	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED at the end of the day
Whole blood	Co, Hg	Cr
Plasma	Cs, Cu, Fe, Hg, Sb, Zn	Rb
Serum	Hg	Cu, Cr, Rb, Zn
Urine	Zn	Cr, Cs, Cu, Rb

All the other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.2(ii)

Insulin requiring Diabetics show no diurnal variation in their circulating trace element concentrations.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AT THE END OF THE DAY	TRACE ELEMENTS SIGNIFICANTLY* HIGHER AT THE END OF THE DAY
Whole blood	-	Zn, Hg
Plasma	Co, Cr, Cu	Se
Serum	Co, Cr, Cs, Rb, Sb, Zn	Fe, Hg, Se
Urine	Co	Cr, Cs, Hg, Rb, Sb, Se, Zn

All the other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.2(iii)

Maturity onset Diabetics show no diurnal variation in their circulating trace element concentrations.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AT THE END OF THE DAY	TRACEELEMENTS SIGNIFICANTLY* HIGHER AT THE END OF THE DAY
Whole blood	Cu, Rb, Zn	Cr, Hg, Sb, Se
Plasma	Cu, Fe, Rb, Se, Zn	Cr, Hg, Sb
Serum	Co, Fe, Rb, Zn	Cr, Hg
Urine	-	Co, Cs, Hg, Rb, Sb

All the other trace elements show no differences between the two groups.

Section 3.3

Table 5 (a) Trace Element Concentration in Whole Blood and Plasma of a Control Subject during an Intravenous Glucose Tolerance Test.

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in whole blood									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.60	0.004	7.79	0.12	470.6	2.85	0.015	0.013	0.007	0.009
10	0.72	0.006	8.25	0.14	424.7	3.47	0.021	0.035	0.009	0.011
30	0.69	0.007	9.45	0.14	513.6	3.26	0.018	0.036	0.007	0.009
60	0.86	0.005	8.44	0.13	447.8	3.11	0.015	0.029	0.006	0.008
90	0.90	0.002	7.35	0.18	439.5	2.98	0.009	0.029	0.006	0.007

Table 5 (b)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in plasma									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.88	0.006	1.04	0.16	2.59	0.76	0.035	0.062	0.024	0.010
10	0.95	0.015	0.57	0.18	1.91	0.86	0.040	0.061	0.088	0.010
30	1.14	0.020	2.24	0.31	0.50	1.22	0.050	0.060	0.020	0.012
60	0.70	0.008	1.91	0.15	1.61	0.48	0.030	0.058	0.019	0.011
90	1.06	0.011	1.00	0.14	1.80	0.95	0.035	0.045	0.009	0.004

Table 6 Plasma glucose concentration of a control subject during an intravenous glucose tolerance test.

Time of Sampling (mins)	Plasma Glucose Concentration ($mmol.L^{-1}$)
0	5.0
10	17.5
30	13.2
60	9.6
90	6.8

Figure 6

Plasma Glucose Concentration of a Control Subject During an Intravenous Glucose Tolerance Test.

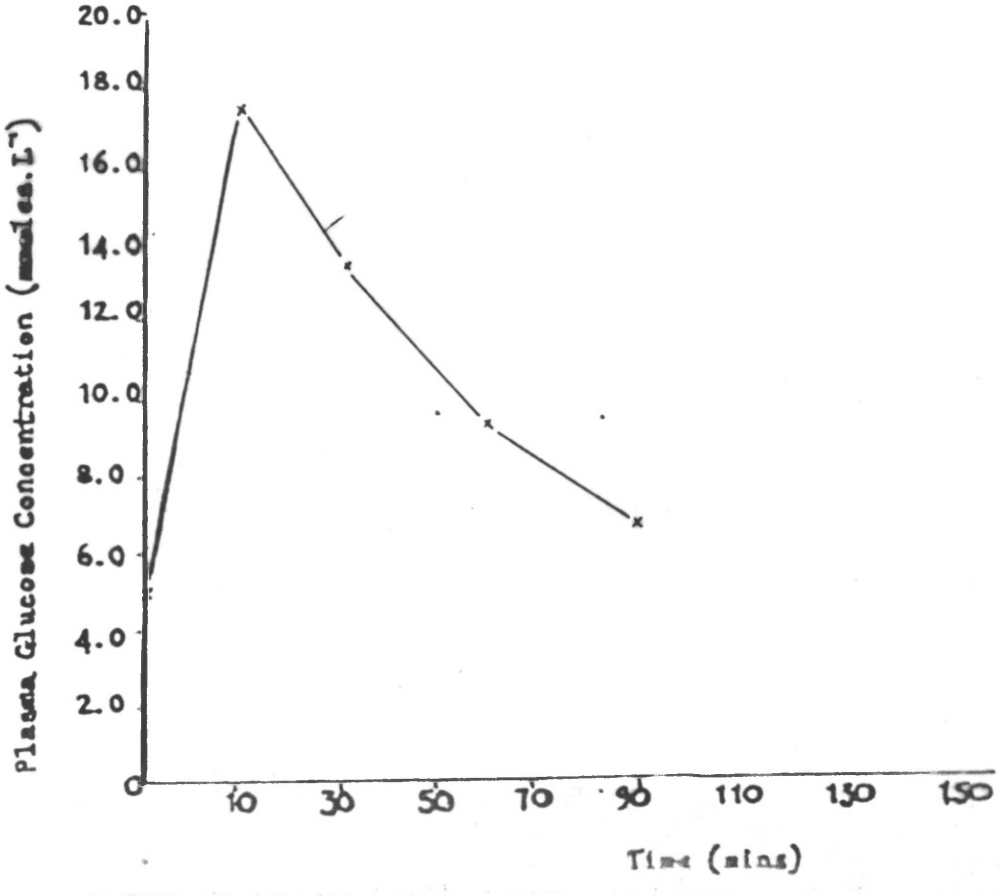


Figure 5(a)

Trace Element Concentration in Whole Blood of a Control Subject During an Intravenous Glucose Tolerance Test.

Figure 6

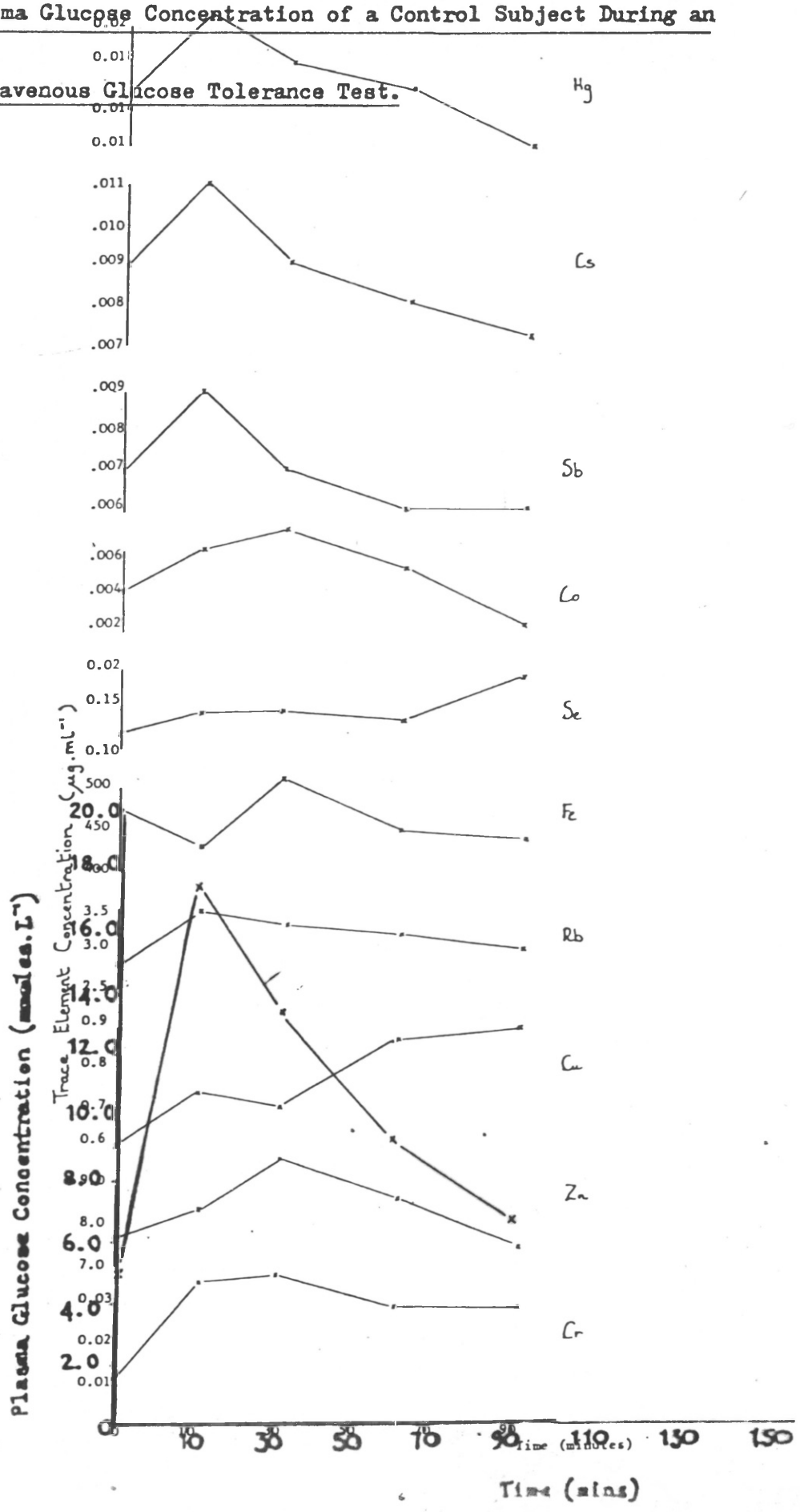


Figure 5(a)

Trace Element Concentration in Whole Blood of a Control Subject During an Intravenous Glucose Tolerance Test.

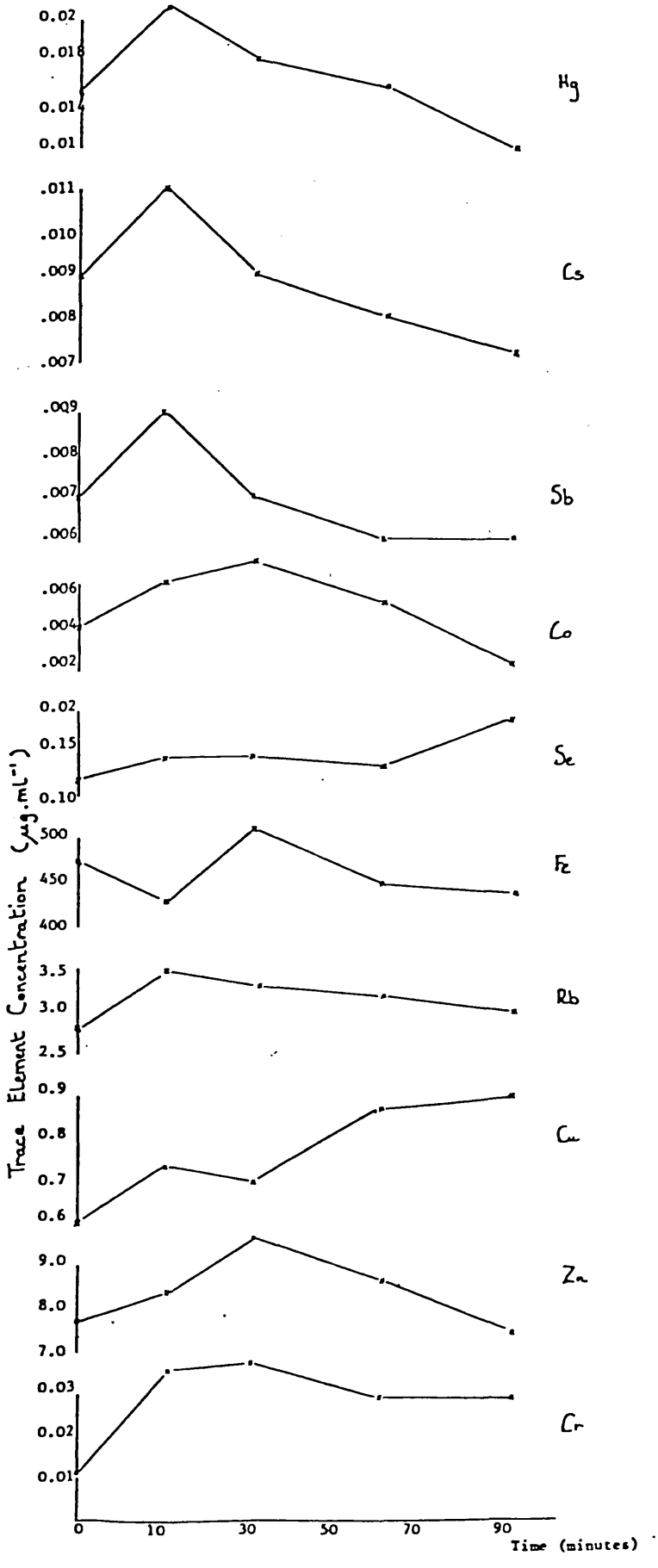


Figure 6

Plasma Glucose Concentration of a Control Subject During an Intravenous Glucose Tolerance Test.

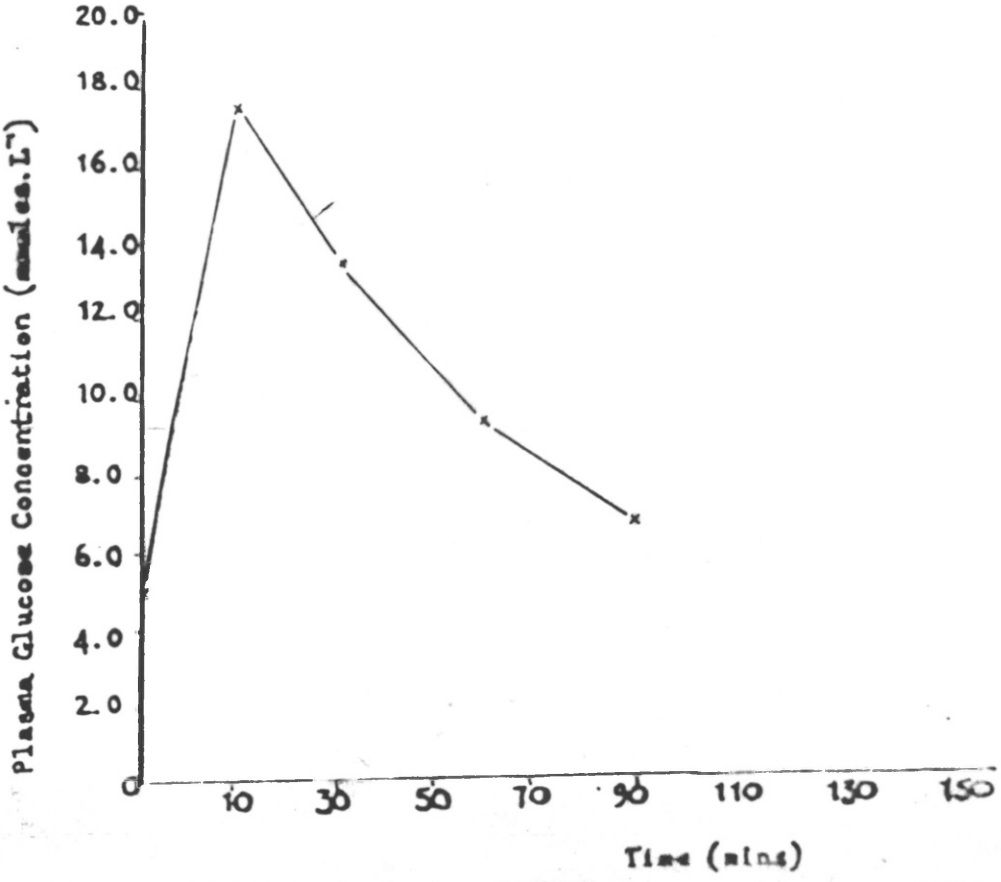


Figure 5(b)

Trace Element Concentration in Plasma of a Control Subject During an Intravenous Glucose Tolerance Test

Figure 6

Plasma Glucose Concentration of a Control Subject During an Intravenous Glucose Tolerance Test.

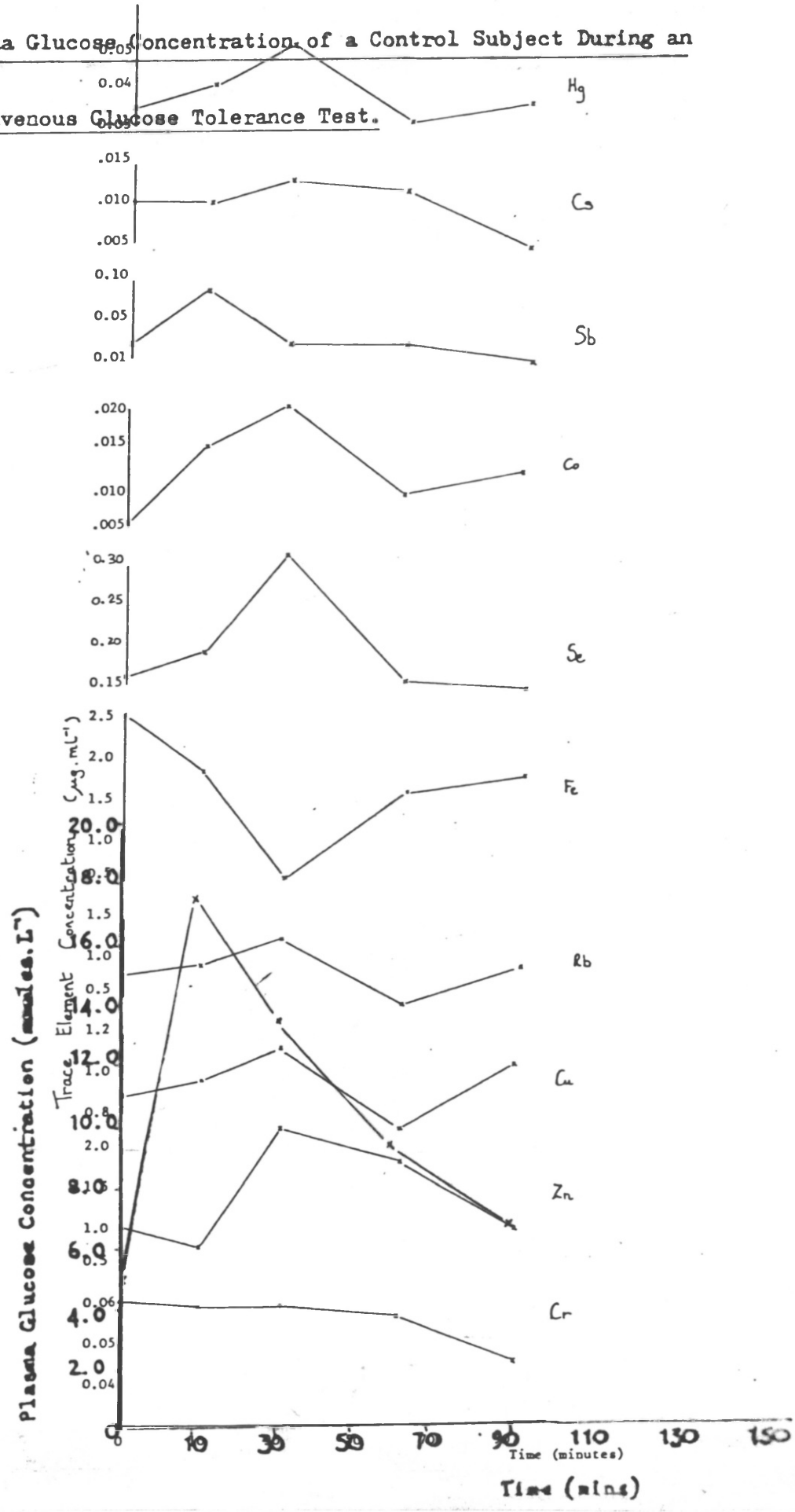
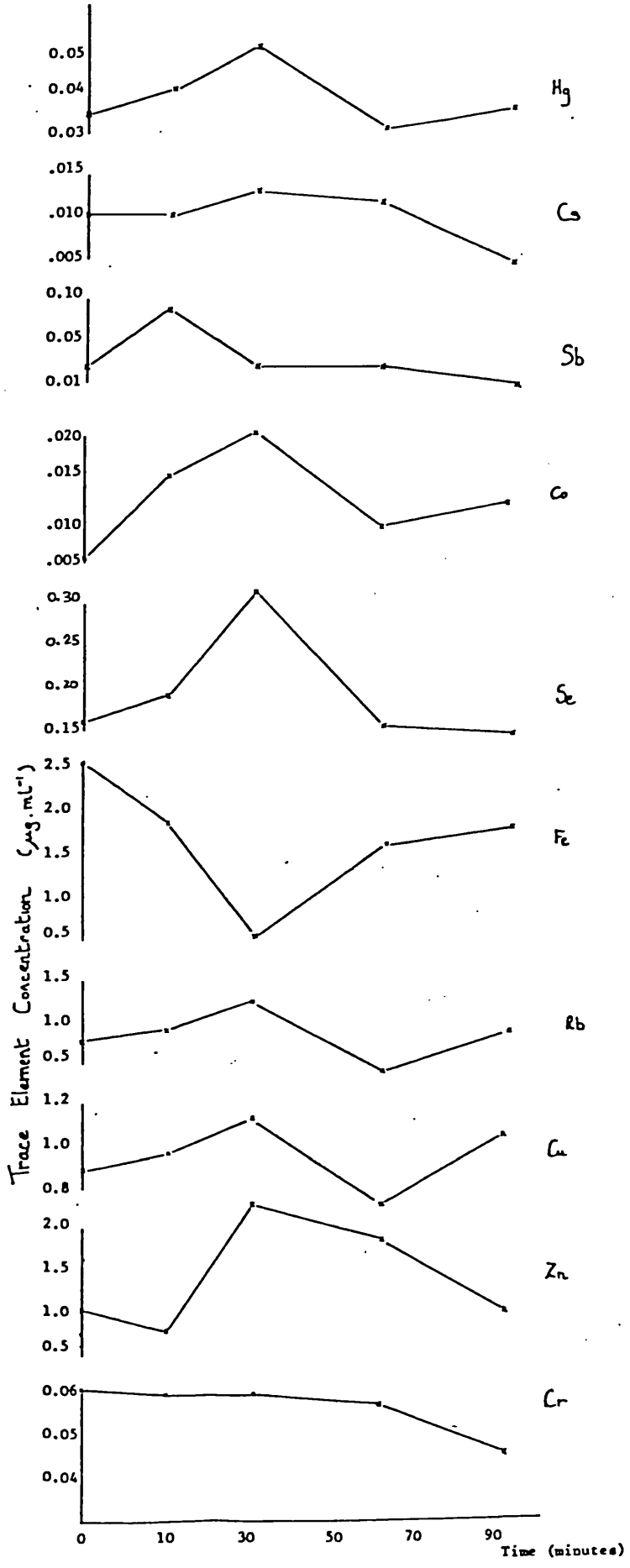


Figure 5(b)

Trace Element Concentration in Plasma of a Control Subject During an Intravenous Glucose Tolerance Test



An Investigation into the Effect of Hypoglycaemia on Circulating trace elements

An experiment to study the effect of a 100 oral glucose load on an hypoglycaemic subject.

Table 7 (a)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in whole blood									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.90	0.003	5.3	0.11	390.3	2.88	0.017	0.002	0.004	0.007
30	1.26	0.002	4.3	0.08	330.2	2.54	0.012	0.001	0.002	0.006
60	1.65	0.005	5.3	0.06	412.1	2.64	0.018	0.002	0.004	0.007
90	1.15	0.008	6.1	0.12	442.5	2.17	0.022	0.003	0.004	0.005
120	0.77	0.006	5.6	0.12	431.7	2.53	0.022	0.003	0.010	0.009

Table 7 (b)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in plasma									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.88	0.006	1.86	0.23	1.14	0.82	0.056	0.003	0.020	0.012
30	0.77	0.007	1.53	0.13	1.02	0.27	0.037	0.003	0.014	0.007
60	0.77	0.009	1.36	0.21	1.24	0.18	0.046	0.004	0.020	0.012
90	0.66	0.005	1.14	0.21	0.93	0.20	0.048	0.010	0.020	0.012
120	0.77	0.005	1.23	0.21	1.10	0.39	0.059	0.009	0.041	0.017

Plasma glucose and insulin concentrations of a control subject during an oral glucose tolerance test.

Table 8

Time of Sampling (mins)	Plasma Glucose Concentration ($mmol.L^{-1}$)	Plasma Insulin Concentration ($mU.L^{-1}$)
0	5.3	3.0
30	3.1	32.4
60	3.5	53.0
90	4.0	61.0
120	3.8	57.0

Figure 8

Plasma Glucose(—x—) and Insulin(—Δ—) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

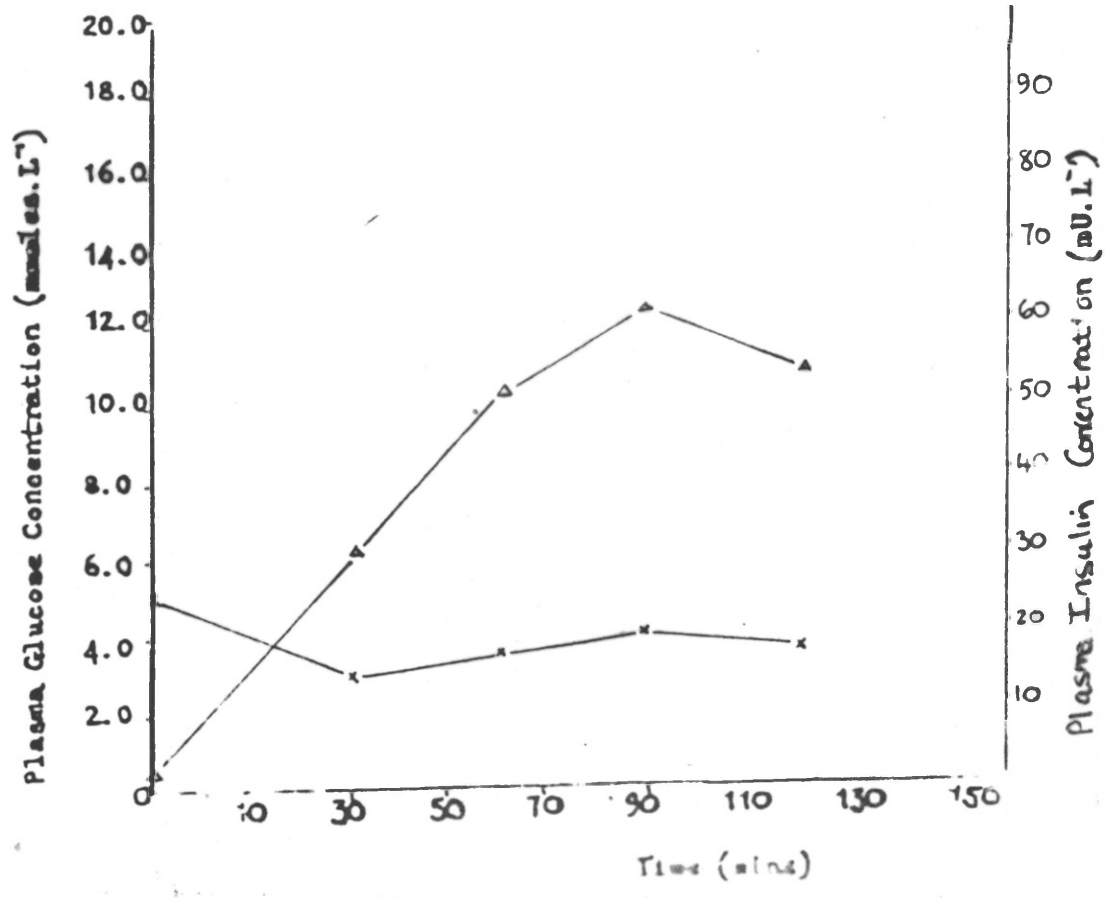


Figure 7(a)

Trace element Concentration in Whole Blood of an Hypoglcaemic Subject During an Oral Glucose Tolerance Test

Figure 8

Plasma Glucose (— x —) and Insulin (— Δ —) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

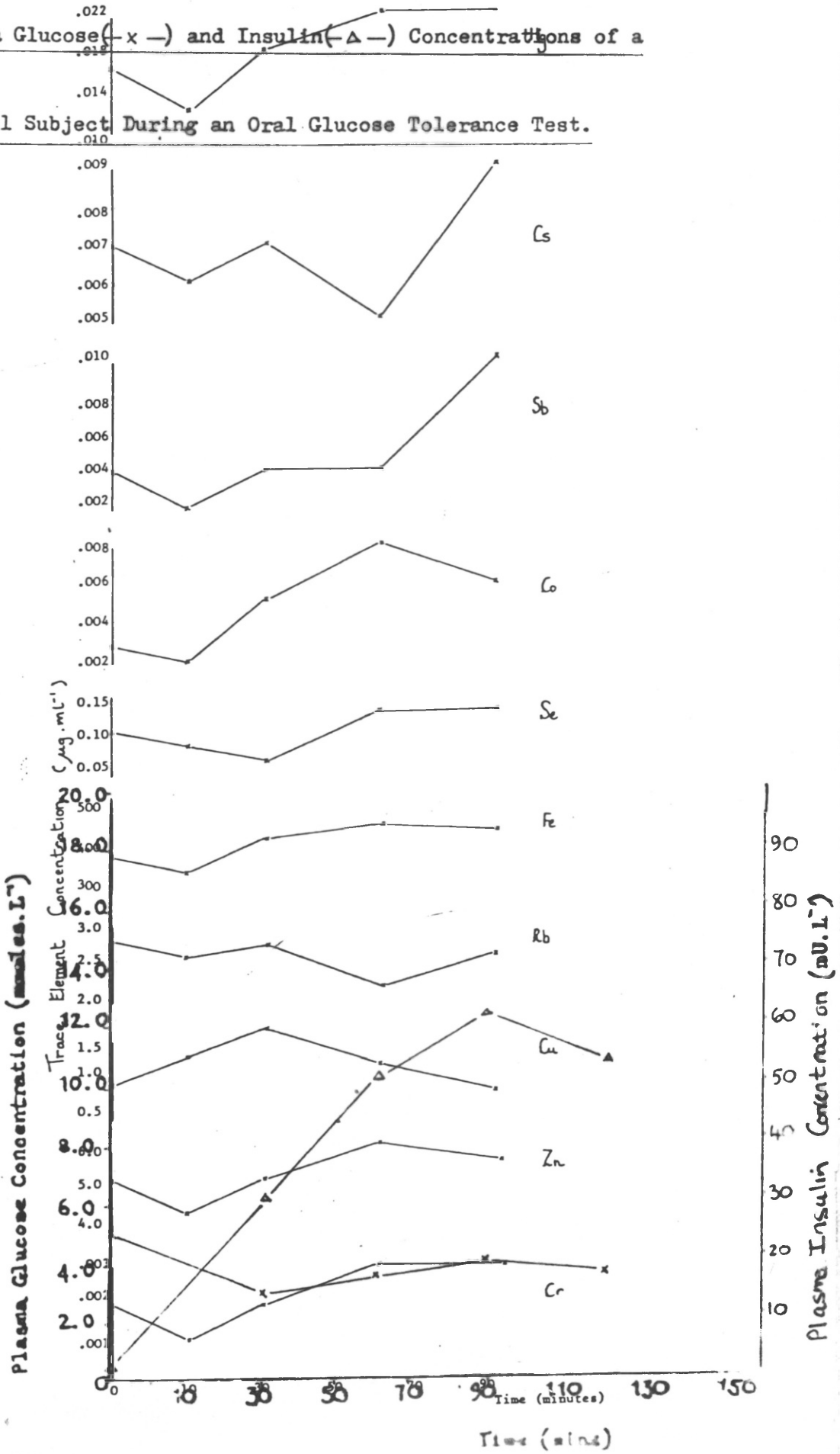


Figure 7(a)

Trace element Concentration in Whole Blood of an Hypoglcaemic Subject During an Oral Glucose Tolerance Test

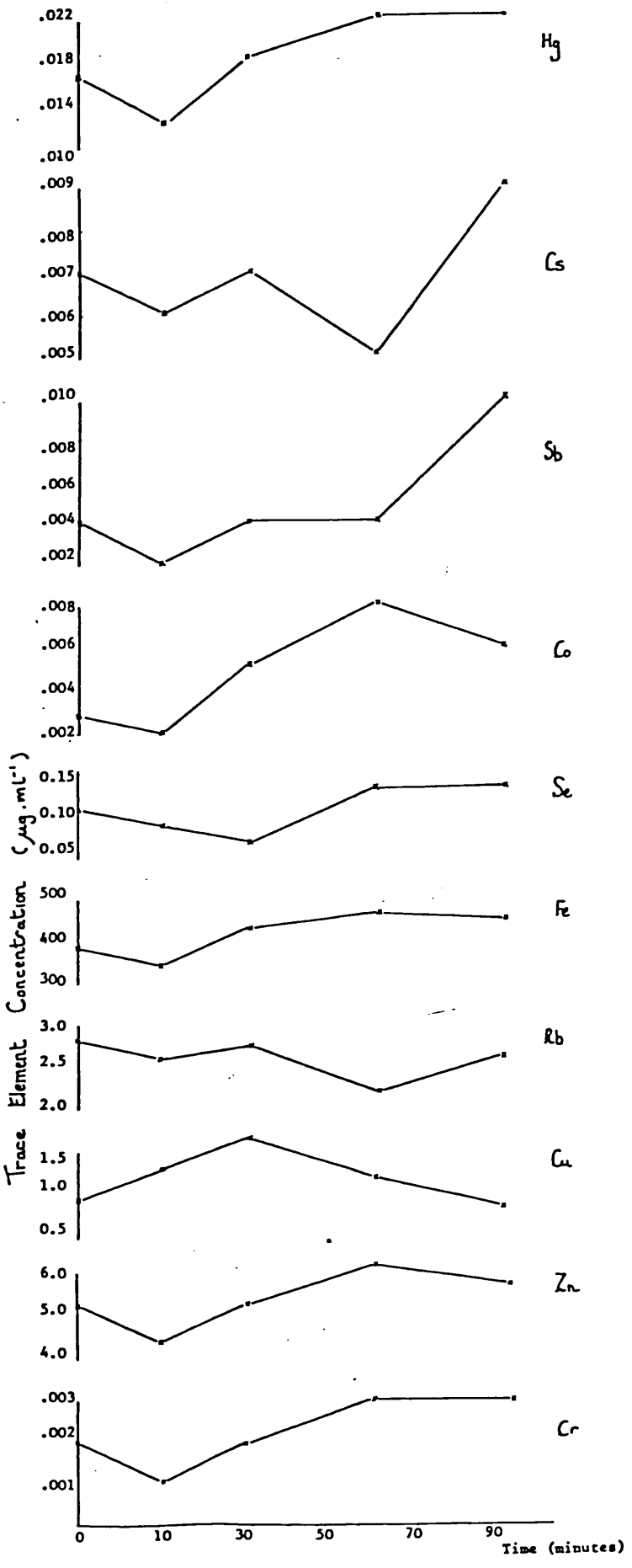


Figure 8

Plasma Glucose(— x —) and Insulin(— Δ —) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

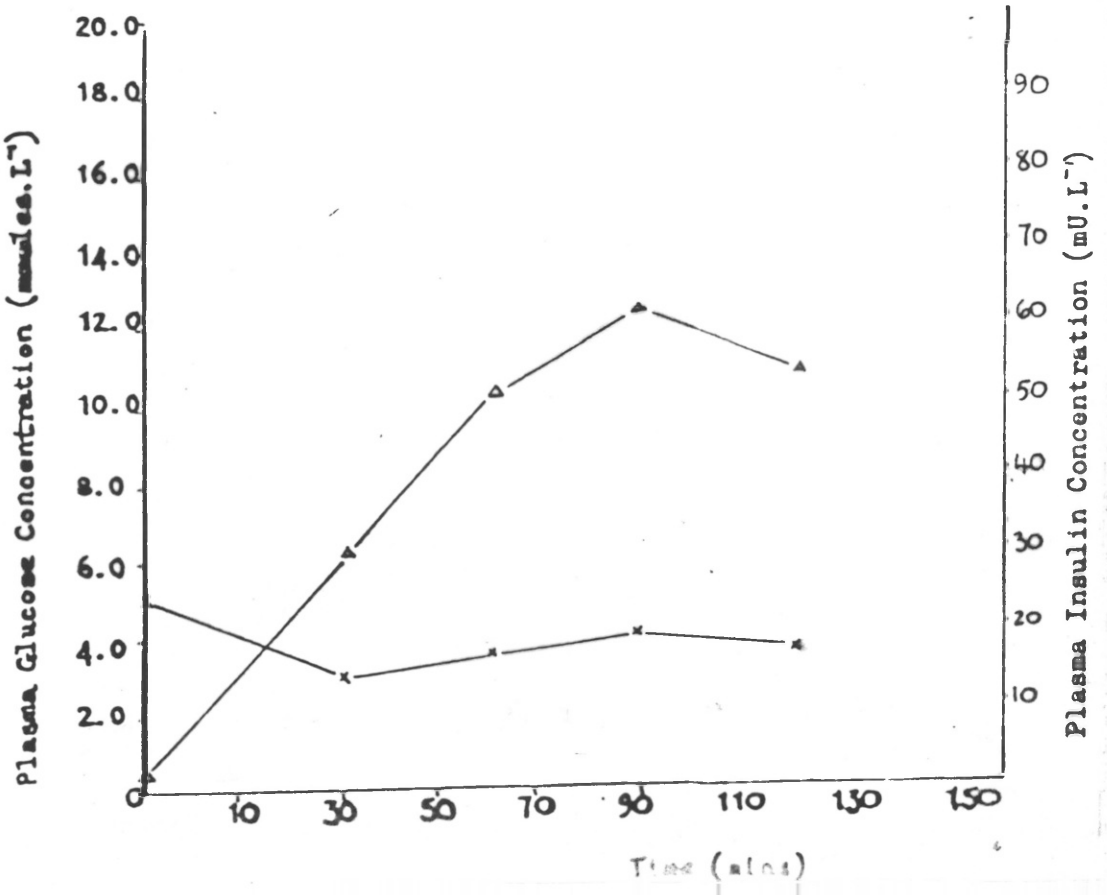


Figure 7(b)

Trace Element Concentration in Plasma of an Hypoglycaemic Subject During an Oral Glucose Tolerance Test

Figure 8

Plasma Glucose(—x—) and Insulin(—Δ—) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

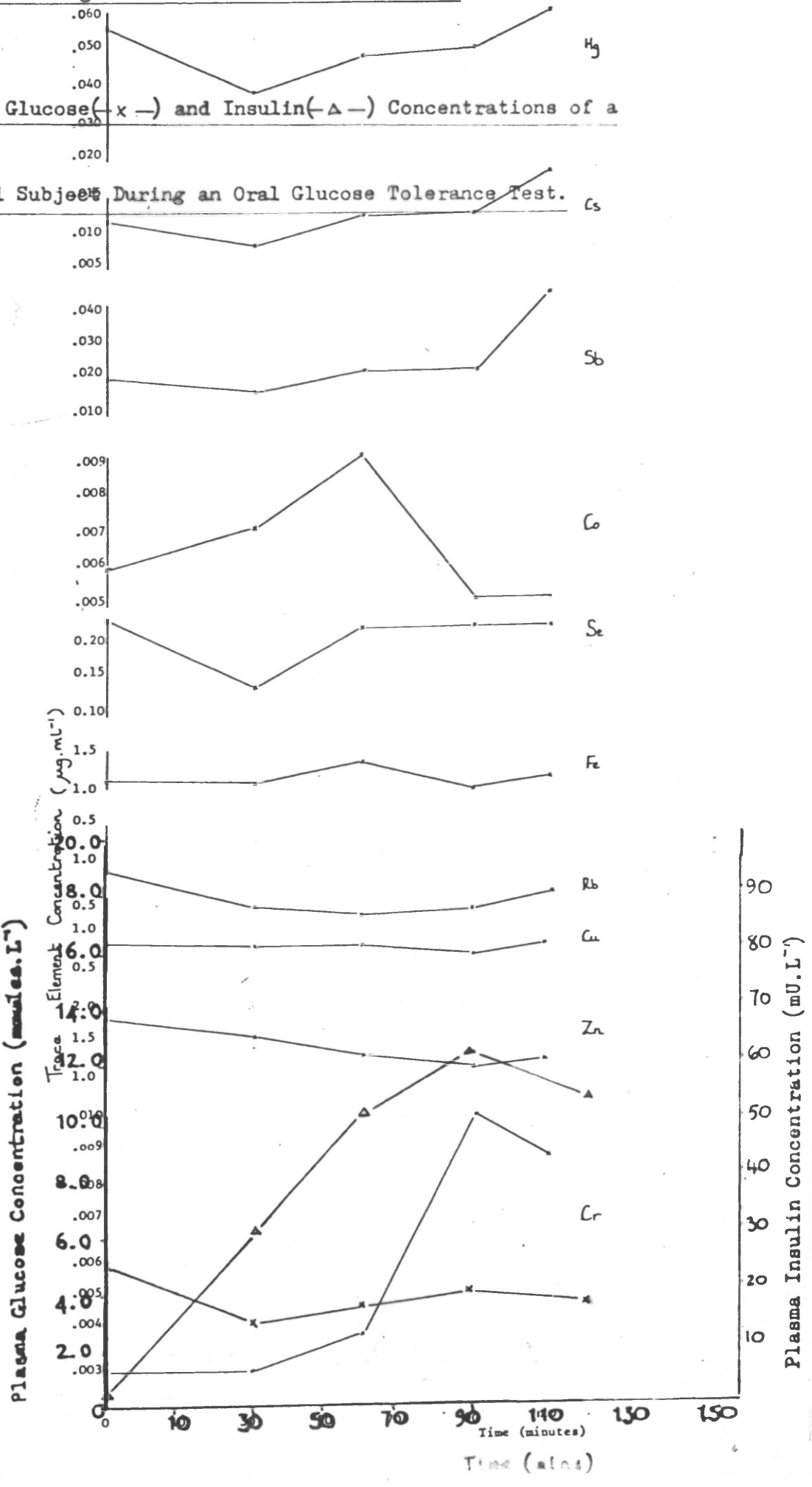
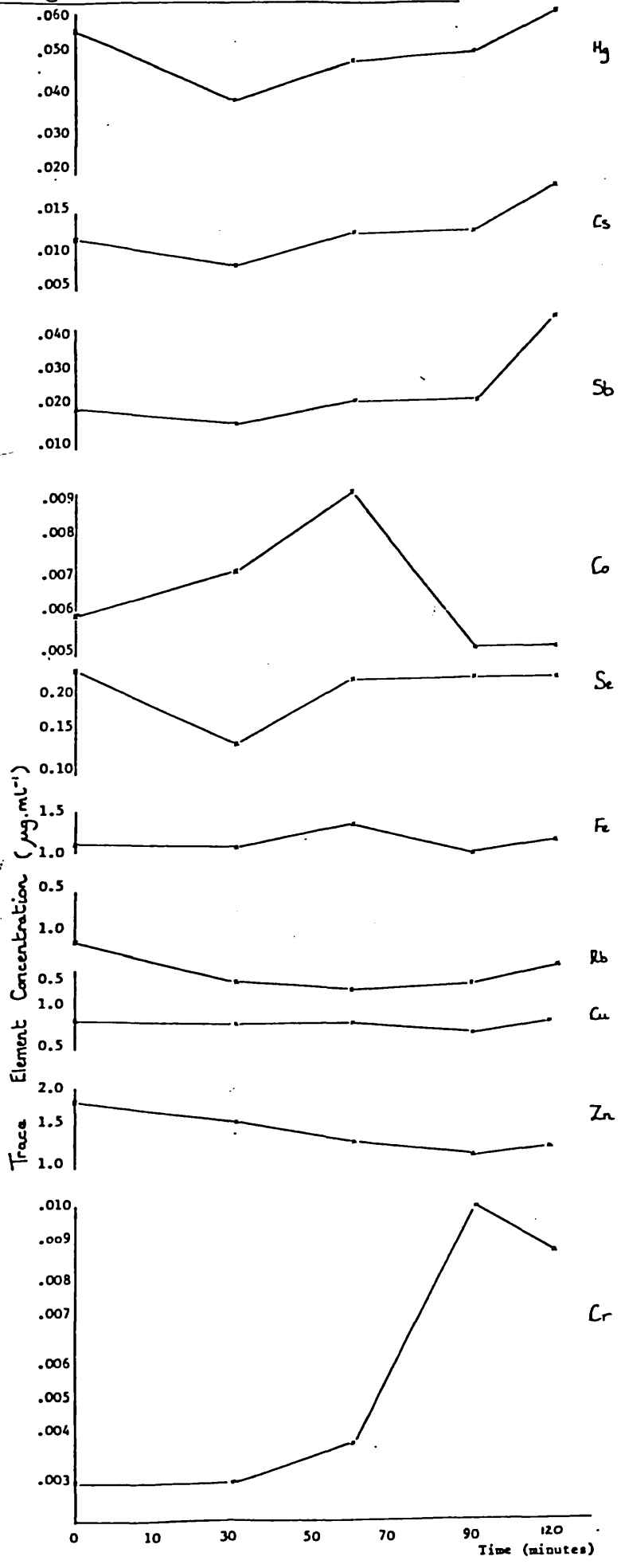


Figure 7(b)

Trace Element Concentration in Plasma of an Hypoglycaemic Subject During an Oral Glucose Tolerance Test



An Experiment to study the effect of an intravenous glucose load on an hypoglycaemic subject

Table 9 (a)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in whole blood									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.60	0.002	5.92	0.14	482.3	2.30	0.020	0.003	0.002	0.009
30	0.69	0.004	5.99	0.13	467.2	1.99	0.019	0.003	0.001	0.009
60	0.56	0.003	5.85	0.14	467.5	2.15	0.016	0.003	0.001	0.008
90	0.54	0.008	9.30	0.18	621.3	3.02	0.023	0.004	0.003	0.012
120	0.60	0.002	6.88	0.15	530.4	2.40	0.017	0.003	0.007	0.005
150	0.60	0.005	5.05	0.15	507.9	3.67	0.031	0.004	0.010	0.007

Table 9 (b)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in plasma									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.77	0.010	1.52	0.27	1.01	0.42	0.026	0.004	0.003	0.008
30	0.75	0.008	1.07	0.30	1.70	0.85	0.035	0.006	0.004	0.013
60	0.74	0.007	1.52	0.32	1.98	0.67	0.031	0.005	0.003	0.010
90	0.63	0.003	1.57	0.49	1.30	0.87	0.033	0.007	0.006	0.015
120	0.84	0.006	1.95	0.29	1.74	0.54	0.035	0.004	0.003	0.008
150	0.75	0.007	1.03	0.64	1.66	0.55	0.048	0.004	0.002	0.001

Plasma glucose and insulin concentrations of a control subject during an intravenous glucose load

Table 10

Time of Sampling (mins)	Plasma Glucose Concentration ($mmol.L^{-1}$)	Plasma Insulin Concentration ($mU.L^{-1}$)
0	5.8	2.6
30	5.1	30.6
60	3.2	61.0
90	4.5	46.3
120	3.9	75.3
150	3.3	55.6

Figure 10

Plasma Glucose(—x—) and Insulin(—Δ—) Concentrations of a Control Subject During an Intravenous Glucose Tolerance Test.

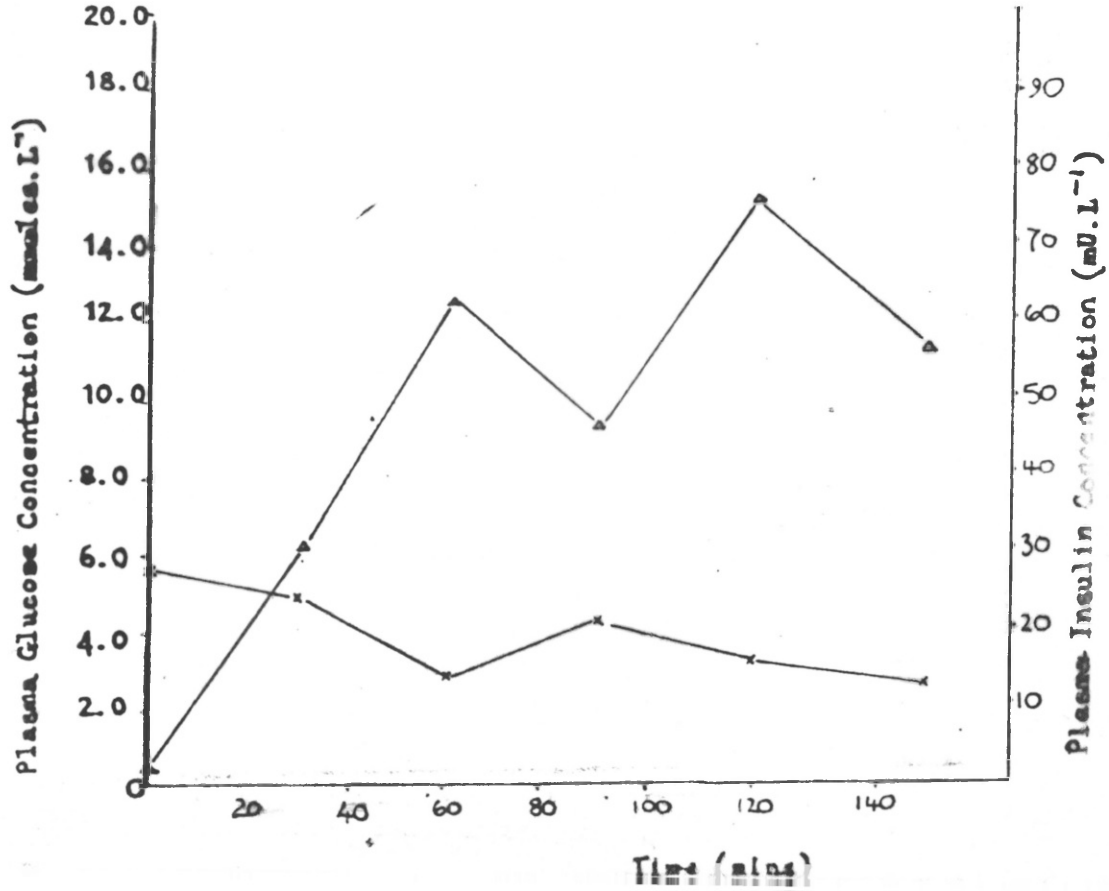


Figure 9(a)

Trace Element Concentration in Whole Blood of an Hypoglycaemic Subject During an Intravenous Glucose Tolerance Test

Figure 10

Plasma Glucose (—x—) and Insulin (—Δ—) Concentrations of a Control Subject During an Intravenous Glucose Tolerance Test.

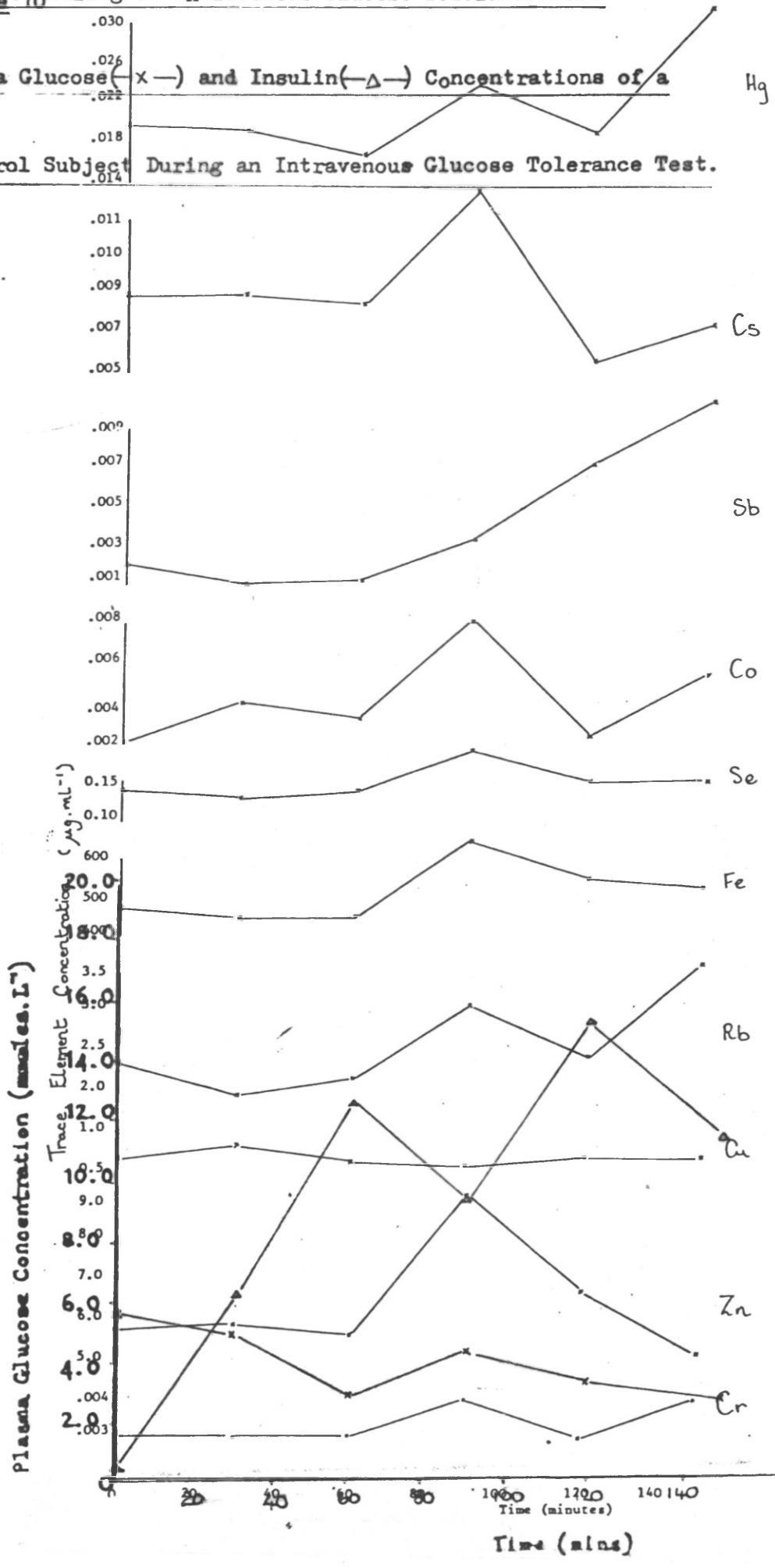


Figure 9(a)

Trace Element Concentration in Whole Blood of an Hypoglycaemic Subject During an Intravenous Glucose Tolerance Test

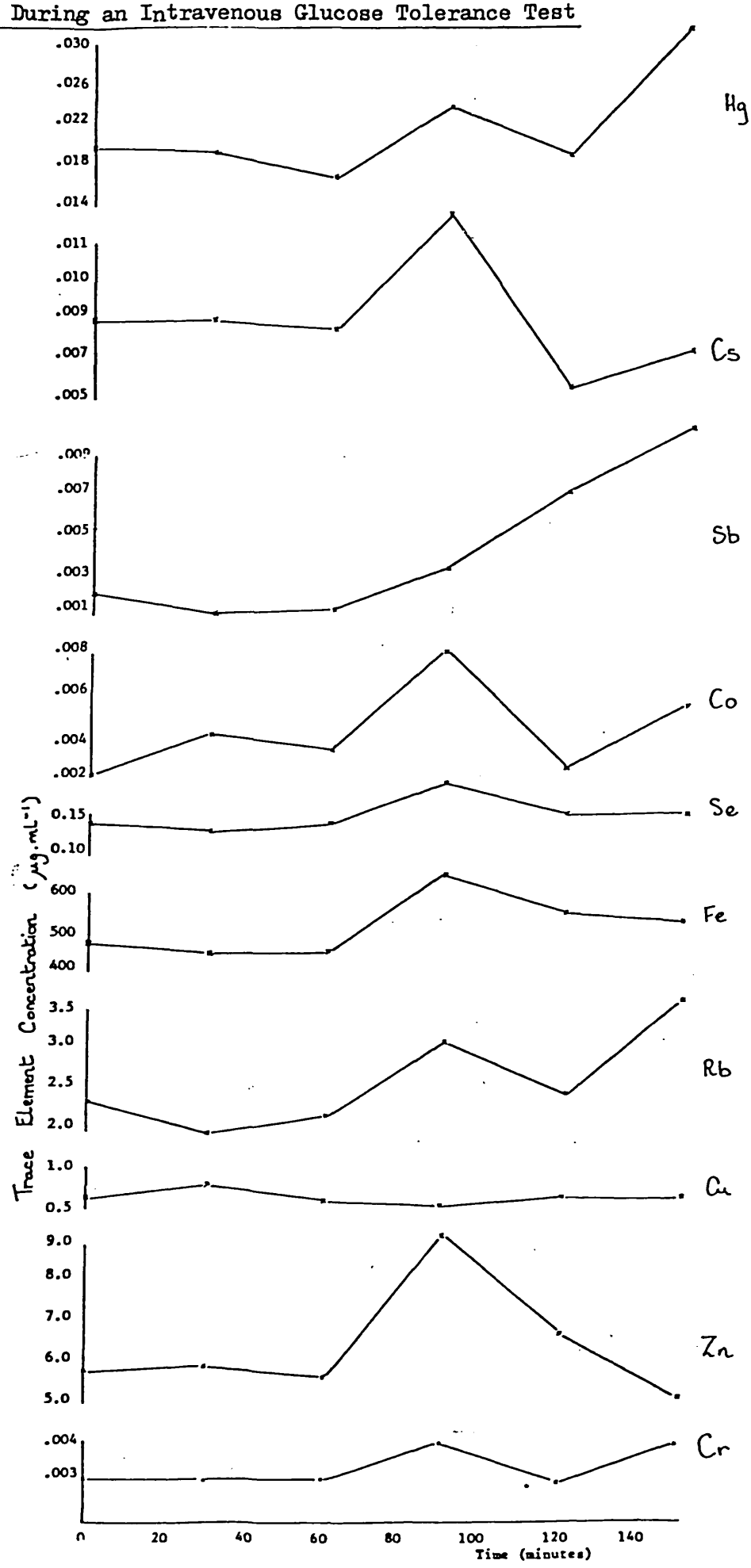


Figure 10

Plasma Glucose(—x—) and Insulin(—△—) Concentrations of a Control Subject During an Intravenous Glucose Tolerance Test.

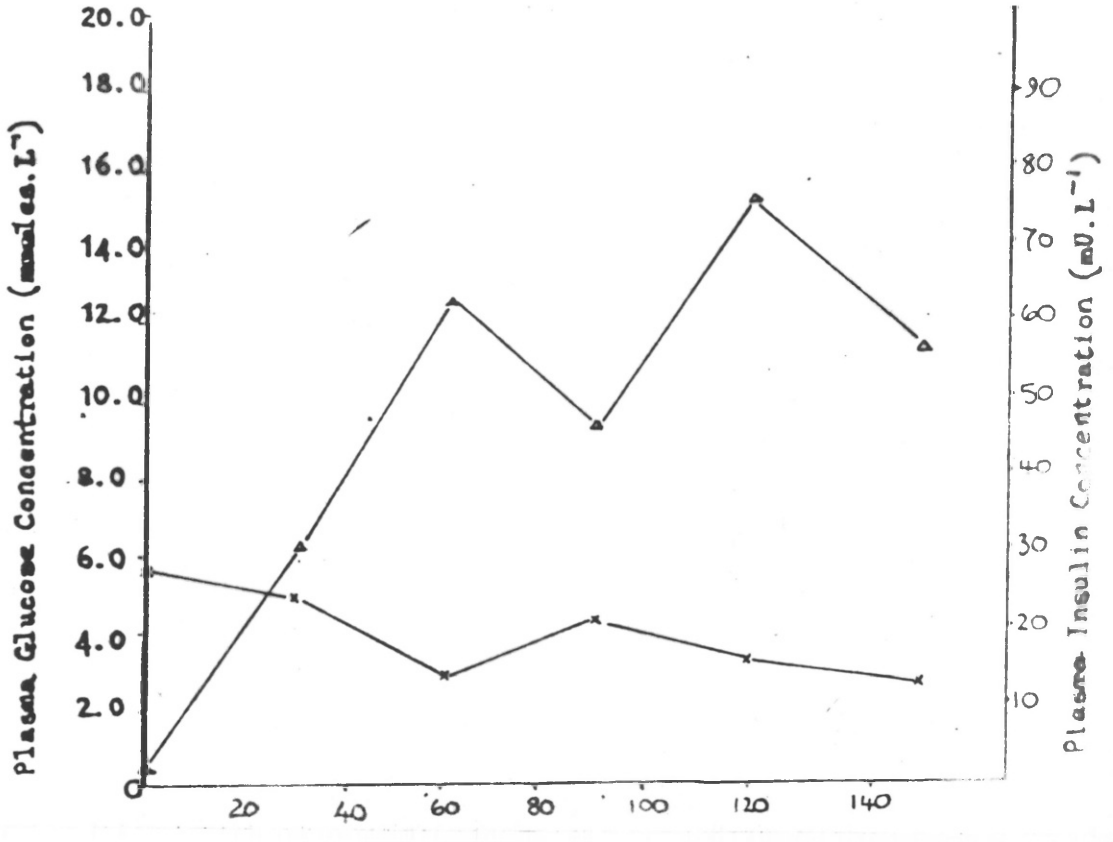


Figure 9(b)

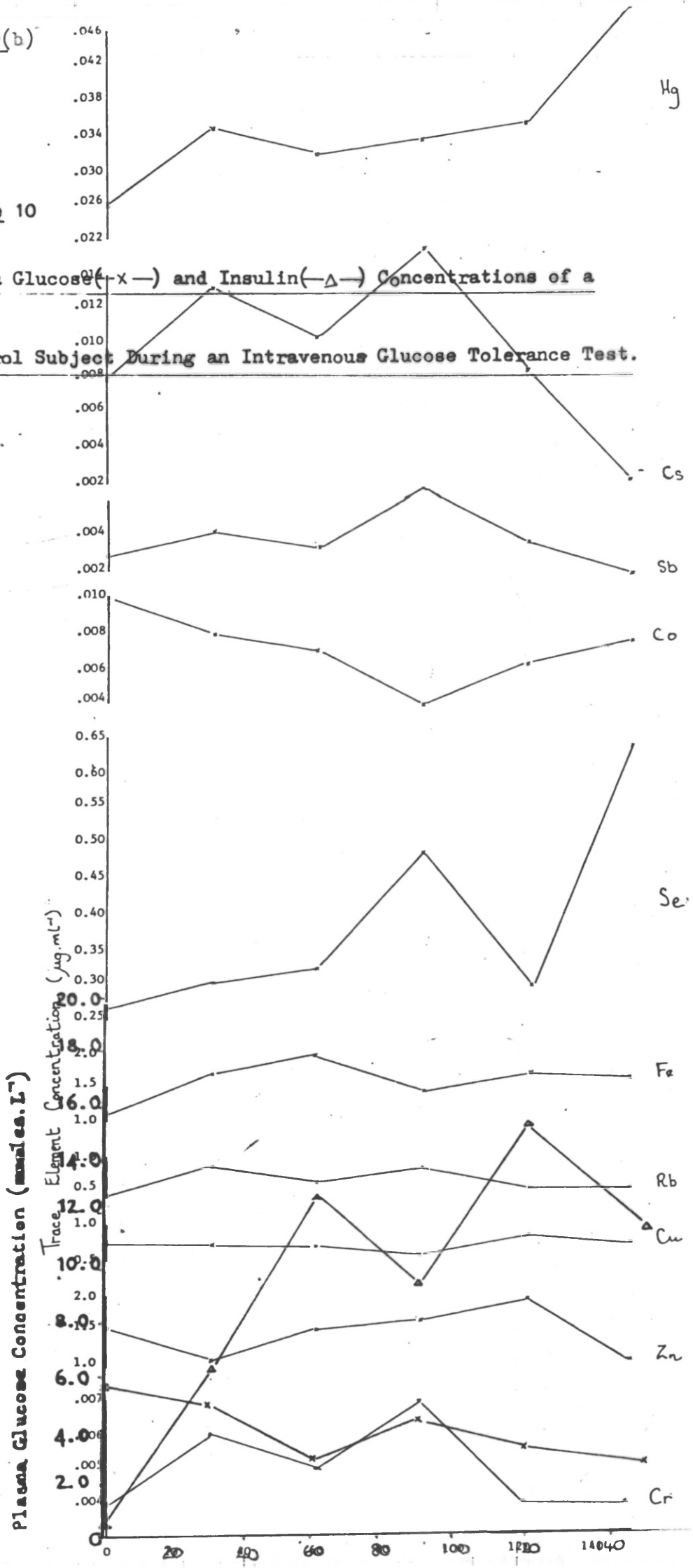
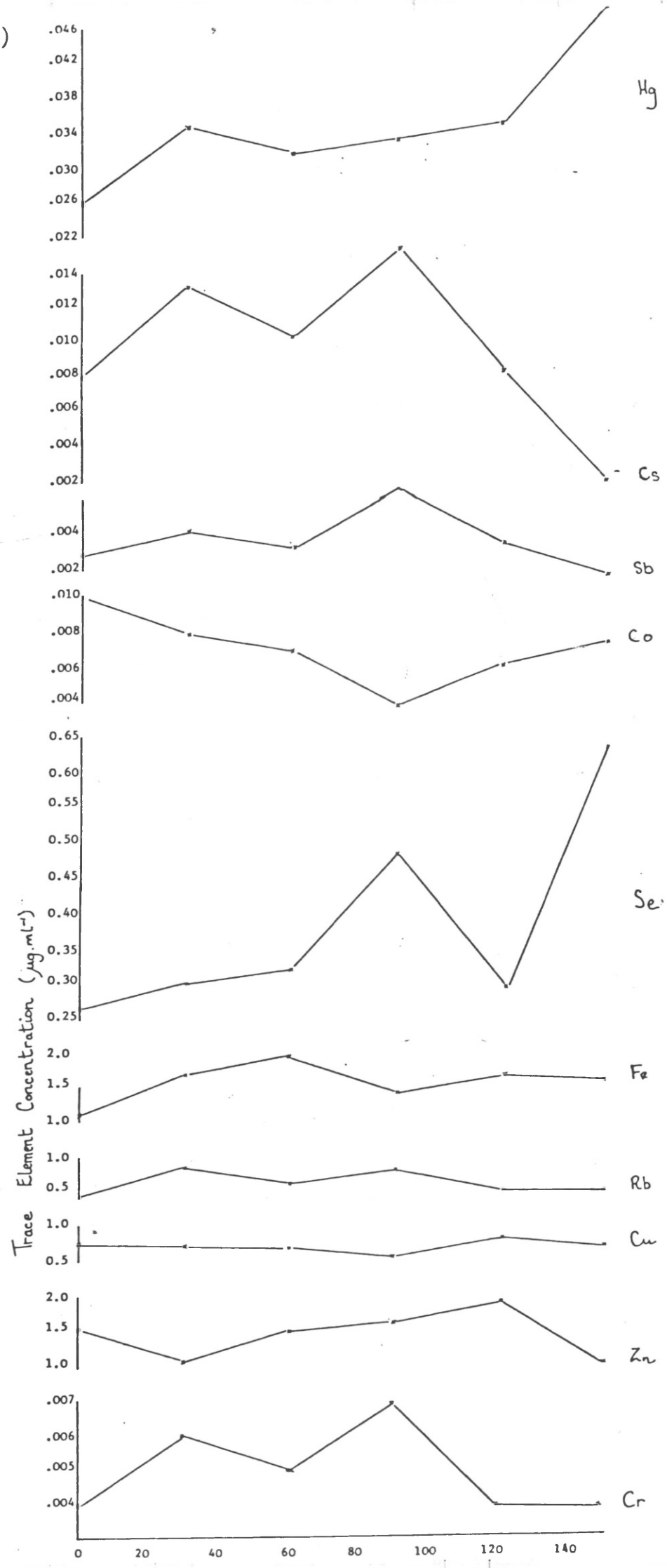


Figure 10

Plasma Glucose(—x—) and Insulin(—Δ—) Concentrations of a Control Subject During an Intravenous Glucose Tolerance Test.

Figure 9(b)


Trace Element Concentration (μg.ml⁻¹)
.046
.042
.038
.034
.030
.026
.022
Hg
.014
.012
.010
.008
.006
.004
.002
Cs
.004
.002
Sb
.010
.008
.006
.004
Co
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
Se
2.0
1.5
1.0
Fe
1.0
0.5
Rb
1.0
0.5
Cu
2.0
1.5
1.0
Zn
.007
.006
.005
.004
Cr
0
20
40
60
80
100
120
140

An Experiment to Study the Effect of 10g Guar (oral dose) on an hypoglycaemic subject

Table 11 (a)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in whole blood									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.72	0.004	5.27	0.12	394.1	2.53	0.019	0.001	0.006	0.008
30	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
60	0.69	0.006	5.49	0.12	411.3	3.17	0.021	0.002	0.006	0.008
90	0.68	0.004	5.44	0.13	410.9	3.09	0.022	0.002	0.006	0.008

Table 11 (b)

Time of Sampling (mins)	Trace Element Concentration ($\mu g.ml^{-1}$) in plasma									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.02	0.002	1.64	0.07	0.85	0.70	0.033	0.005	0.001	0.009
30	0.84	0.005	2.01	0.12	1.44	0.76	0.043	0.004	0.002	0.008
60	0.84	0.007	1.38	0.14	1.54	1.17	0.043	0.004	0.002	0.009
90	0.77	0.008	1.98	0.14	0.44	0.75	0.040	0.003	0.006	0.004

Plasma glucose and insulin concentrations of a control subject during an oral Guar load

Table 12

Time of Sampling (mins	Plasma Glucose Concentration ($mmol.L^{-1}$)	Plasma Insulin Concentration ($mU.L^{-1}$)
	5.0	4.7
30	4.0	40.0
60	2.8	27.0
90	3.4	47.0

N.D. Not determined

Figure 12

Plasma Glucose(—x—) and Insulin (—Δ—) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

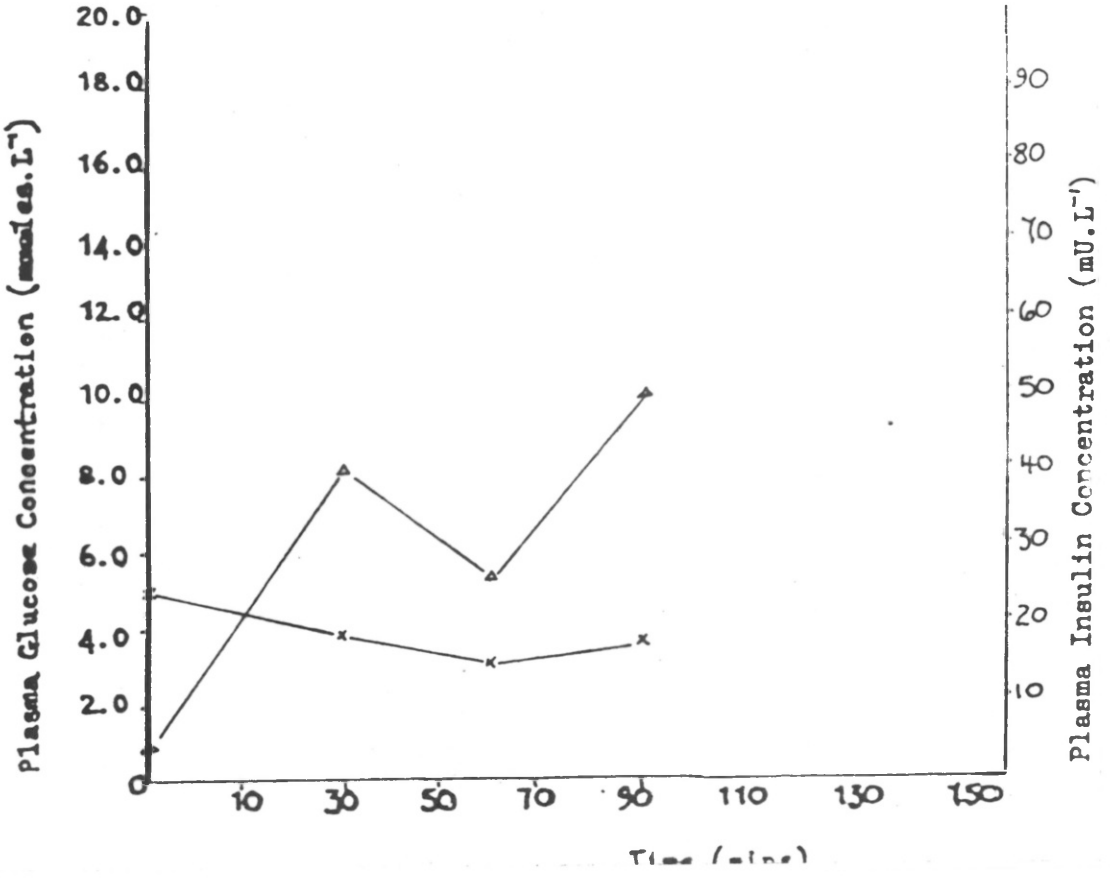


Figure 11(a)

Figure 12

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Trace Element Concentration in Whole Blood of an Hypoglcaemic Plasma Glucose(—x—) and Insulin (—Δ—) Concentrations of a Subject(Given 10g Guar) During a Glucose Tolerance Test.

Control Subject During an Oral Glucose Tolerance Test.

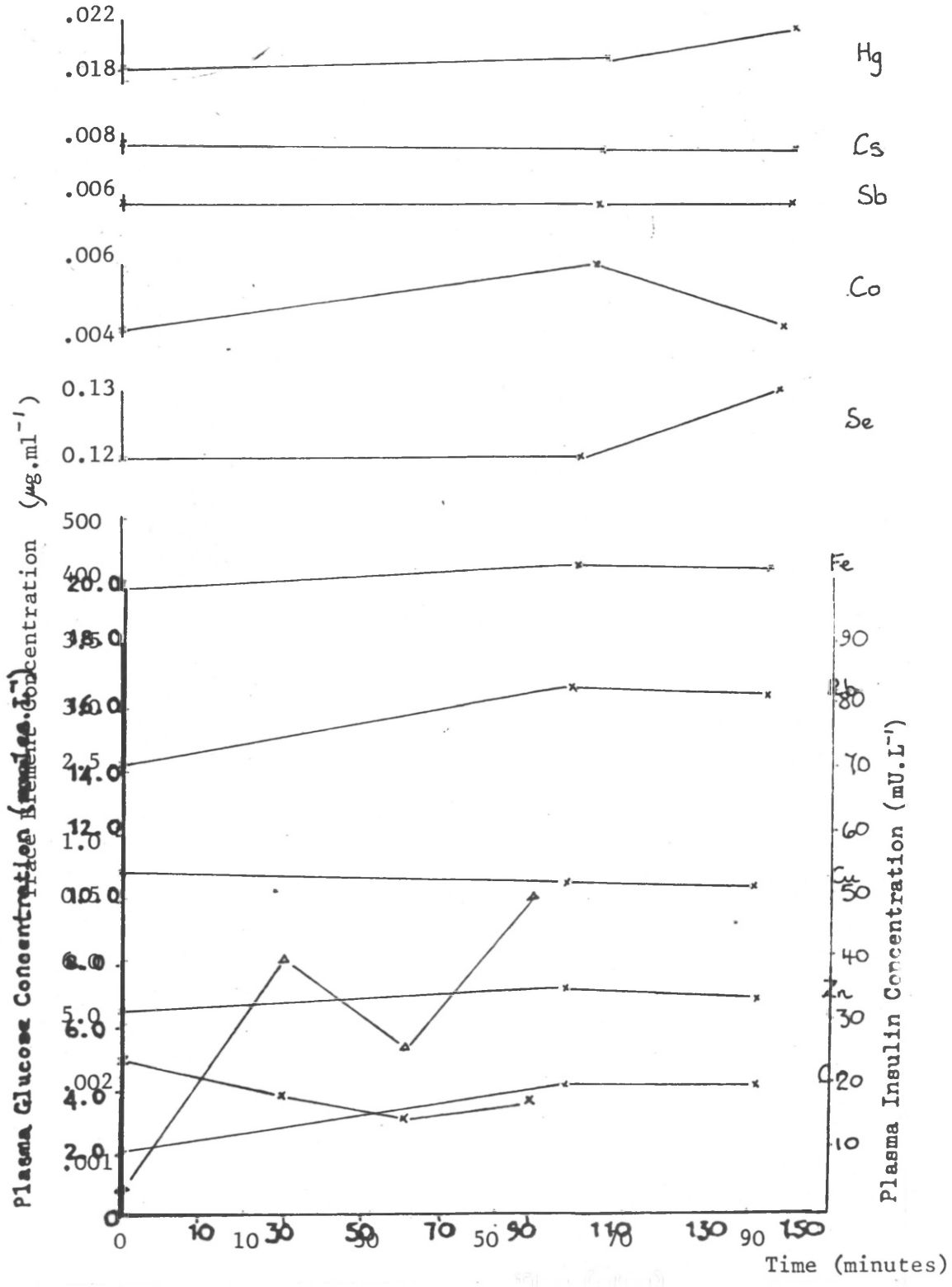


Figure 11(a)

Trace Element Concentration in Whole Blood of an Hypoglcaemic Subject(Given 10g Guar) During a Glucose Tolerance Test.

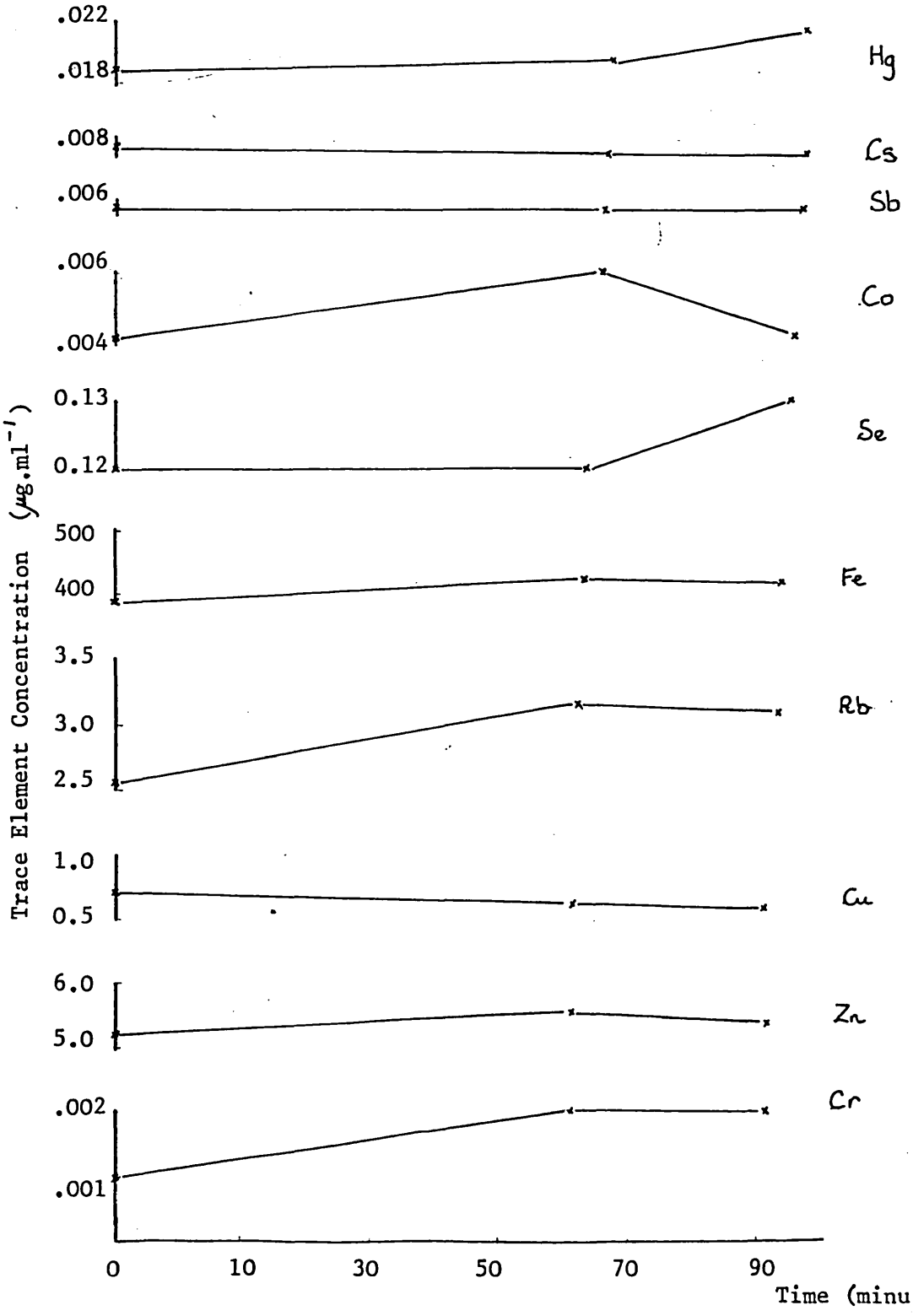


Figure 12

Plasma Glucose (–x–) and Insulin (–Δ–) Concentrations Of a Control Subject During an Oral Glucose Tolerance Test.

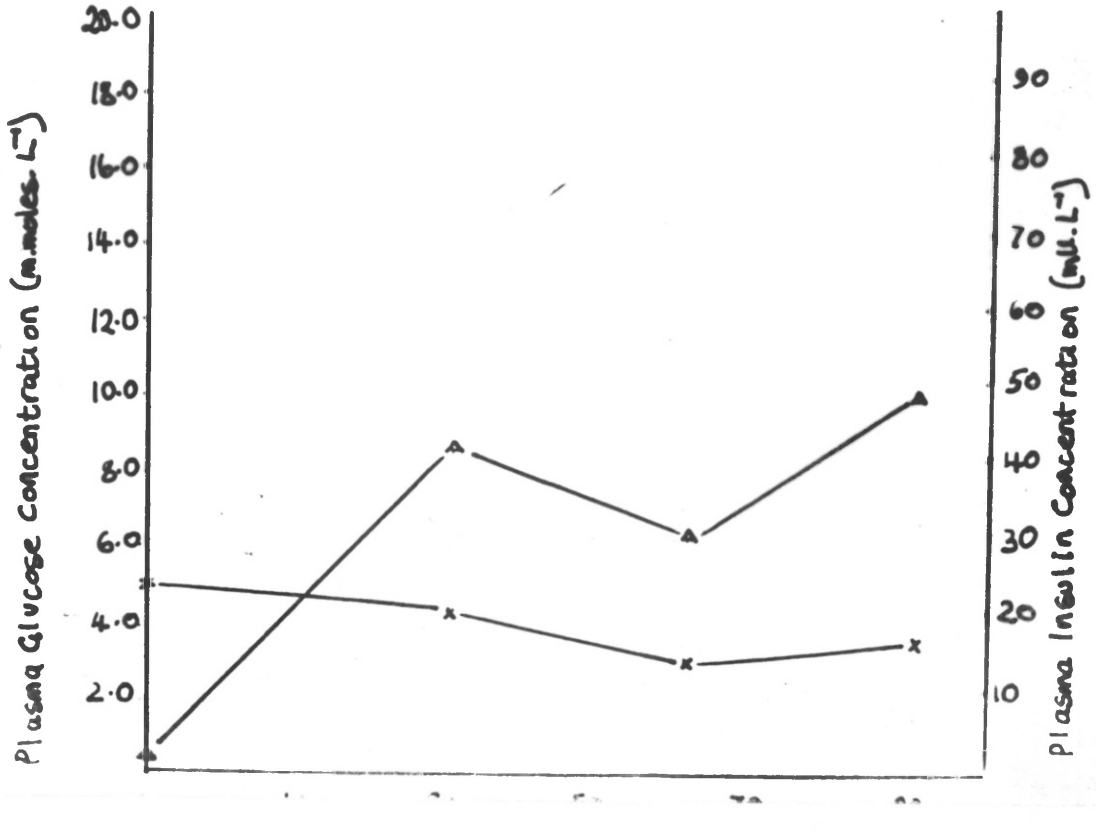


Figure 11(b)

Trace Element Concentration in Plasma of an Hypoglycaemic Subject

(Given 10g ~~Lævu~~) During a Glucose Tolerance Test.

Figure 12

Plasma Glucose (—x—) and Insulin (—Δ—) Concentrations of a Control Subject During an Oral Glucose Tolerance Test.

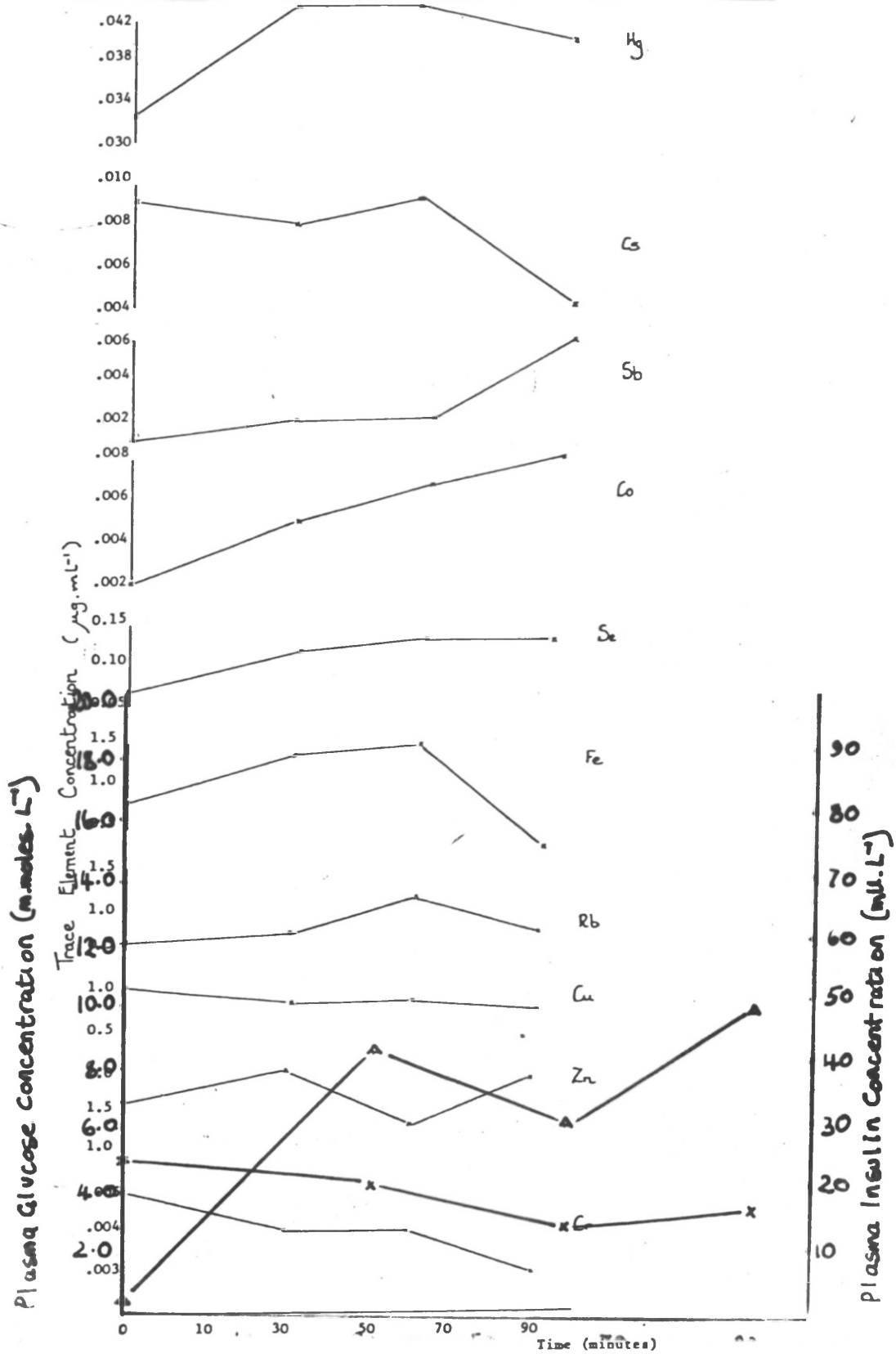
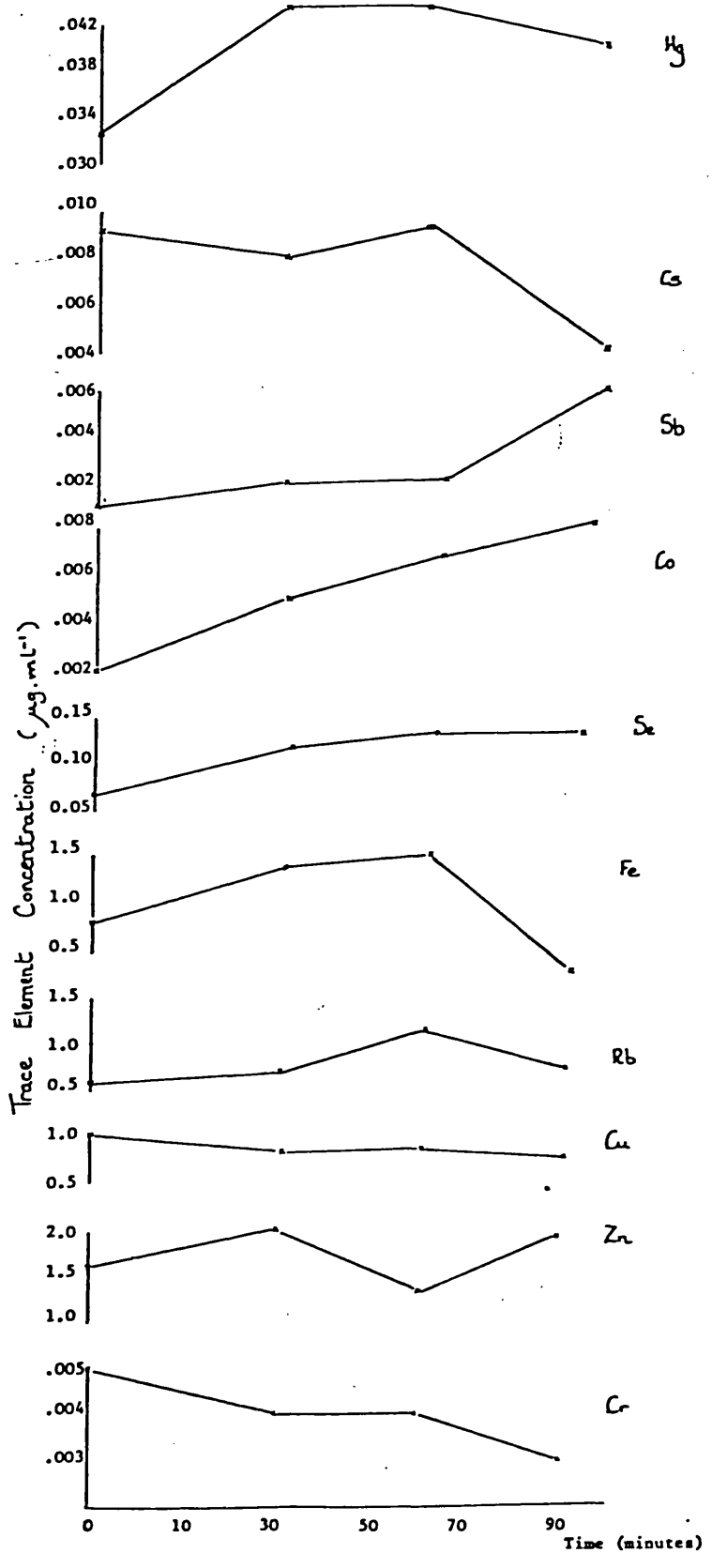


Figure 11(b)

Trace Element Concentration in Plasma of an Hypoglycaemic Subject

(Given 10g Guar) During a Glucose Tolerance Test.



Section 3.4

Table 13 Individuals Participating in Oral Glucose Tolerance Study

Subject Group	Mean Age (years) ($SD\bar{x}$)	Mean Weight (kg) ($SD\bar{x}$)	Mean Height (m) ($SD\bar{x}$)	Duration of disease (years) ($SD\bar{x}$)
Lean Controls n = 5	52.2 ± 4.9	77.6 ± 7.49	1.75 ± 0.06	-
Lean Diabetics n = 5	59 ± 4.2	72.9 ± 10.6	1.71 ± 0.06	12.3 ± 4.8
Obese Controls n = 8	44.9 ± 5.3	102.1 ± 24.9	1.63 ± 0.05	-
Obese Diabetics n = 8	42.8 ± 4.5	89.8 ± 16.6	1.55 ± 0.08	3.5 ± 1.8

Table 14 Weight Loss Achieved by Obese Individuals during one month on the Cambridge Diet

Subject Group	Mean Weight loss (kg) ($SD\bar{x}$)
Obese Controls (n = 6)	9.72 ± 2.02
Obese Diabetics (n = 6)	6.83 ± 3.46

Trace Element Content of the Erythrocytes of Lean Individuals after a 75g oral Glucose challenge

Table 15: Lean Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.46 (.05)	0.002 (.0003)	8.13 (1.52)	0.13 (.05)	695.8 (92.2)	3.14 (.47)	0.020 (.0009)	0.0044 (.001)	0.0025 (.0006)	0.005 (.0008)
30	0.57 (.11)	0.001 (.0001)	7.81 (.98)	0.12 (.03)	684.5 (49.3)	2.93 (.25)	0.021 (.0008)	0.0077 (.002)	0.0022 (.0006)	0.004 (.0005)
60	0.49 (.02)	0.002 (.0003)	8.83 (1.26)	0.11 (.02)	811.1 (124.8)	3.18 (.51)	0.015 (.0007)	0.0035 (.001)	0.0014 (.0004)	0.0052 (.0008)
90	0.48 (.06)	0.002 (.0002)	8.36 (1.04)	0.13 (.02)	855.3 (99.6)	3.09 (.25)	0.018 (.0007)	0.0067 (.0005)	0.0014 (.0004)	0.0048 (.0004)
120	0.52 (.07)	0.002 (.0003)	8.66 (1.14)	0.12 (.02)	869.1 (63.9)	3.31 (.36)	0.022 (.0009)	0.0075 (.0005)	0.0015 (.0004)	0.0039 (.0008)
150	0.56 (.06)	0.002 (.0002)	8.51 (1.16)	0.12 (.03)	852.4 (99.6)	3.19 (.30)	0.026 (.001)	0.0033 (.001)	0.0018 (.0007)	0.0051 (.0006)
180	0.47 (.09)	0.002 (.0002)	9.10 (.36)	0.08 (.02)	721.1 (9.59)	3.35 (.01)	0.010 (.0004)	0.0013 (.0005)	0.0012 (.00005)	0.0056 (.0009)

Table 16: Lean Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.61 (.12)	0.002 (.0004)	10.42 (1.04)	0.14 (.01)	911.7 (44.5)	4.17 (.36)	0.025 (.009)	0.0085 (.003)	0.0028 (.0007)	0.0041 (.0004)
30	0.59 (.09)	0.003 (.0003)	11.43 (1.27)	0.15 (.02)	994.5 (71.2)	4.45 (.31)	0.035 (.01)	0.0088 (.003)	0.0021 (.0007)	0.0045 (.0004)
60	0.50 (.04)	0.003 (.0007)	9.81 (1.76)	0.15 (.01)	896.1 (73.6)	3.84 (.08)	0.025 (.009)	0.0079 (.003)	0.0016 (.0004)	0.0039 (.0005)
90	0.56 (.10)	0.002 (.0004)	9.72 (1.26)	0.14 (.02)	992.5 (85.8)	4.28 (.87)	0.023 (.008)	0.010 (.005)	0.0018 (.0003)	0.0046 (.0007)
120	0.53 (.06)	0.002 (.0003)	8.31 (.62)	0.11 (.009)	661.6 (49.3)	3.40 (.24)	0.016 (.006)	0.0086 (.0018)	0.0013 (.0003)	0.0039 (.0002)
150	0.47 (.04)	0.001 (.0005)	8.62 (.87)	0.12 (.007)	674.8 (35.9)	3.49 (.25)	0.011 (.005)	0.0087 (.002)	0.0011 (.0004)	0.004 (.0002)
180	0.56 (.04)	0.002 (.0006)	13.53 (.30)	0.14 (.01)	841.3 (98.0)	3.67 (.42)	0.005 (.002)	0.0123 (.0001)	0.0013 (.00007)	0.0053 (.0004)

Trace Element Content of the Plasma of Lean Individuals after a 75g oral Glucose Challenge

Table 17 : Lean Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.76 (.06)	0.003 (.001)	0.99 (.09)	0.11 (.02)	1.43 (.21)	0.17 (.02)	0.014 (.006)	0.0032 0.0007)	0.0013 (.0004)	0.0012 (.0001)
30	0.81 (.04)	0.001 (.00007)	1.04 (.03)	0.12 (.03)	1.61 (.09)	0.14 (.01)	0.013 (.005)	0.0052 (.001)	0.0013 (.0003)	0.0012 (.0002)
60	0.80 (.04)	0.002 (.0003)	0.95 (.04)	0.13 (.02)	2.08 (.13)	0.14 (.01)	0.013 (.005)	0.0054 (.002)	0.0014 (.0004)	0.0011 (.00006)
90	0.78 (.06)	0.001 (.0001)	1.09 (.07)	0.12 (.03)	1.78 (.17)	0.18 (.01)	0.016 (.006)	0.0039 (.001)	0.0014 (.0004)	0.0012 (.00007)
120	0.76 (.04)	0.002 (.0004)	0.90 (.05)	0.11 (.02)	1.63 (.19)	0.16 (.02)	0.013 (.006)	0.0078 (.0036)	0.0014 (.0005)	0.0014 (.0001)
150	0.86 (.04)	0.003 (.001)	0.89 (.04)	0.11 (.02)	1.26 (.13)	0.15 (.009)	0.015 (.006)	0.0044 (.0007)	0.0015 (.0005)	0.0014 (.0002)
180	0.92 (.02)	0.002 (.0009)	1.07 (.09)	0.08 (.005)	1.21 (.12)	0.16 (.02)	0.007 (.001)	0.007 (.0006)	0.0008 (.0001)	0.0012 (.0001)

Table 18 : Lean Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.17 (.14)	0.0013 (.0003)	1.10 (.13)	0.13 (.03)	1.98 (.41)	0.24 (.04)	0.017 (.007)	0.0044 (.001)	0.0016 (.0002)	0.0015 (.0002)
30	1.19 (.23)	0.0023 (.0007)	1.26 (.12)	0.12 (.02)	1.54 (.23)	0.36 (.01)	0.015 (.006)	0.0035 (.0004)	0.0013 (.0002)	0.0012 (.0001)
60	1.04 (.15)	0.001 (.0001)	0.93 (.09)	0.12 (.02)	1.76 (.42)	0.22 (.04)	0.015 (.006)	0.0034 (.0004)	0.0014 (.0003)	0.0012 (.0001)
90	0.92 (.12)	0.0009 (.00009)	0.97 (.13)	0.11 (.02)	1.10 (.14)	0.19 (.03)	0.012 (.005)	0.0041 (.0007)	0.0010 (.0006)	0.0010 (.0008)
120	0.89 (.07)	0.001 (.0002)	0.89 (.05)	0.11 (.02)	1.17 (.19)	0.21 (.02)	0.012 (.004)	0.0046 (.0009)	0.0011 (.0003)	0.0010 (.0001)
150	1.09 (.17)	0.001 (.0001)	0.89 (.07)	0.12 (.02)	1.31 (.16)	0.20 (.02)	0.013 (.005)	0.0038 (.0005)	0.0012 (.0002)	0.0012 (.0001)
180	1.24 (.13)	0.002 (.0005)	0.99 (.05)	0.07 (.01)	1.30 (.20)	0.14 (.04)	0.002 (.0005)	0.0048 (.0003)	0.0005 (.0002)	0.0013 (.0006)

Figure 19(a)

Plasma Glucose(—x—) and Insulin(—△—) Concentrations (+/-S.D.X)

of Lean Controls During an Oral Glucose Tolerance Test.

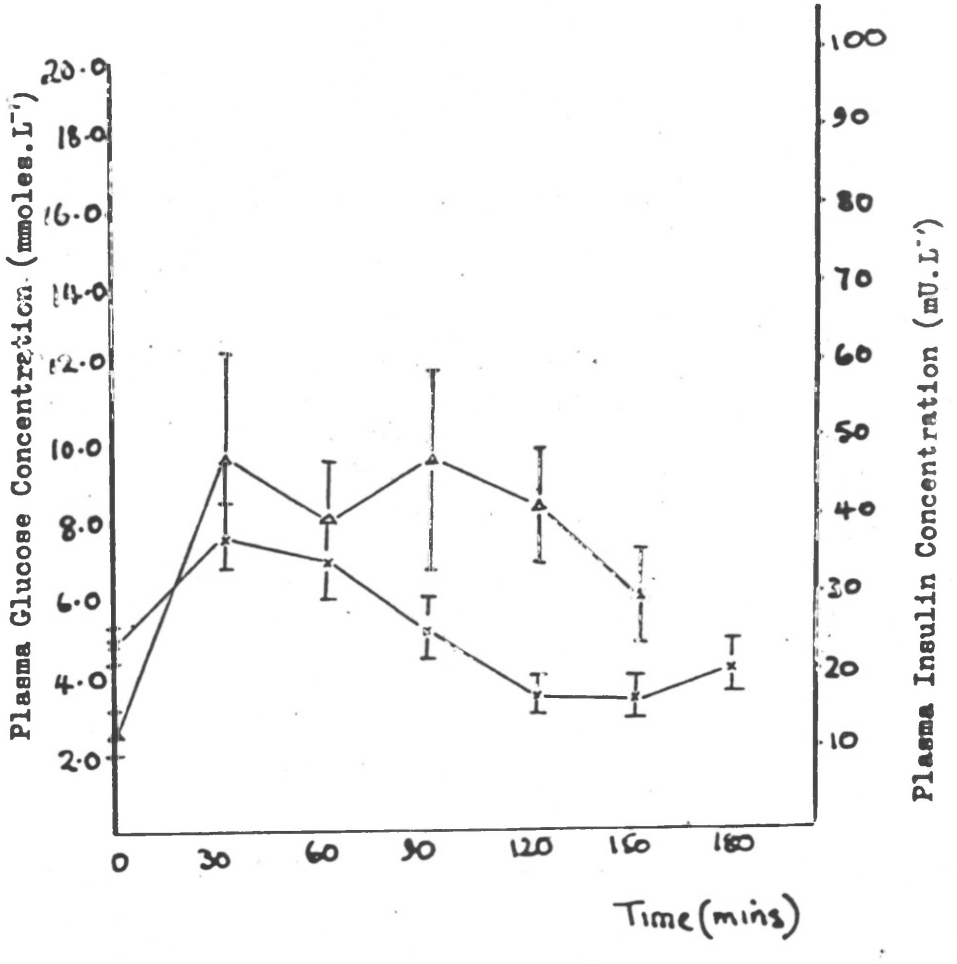


Figure 15
Trace Element Concentration in Erythrocytes of Lean Controls

Figure 19(a) During an Oral Glucose Tolerance Test

Plasma Glucose (—x—) and Insulin (—Δ—) Concentrations (+/-S.D.X)

of Lean Controls During an Oral Glucose Tolerance Test.

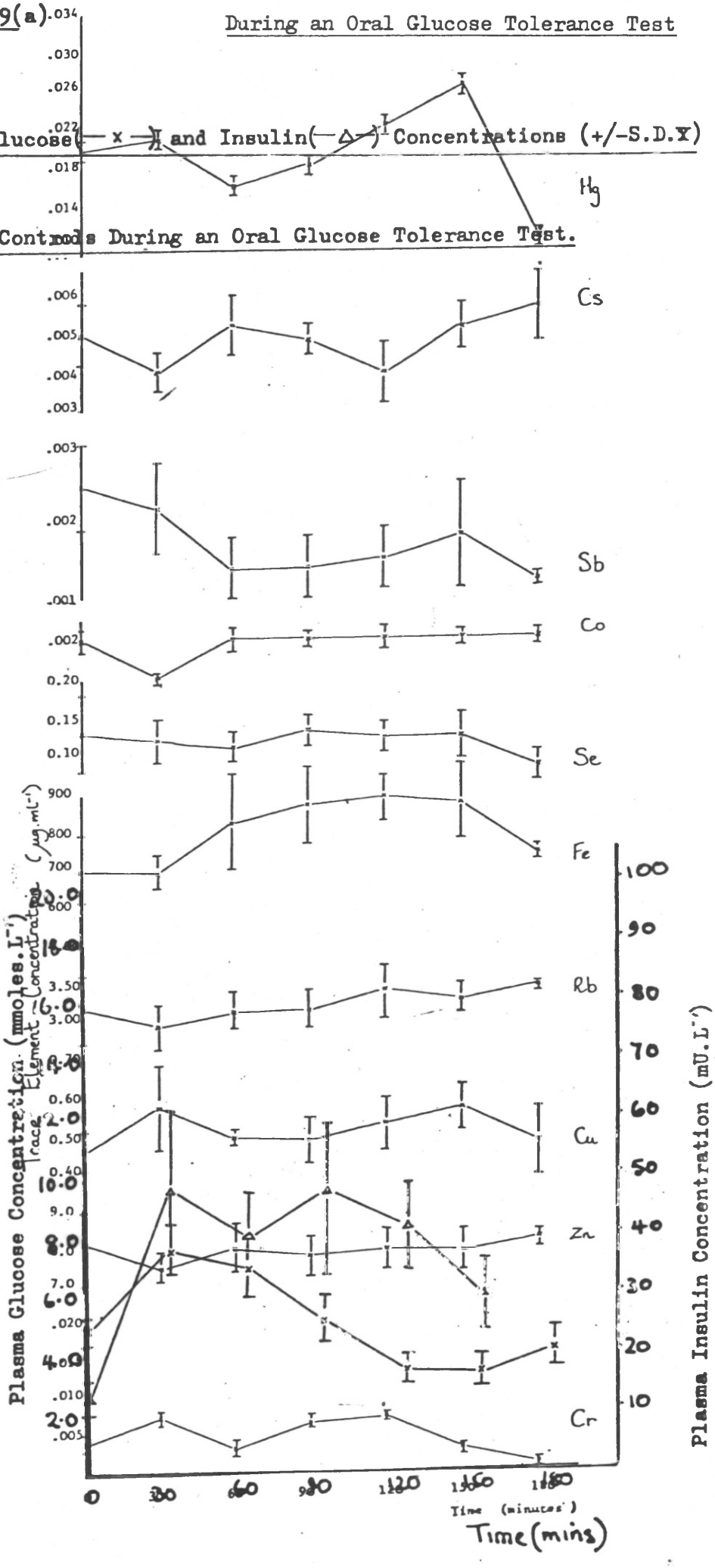


Figure 15
Trace Element Concentration in Erythrocytes of Lean Controls

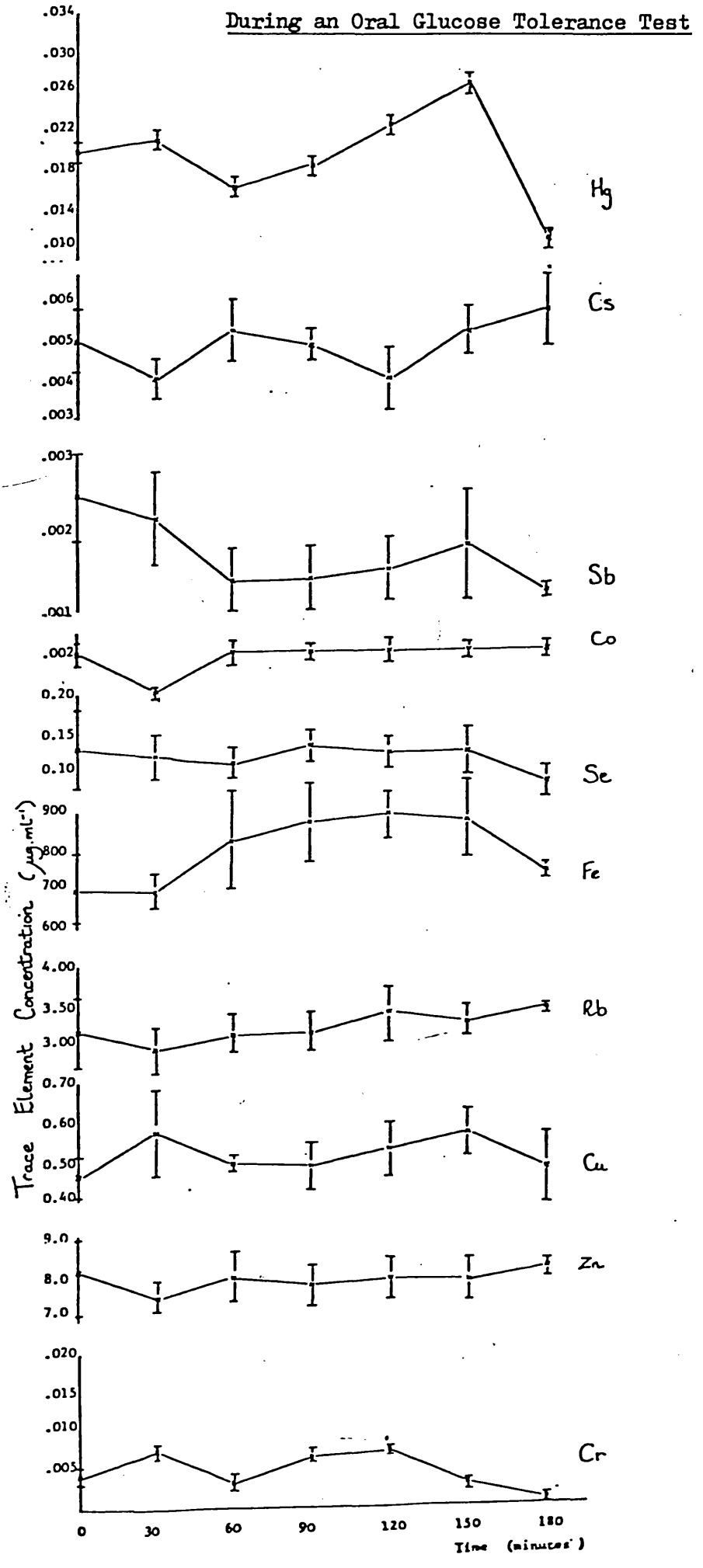


Figure 19(b)

Plasma Glucose (— x —) and Insulin(—△—) Concentrations(+/-S.D.$\bar{x}$)

of Lean Diabetics During an Oral Glucose Tolerance Test.

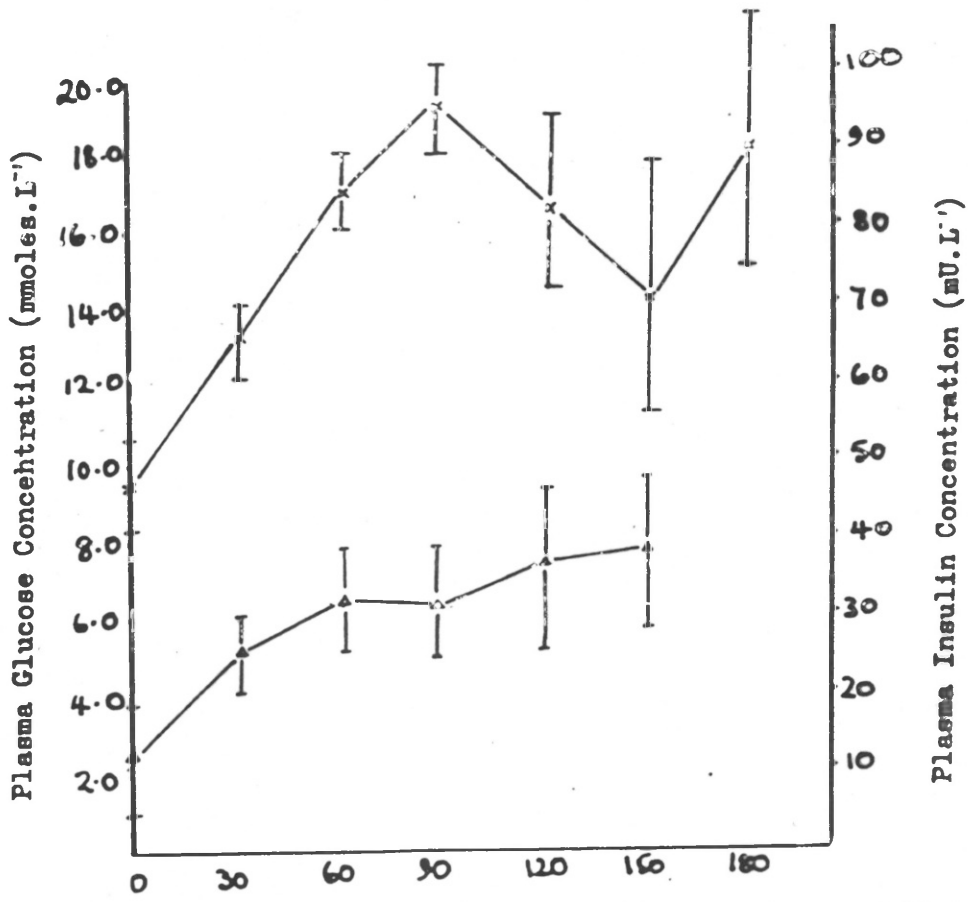


Figure 16

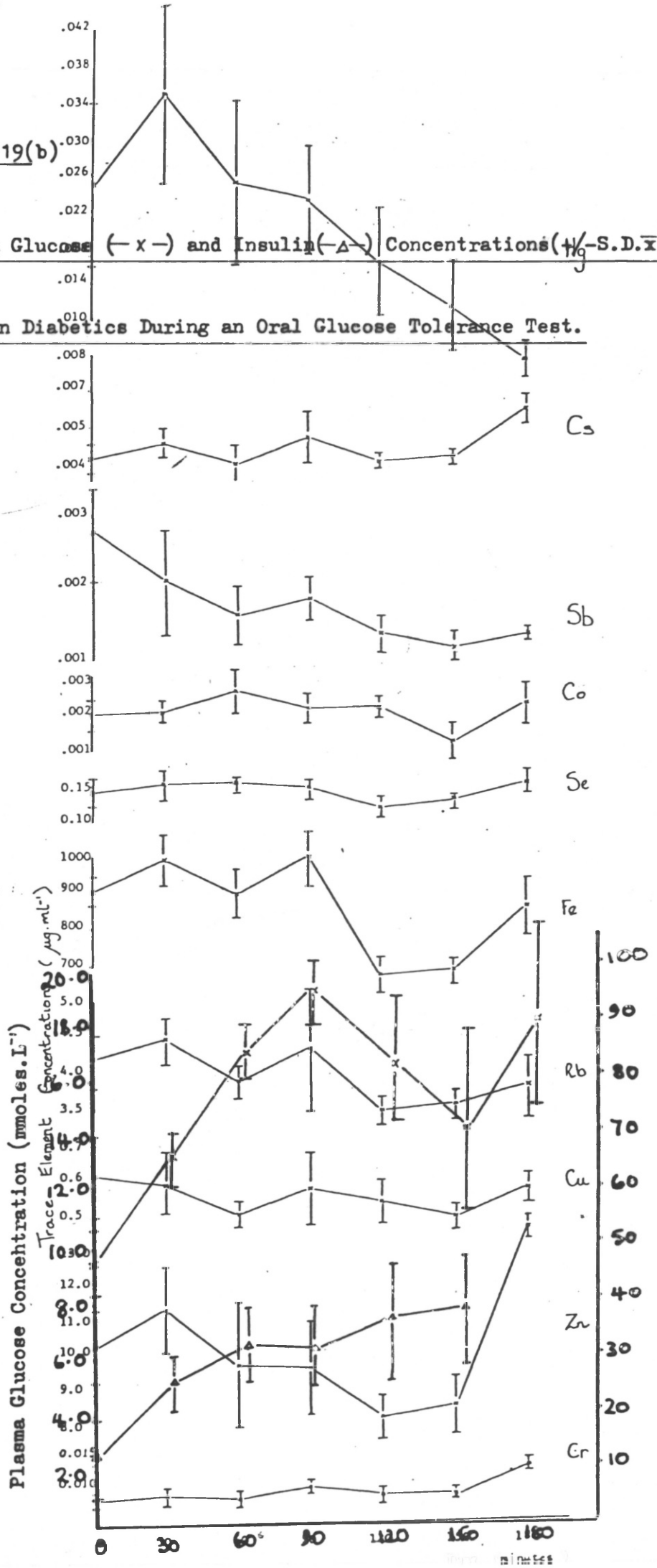
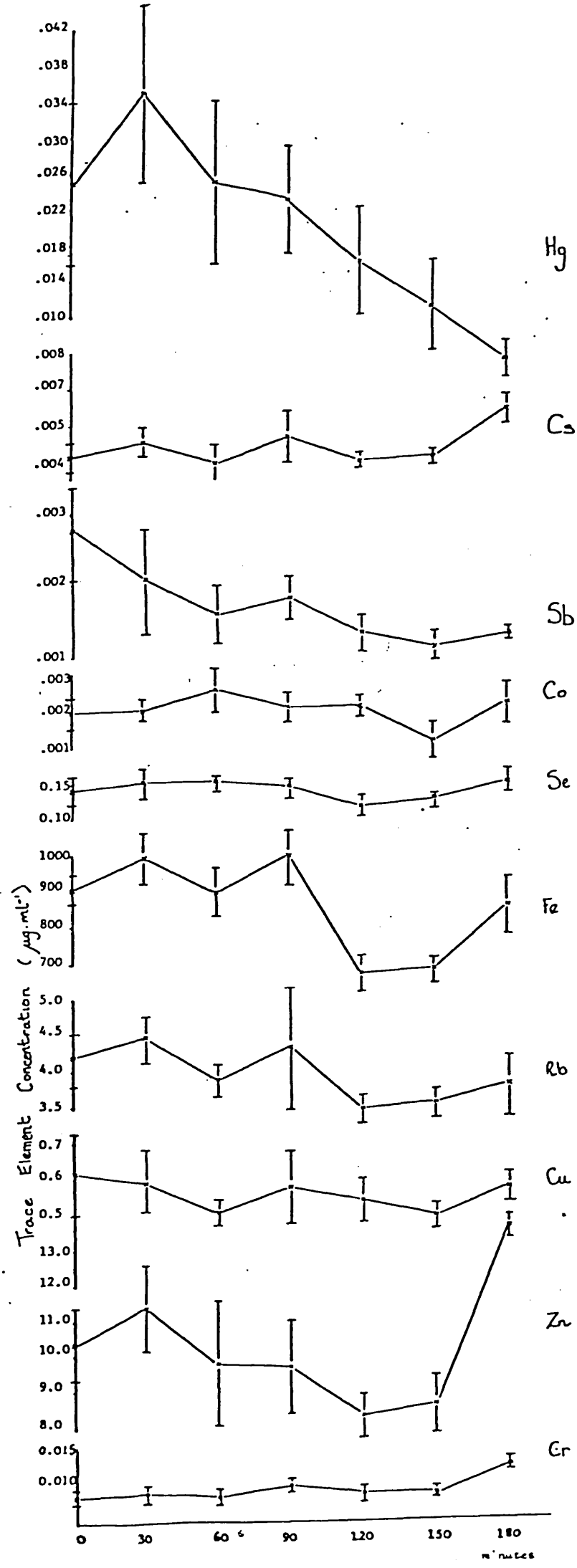


Figure 19(b)

Plasma Glucose (— x —) and Insulin (— Δ —) Concentrations (±S.D.$\bar{x}$) of Lean Diabetics During an Oral Glucose Tolerance Test.

Plasma Insulin Concentration in Erythrocytes of Lean Diabetics During an Oral Glucose Tolerance Test

Figure 16



Trace Element Concentration in Erythrocytes of Lean Diabetics During an Oral Glucose Tolerance Test

Figure 19(a)

Plasma Glucose(— x —) and Insulin(—△—) Concentrations (+/-S.D.x)

of Lean Controls During an Oral Glucose Tolerance Test.

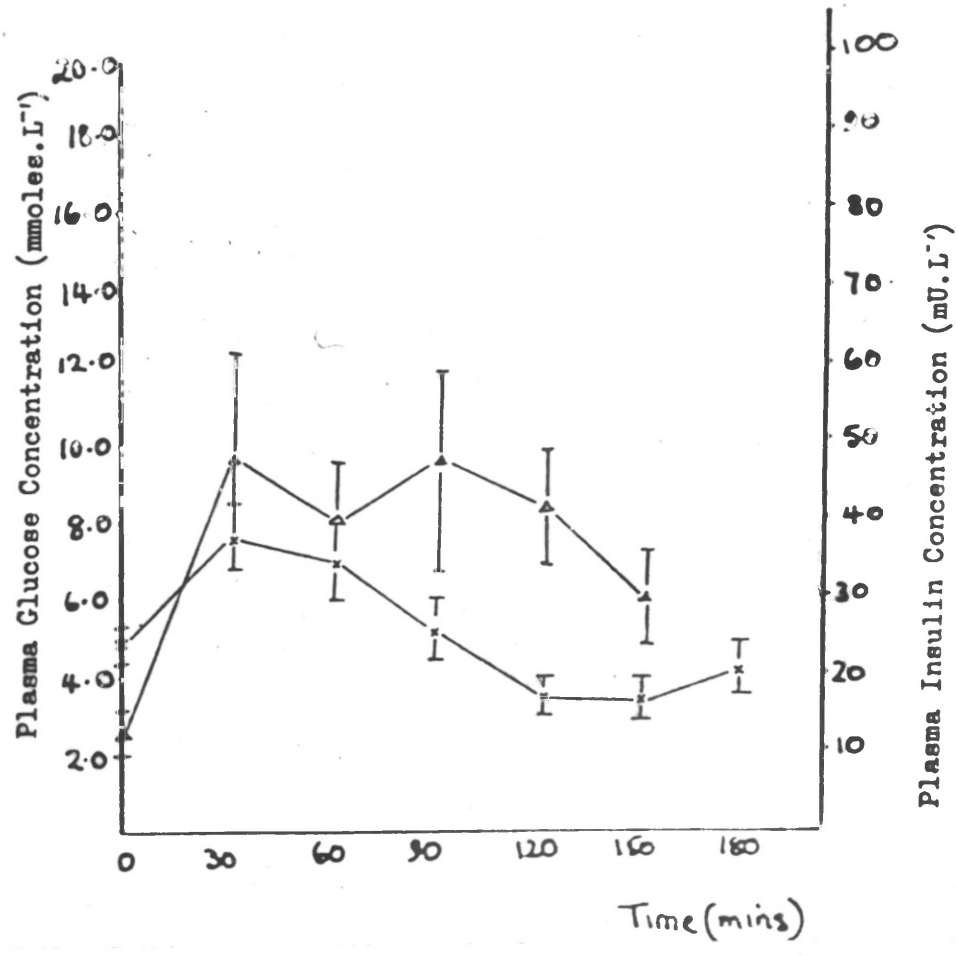


Figure 19

Trace Element Concentration in Plasma of Lean Controls During an Oral Glucose Tolerance Test

Figure 19(a)

Plasma Glucose (— x —) and Insulin (— Δ —) Concentrations (+/- S.D.x) of Lean Controls During an Oral Glucose Tolerance Test.

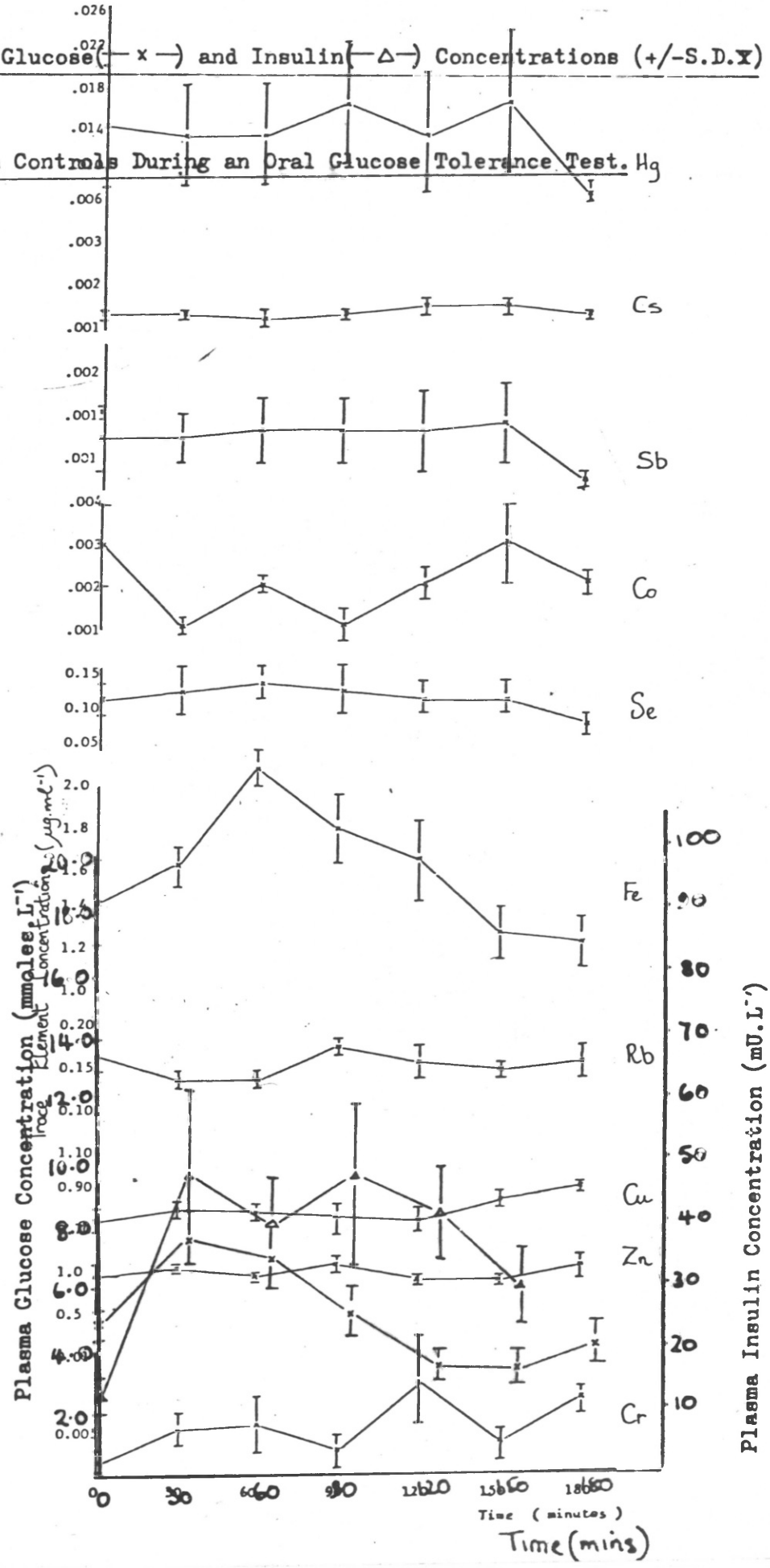


Figure 14

Trace Element Concentration in Plasma of Lean Controls During an Oral Glucose Tolerance Test

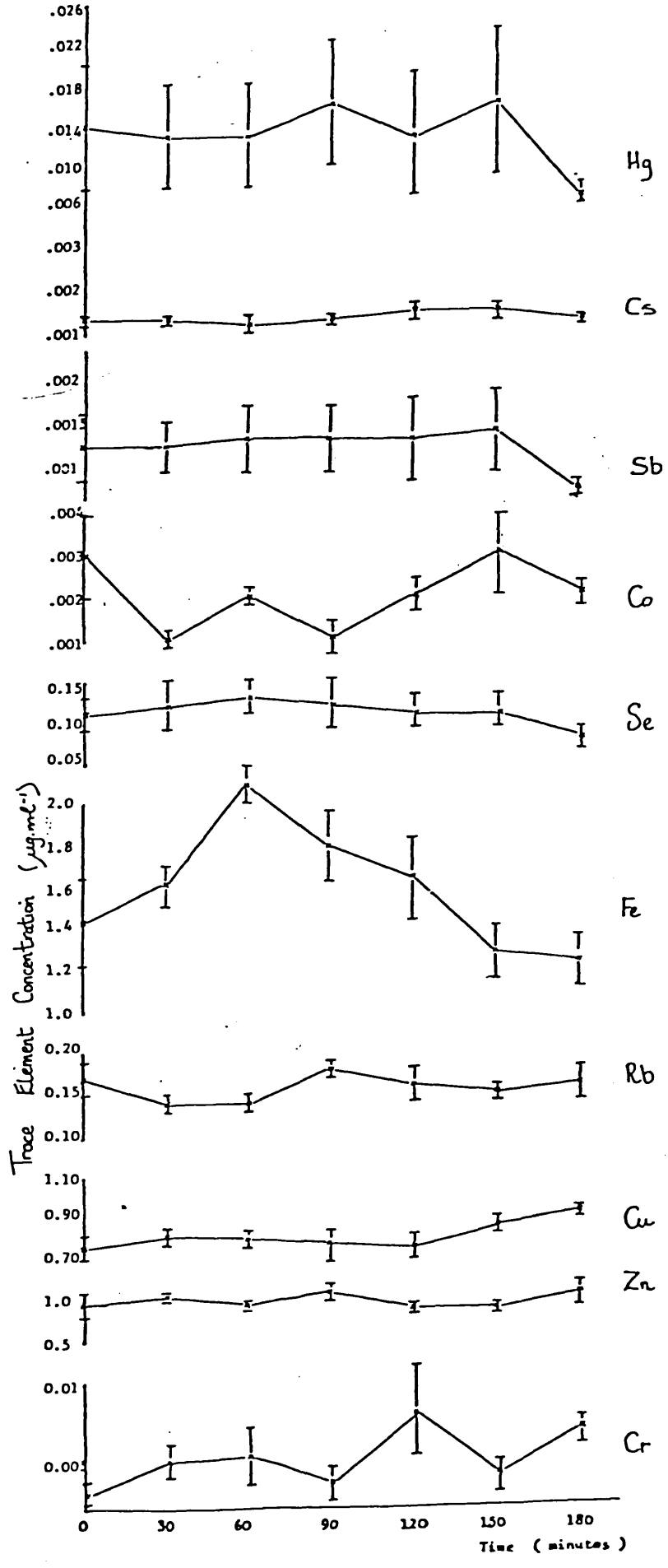


Figure 19(b)

Plasma Glucose (— x —) and Insulin(—Δ—) Concentrations(+/-S.D.$\bar{x}$)

of Lean Diabetics During an Oral Glucose Tolerance Test.

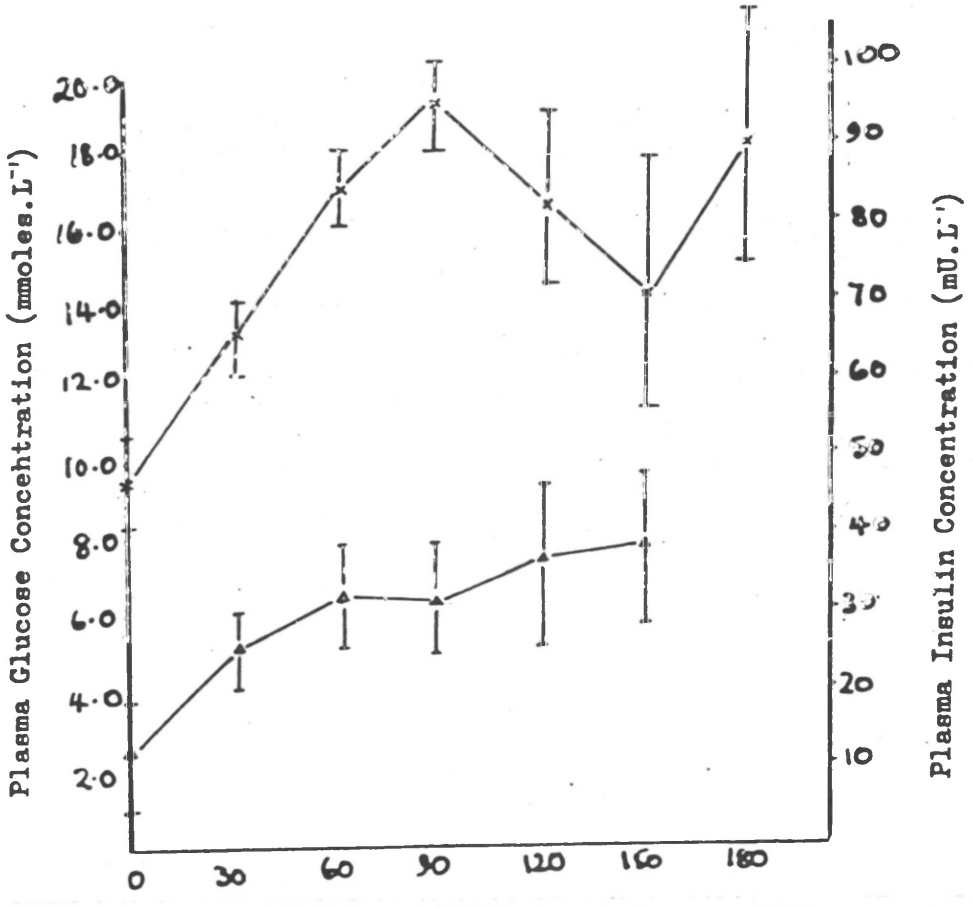


Figure 18

Trace Element Concentration in Plasma of Lean Diabetics During an Oral Glucose Tolerance Test

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Figure 19(b)

Plasma Glucose (— x —) and Insulin(—Δ—) Concentrations(+/-S.D.$\bar{x}$) of Lean Diabetics During an Oral Glucose Tolerance Test.

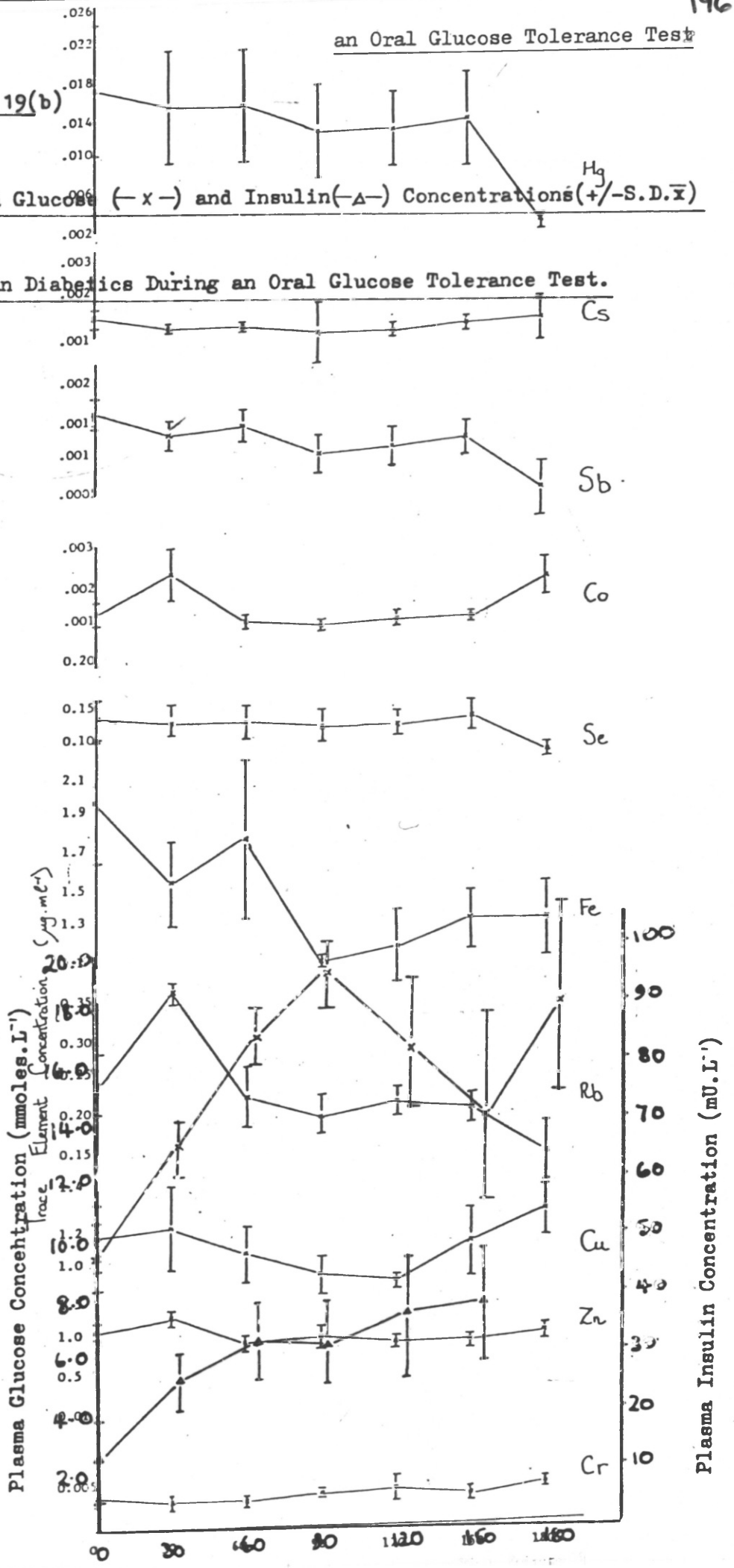
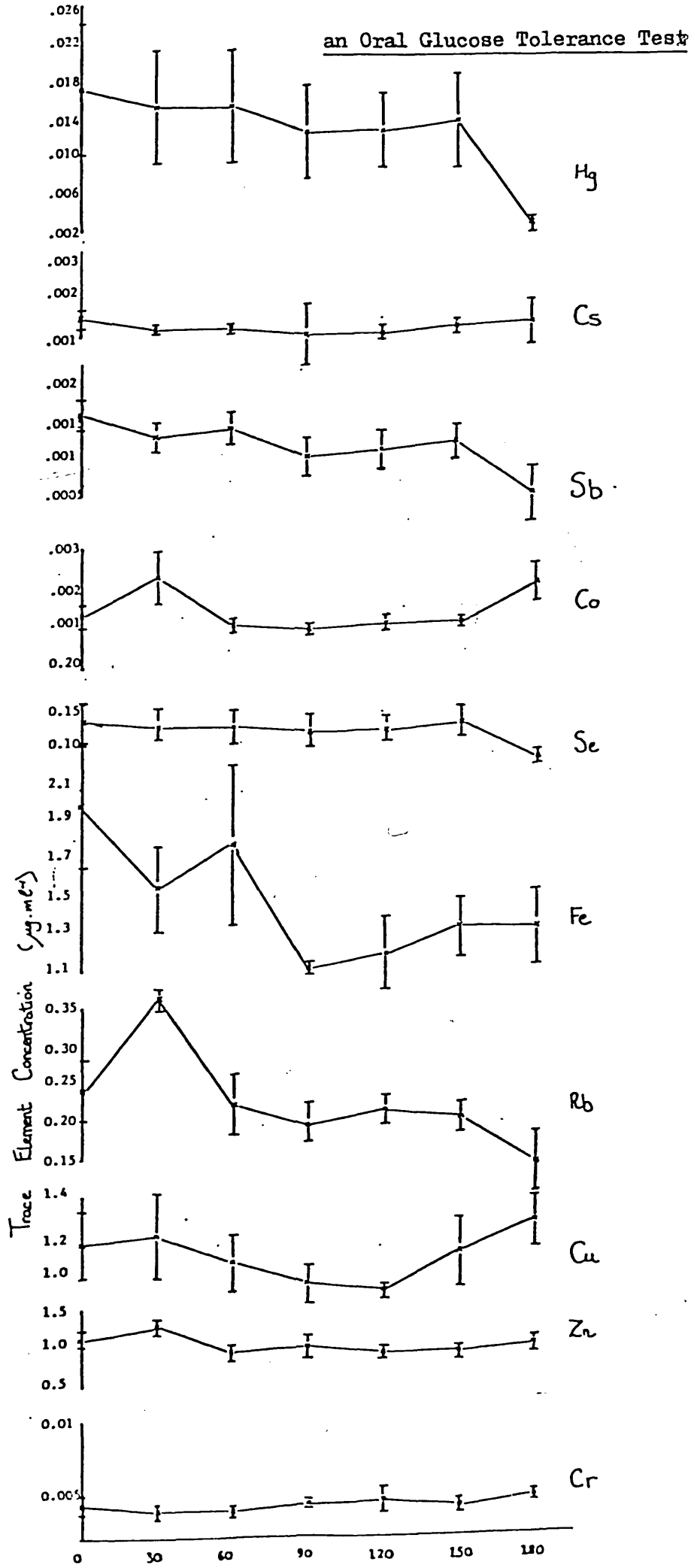


Figure 18

Trace Element Concentration in Plasma of Lean Diabetics During an Oral Glucose Tolerance Test



Plasma Glucose Concentration ($mmol.L^{-1}$) of Lean Controls

Table 19 (a)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	4.7	6.9	8.7	5.4	2.4	2.2	N.D.
2	4.8	6.9	5.5	5.8	4.4	3.9	N.D.
3	3.8	6.4	3.2	3.5	3.5	4.4	N.D.
4	4.7	7.4	6.5	5.3	5.2	2.7	3.7
5	5.9	10.9	10.8	7.1	2.7	4.2	4.6
$\bar{x}$	4.8	7.7	6.9	5.4	3.6	3.5	4.2
$SD\bar{x}$	0.3	0.8	1.3	0.6	0.5	0.4	0.5

Plasma Glucose Concentration ($mmol.L^{-1}$) of Lean Diabetics

Table 19 (b)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	8.5	11.2	14.7	19.2	18.8	19.7	N.D.
2	10.9	15.1	17.9	19.8	15.9	16.3	N.D.
3	7.5	12.4	14.7	15.0	7.9	3.1	N.D.
4	13.5	16.0	21.2	23.1	23.0	22.1	21.3
5	7.1	10.3	16.1	18.8	17.7	11.2	15.3
$\bar{x}$	9.5	13.0	16.9	19.2	16.7	14.5	18.3
$SD\bar{x}$	1.2	1.1	1.2	1.3	2.5	3.4	3.3

Plasma Insulin Concentration ($mU.L^{-1}$) of Lean Controls

Table 20 (a)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	14.0	30.0	65.0	23.0	40.0	18.0	N.D.
2	N.D.	82.0	48.0	58.0	68.0	36.0	N.D.
3	6.0	23.0	31.0	10.0	46.0	51.0	N.D.
4	14.0	82.0	24.0	66.0	30.0	32.0	22.0
5	18.0	20.0	36.0	72.0	12.0	2.0	1.0
$\bar{x}$	13.0	47.4	40.8	45.8	39.2	27.8	N.D.
$SD\bar{x}$	2.5	14.2	7.2	12.3	9.2	8.3	N.D.

Plasma Insulin Concentration ($mU.L^{-1}$) of Lean Diabetics

Table 20 (b)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	12.0	10.0	49.0	30.0	31.0	3.0	N.D.
2	19.0	19.0	29.0	30.0	29.0	29.0	N.D.
3	24.0	27.0	28.0	20.0	16.0	48.0	N.D.
4	49.0	33.0	36.0	28.0	24.0	38.0	9.0
5	12.0	34.0	22.0	52.0	74.0	60.0	70.0
$\bar{x}$	23.0	24.6	32.8	32.0	34.8	35.6	N.D.
$SD\bar{x}$	6.8	4.5	4.6	5.3	10.1	9.6	N.D.

Trace Element Excretion of Lean Individuals before and after a 75g oral Glucose Load

Table 21 (a) : Lean Controls

Sample	Mean Elemental Concentration (μg.mmol Creat^{-1}) (SD$\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 5)	0.005 (.001)	0.14 (.03)	87.6 (18.6)	3.53 (.47)	121.1 (26.8)	214.4 (30.9)	0.60 (.19)	0.61 (.19)	0.14 (.06)	0.68 (.08)
U2 (n = 5)	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Table 21 (b) : Lean Diabetics

Sample	Mean Elemental Concentration (μg.mmol Creat^{-1}) (SD$\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 5)	0.004 (.001)	0.13 (.05)	88.9 (13.4)	2.80 (1.16)	126.7 (75.1)	176.6 (47.3)	0.39 (.37)	0.45 (.13)	0.14 (.07)	0.61 (.28)
U2 (n = 5)	0.003 (.001)	0.16 (.09)	74.4 (2.50)	1.82 (.71)	55.8 (1.94)	155.5 (66.6)	0.03 (.006)	0.59 (.15)	0.07 (.04)	0.37 (.22)

U1 - Urine collected 24 hours prior to the glucose tolerance test.

U2 - Urine collected 24 hours after the glucose tolerance test.

N.D. - Not determined

Trace Element Content of the Erythrocytes of Obese Individuals after a 75g oral Glucose Challenge

Table 22 : Obese Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.53 (.04)	0.002 (.0005)	9.74 (.86)	0.15 (.01)	743.2 (40.4)	3.21 (.29)	0.015 (.005)	0.020 (.003)	0.0019 (.0004)	0.0064 (.001)
30	0.48 (.04)	0.003 (.0004)	9.91 (.49)	0.14 (.009)	754.5 (17.0)	3.28 (.16)	0.009 (.002)	0.021 (.003)	0.0018 (.0004)	0.0067 (.001)
60	0.44 (.03)	0.004 (.001)	10.38 (.56)	0.14 (.01)	786.1 (24.3)	3.44 (.25)	0.017 (.004)	0.023 (.003)	0.0019 (.0006)	0.0063 (.0009)
90	0.50 (.06)	0.003 (.0007)	10.33 (.65)	0.15 (.01)	784.9 (31.9)	3.72 (.30)	0.013 (.003)	0.023 (.004)	0.0027 (.0009)	0.0071 (.001)
120	0.47 (.02)	0.003 (.0007)	10.31 (.30)	0.16 (.01)	775.8 (11.0)	3.85 (.27)	0.015 (.003)	0.018 (.003)	0.0014 (.0005)	0.0060 (.0008)
150	0.41 (.02)	0.005 (.002	11.33 (.71)	0.18 (.03)	883.0 (12.5)	3.91 (.38)	0.008 (.001)	0.0146 (.0002)	0.0028 (.0006)	0.0083 (.001)
180	0.51 (.10)	0.003 (.0008)	9.84 (.30)	0.15 (.03)	778.9 (3.13)	3.46 (.26)	0.003 (.0001)	0.0095 (.001)	0.0015 (.00003)	0.0079 (.001)

Table 23 : Obese Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.54 (.08)	0.0015 (.0002)	10.71 (.58)	0.15 (.005)	861.0 (59.2)	3.36 (.16)	0.01 (.003)	0.012 (.003)	0.0017 (.0006)	0.0048 (.0003)
30	0.54 (.08)	0.0022 (.0003)	10.67 (.39)	0.15 (.008)	869.2 (60.6)	3.21 (.29)	0.01 (.003)	0.011 (.002)	0.0018 (.0005)	0.0055 (.0007)
60	0.53 (.04)	0.003 (.0007)	10.82 (.51)	0.15 (.005)	791.3 (25.3)	3.05 (.31)	0.0097 (.003)	0.013 (.003)	0.0015 (.0003)	0.0056 (.0005)
90	0.52 (.04)	0.004 (.0004)	10.81 (.30)	0.15 (.009)	863.9 (71.7)	2.91 (.29)	0.008 (.002)	0.013 (.003)	0.002 (.0004)	0.0055 (.0006)
120	0.52 (.05)	0.002 (.0008)	9.46 (.38)	0.14 (.008)	790.4 (61.8)	2.91 (.14)	0.007 (.002)	0.010 (.001)	0.0023 (.0007)	0.0051 (.0008)
150	0.45 (.04)	0.001 (.0003)	9.30 (.67)	0.13 (.007)	689.8 (50.8)	2.84 (.14)	0.003 (.0007)	0.0078 (.001)	0.0028 (.0012)	0.0013 (.0021)
180	0.55 (.02)	0.008 (.006)	11.05 (1.08)	0.15 (.008)	819.5 (78.9)	3.48 (.37)	0.004 (.0023)	0.0082 (.001)	0.0012 (.00006)	0.0049 (.0003)

Trace Element Content of the Plasma of Obese Individuals after a 75g oral Glucose Challenge

Table 24 : Obese Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.15 (.09)	0.001 (.0004)	1.16 (.07)	0.12 (.02)	1.31 (.15)	0.14 (.01)	0.007 (.002)	0.009 (.002)	0.0011 (.0002)	0.0013 (.0001)
30	1.12 (.09)	0.002 (.0005)	1.25 (.10)	0.11 (.007)	1.33 (.11)	0.13 (.007)	0.008 (.001)	0.0096 (.001)	0.0014 (.0004)	0.0013 (.0001)
60	1.08 (.08)	0.002 (.0005)	1.13 (.08)	0.11 (.008)	1.41 (.13)	0.15 (.01)	0.006 (.001)	0.0095 (.002)	0.0009 (.0002)	0.0013 (.0001)
90	1.17 (.08)	0.002 (.0006)	1.14 (.08)	0.11 (.009)	1.55 (.16)	0.13 (.01)	0.007 (.001)	0.011 (.002)	0.0014 (.0005)	0.0014 (.0001)
120	1.09 (.09)	0.001 (.0006)	1.08 (.07)	0.13 (.009)	1.17 (.06)	0.14 (.008)	0.008 (.001)	0.0067 (.001)	0.0009 (.0002)	0.0015 (.0001)
150	1.02 (.07)	0.001 (.0007)	0.89 (.09)	0.13 (.0008)	1.29 (.03)	0.15 (.03)	0.001 (.0007)	0.0037 (.001)	0.001 (0)	0.0012 (.0007)
180	1.22 (.10)	0.001 (.0003)	1.11 (.29)	0.08 (.01)	1.43 (.23)	0.12 (.01)	0.001 (.0002)	0.0034 (.001)	0.0011 (.0007)	0.0011 (.0001)

Table 25 : Obese Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.27 (.14)	0.001 (.0003)	1.27 0.10)	0.12 (.006)	1.47 (.17)	0.14 (.01)	0.007 (.002)	0.0043 (.0009)	0.0018 (.0005)	0.0012 (.0002)
30	1.25 (.08)	0.002 (.0003)	1.21 (.06)	0.12 (.005)	1.42 (.11)	0.18 (.02)	0.006 (.002)	0.0047 (.00008)	0.0016 (.0005)	0.0009 (.00008)
60	1.19 (.09)	0.001 (.0002)	1.04 (.09)	0.12 (.007)	1.54 (.13)	0.13 (.007)	0.005 (.001)	0.0041 (.0004)	0.0016 (.0004)	0.0013 (.0002)
90	1.20 (.11)	0.002 (.0002)	0.98 (.12)	0.12 (.008)	1.44 (.15)	0.13 (.01)	0.005 (.001)	0.0041 (.001)	0.0017 (.0005)	0.0013 (.0002)
120	1.11 (.12)	0.0018 (.0004)	0.98 (.15)	0.11 (.006)	1.28 (.15)	0.15 (.01)	0.006 (.002)	0.0046 (.0005)	0.0017 (.0005)	0.0012 (.0001)
150	1.14 (.14)	0.0012 (.00005)	1.26 (.11)	0.11 (.003)	1.27 (.03)	0.14 (.01)	0.002 (.0002)	0.0038 (.0005)	0.0028 (.001)	0.0013 (.00009)
180	1.10 (.10)	0.0014 (.00004)	1.26 (.08)	0.11 (.005)	1.25 (.09)	0.12 (.005)	0.002 (.0006)	0.0037 (.0005)	0.0011 (.00009)	0.0011 (.00008)

Figure 26(a)

Plasma Glucose(—x—) and Insulin(—△—) Concentrations (+/-S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test.

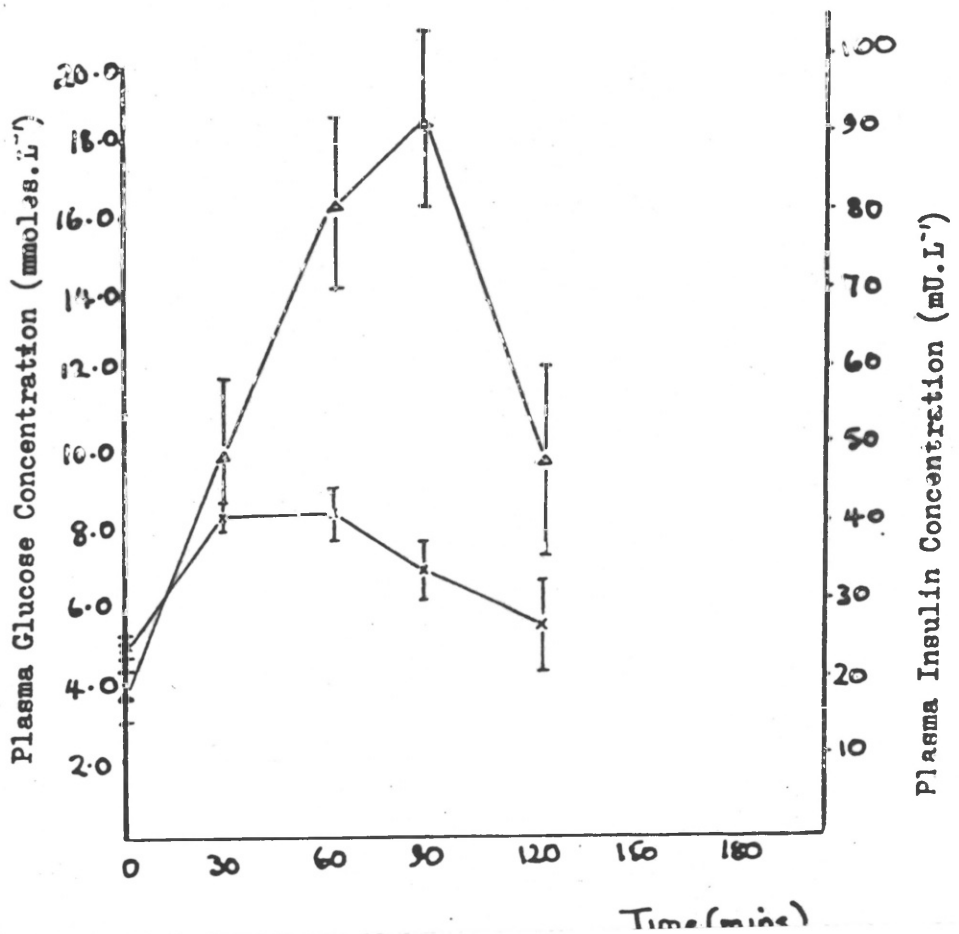


Figure 22

Trace Element Concentration in Erythrocytes of Obese Controls During an Oral Glucose Tolerance Test

Figure 26(a)

Plasma Glucose (— x —) and Insulin (— Δ —) Concentrations (±1/2 S.D.x̄) of Obese Controls During an Oral Glucose Tolerance Test.

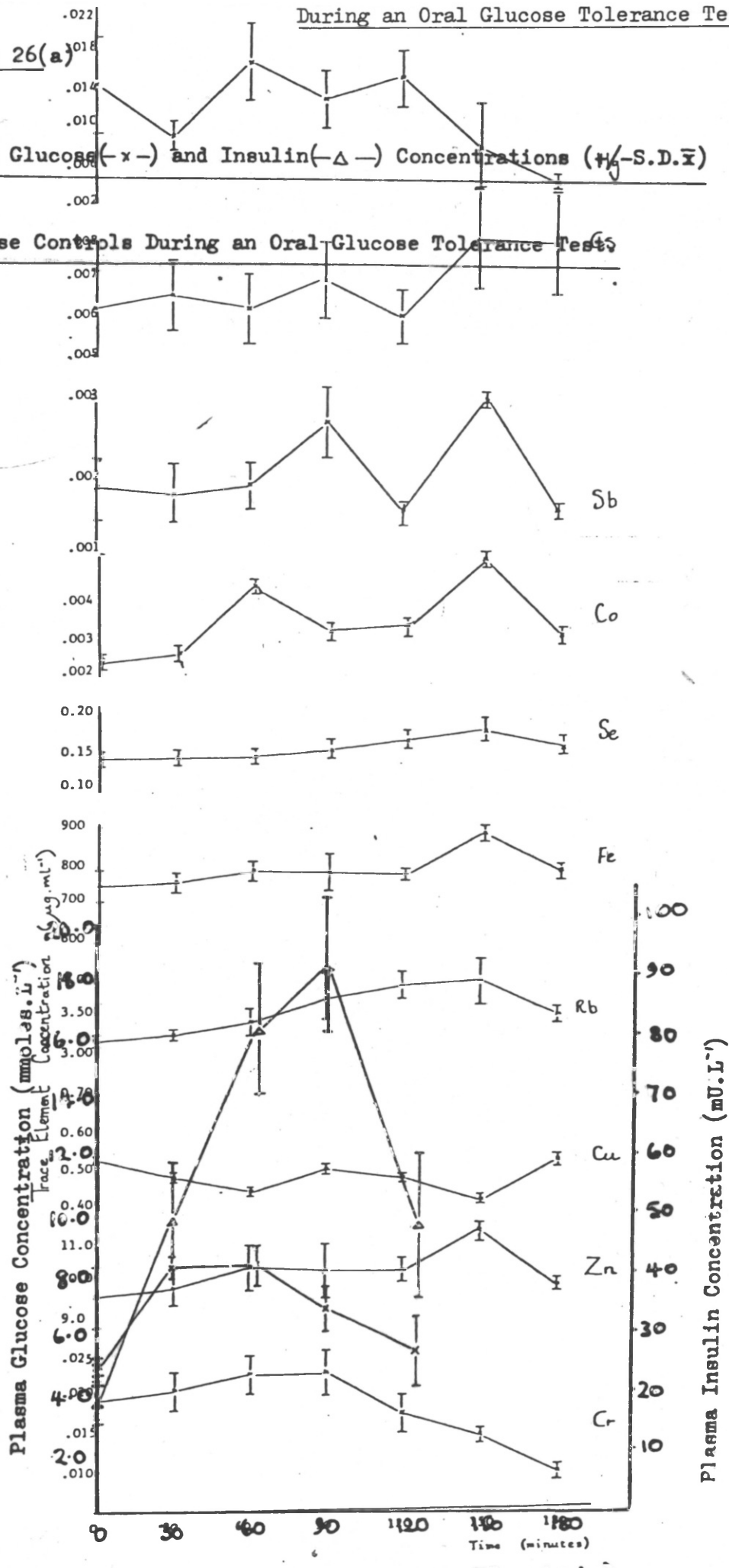


Figure 22

Trace Element Concentration in Erythrocytes of Obese Controls

During an Oral Glucose Tolerance Test

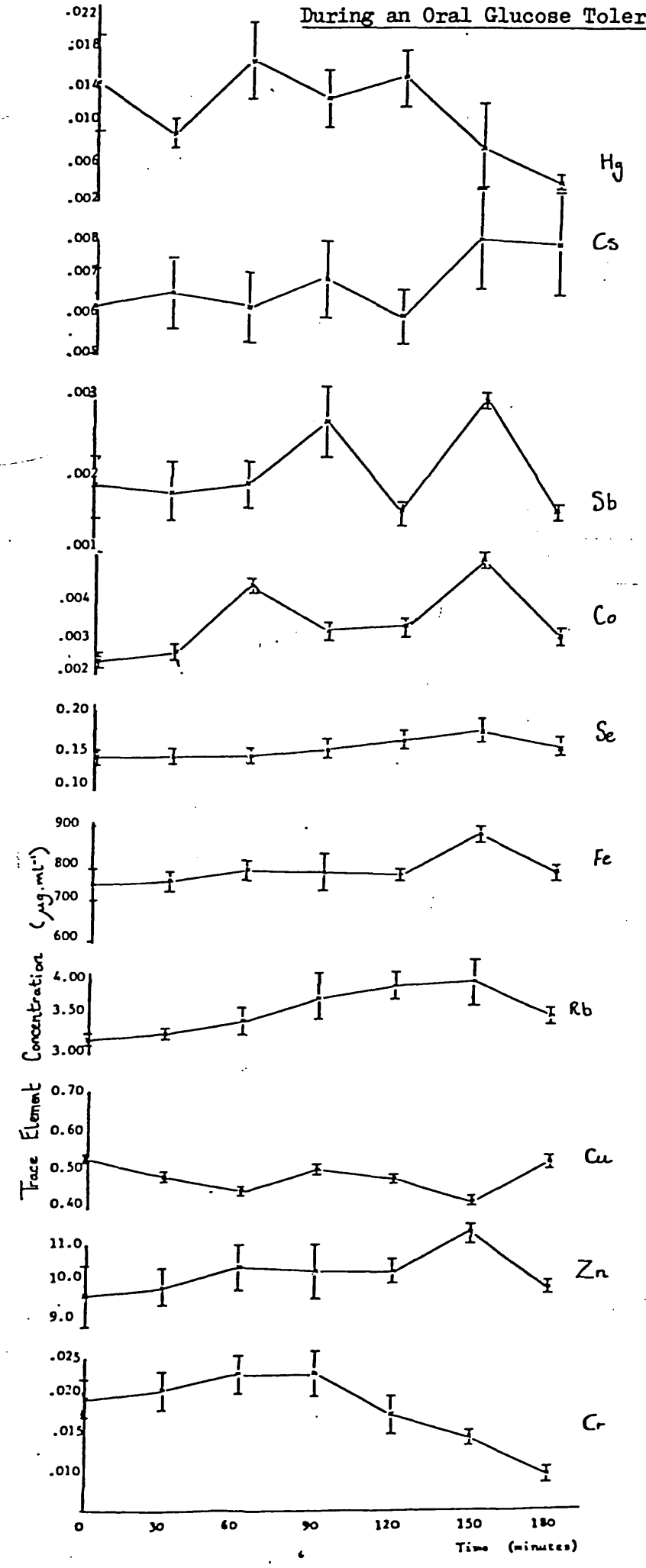


Figure 26(b)

Plasma Glucose (—x—) and Insulin(—Δ—) concentrations (+/-S.D.$\bar{x}$)

of Obese Diabetics During an Oral Glucose Tolerance test.

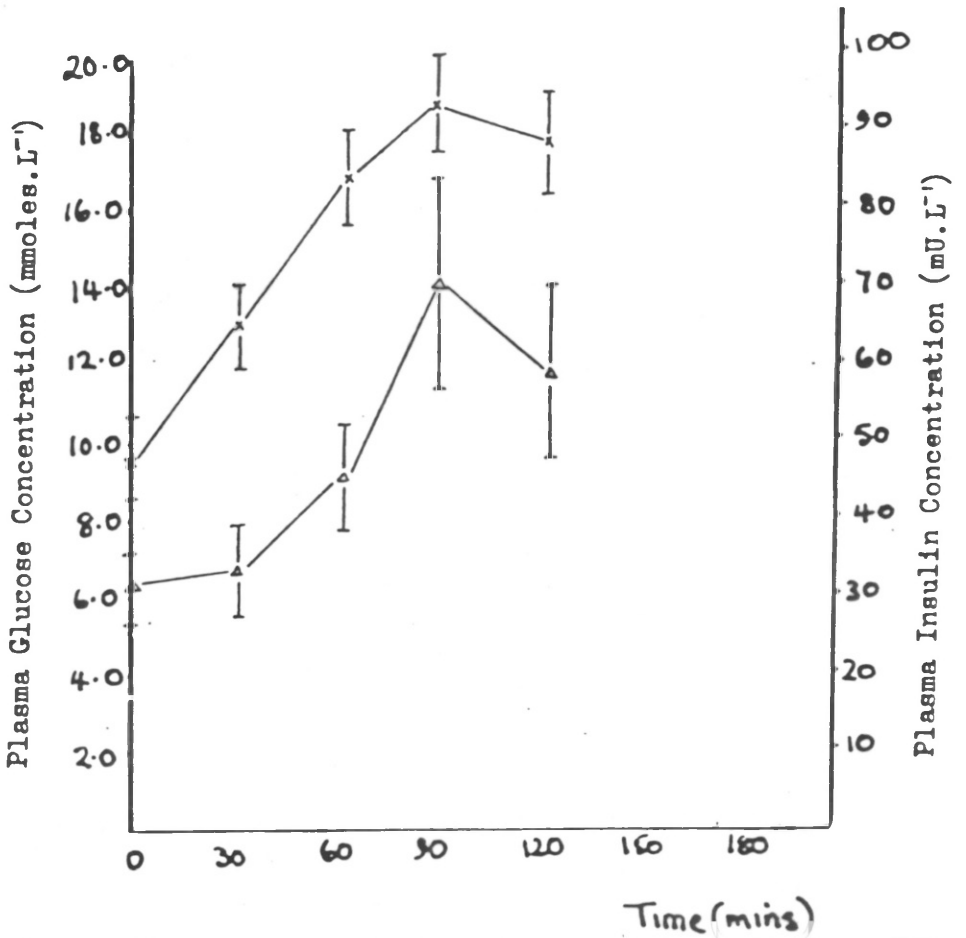


Figure 23

Trace Element Concentration in Erythrocytes of Obese Diabetics

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During an Oral Glucose Tolerance Test

Figure 26(b)

Plasma Glucose (—x—) and Insulin(—Δ—) concentrations (+/-S.D.$\bar{x}$)

of Obese Diabetics During an Oral Glucose Tolerance test.

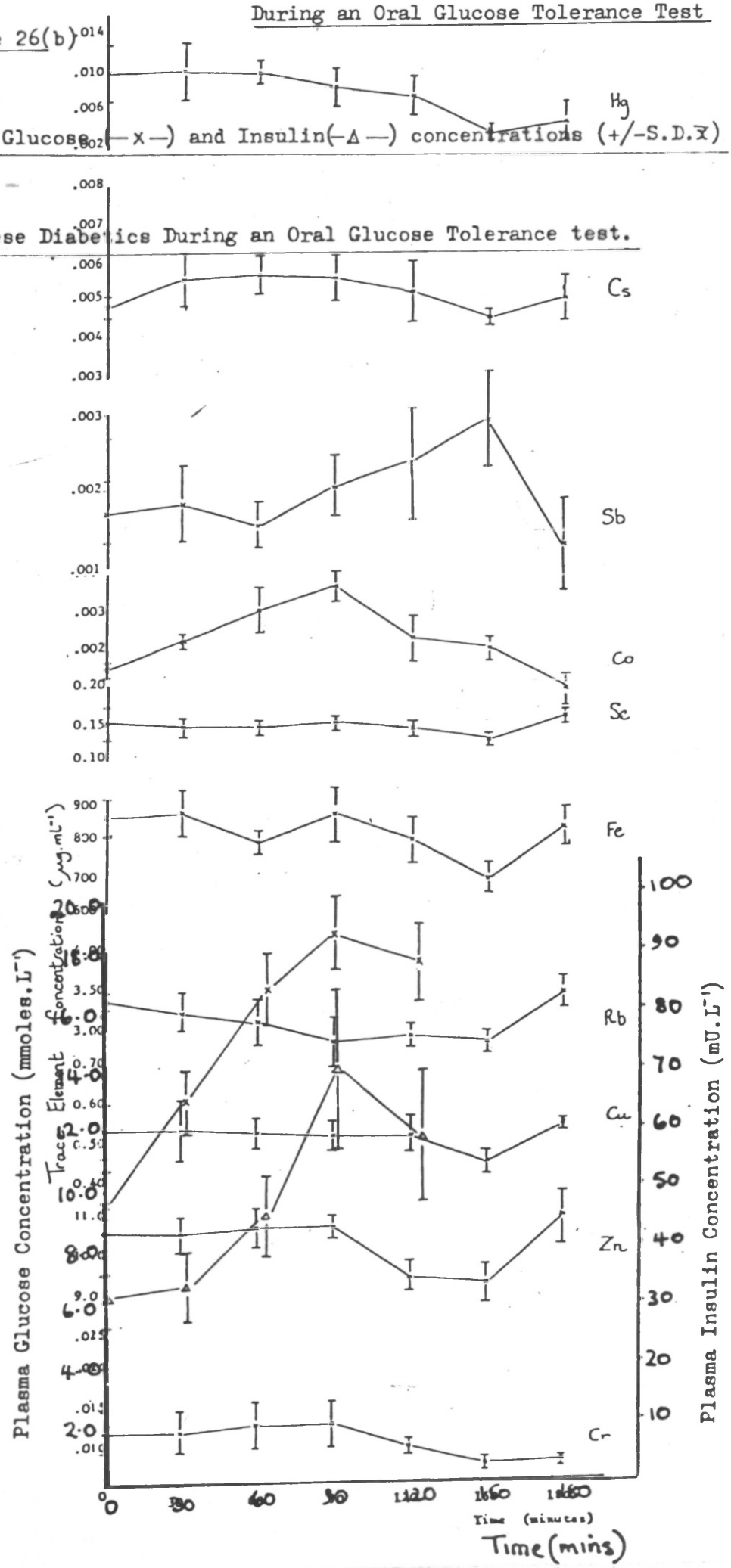


Figure 23

Trace Element Concentration in Erythrocytes of Obese Diabetics During an Oral Glucose Tolerance Test

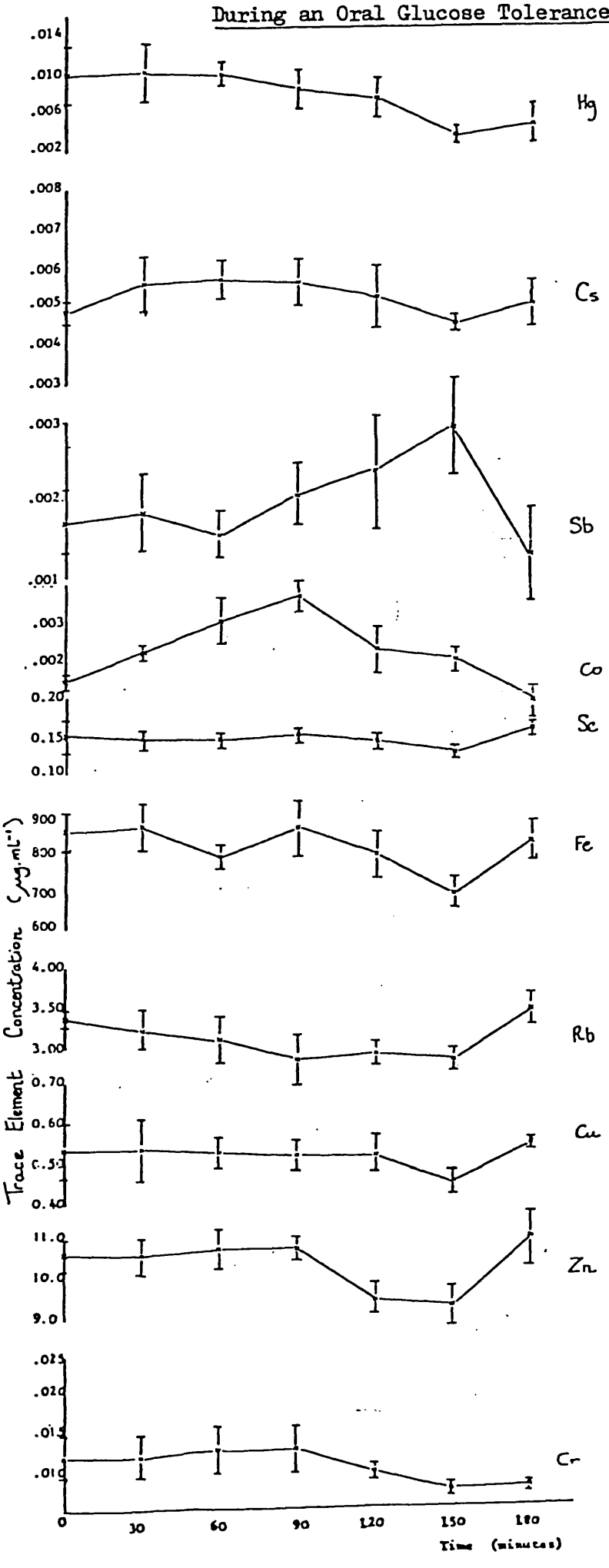


Figure 26(a)

Plasma Glucose(–x–) and Insulin(–△–) Concentrations (+/-S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test.

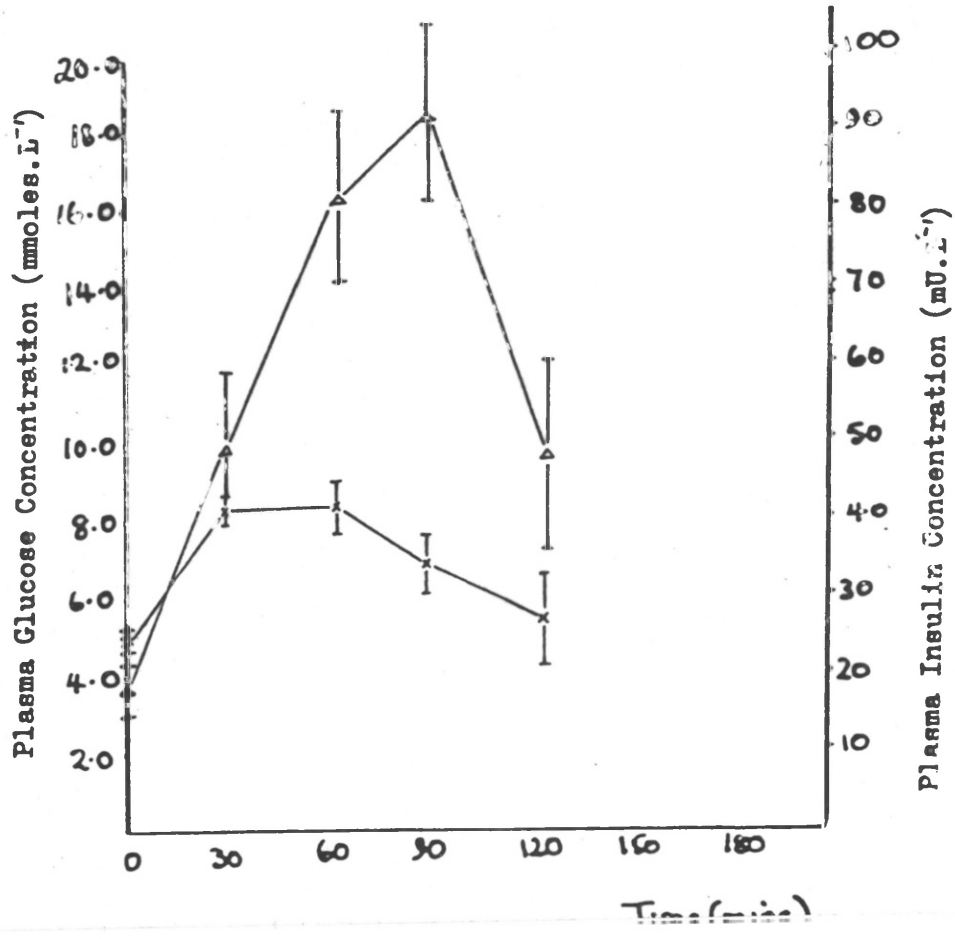


Figure 24

Trace Element Concentration in Plasma of Obese Diabetics During an Oral Glucose Tolerance Test

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Figure 23(a)

Plasma Glucose(-x-) and Insulin(-Δ-) Concentrations (±S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test.

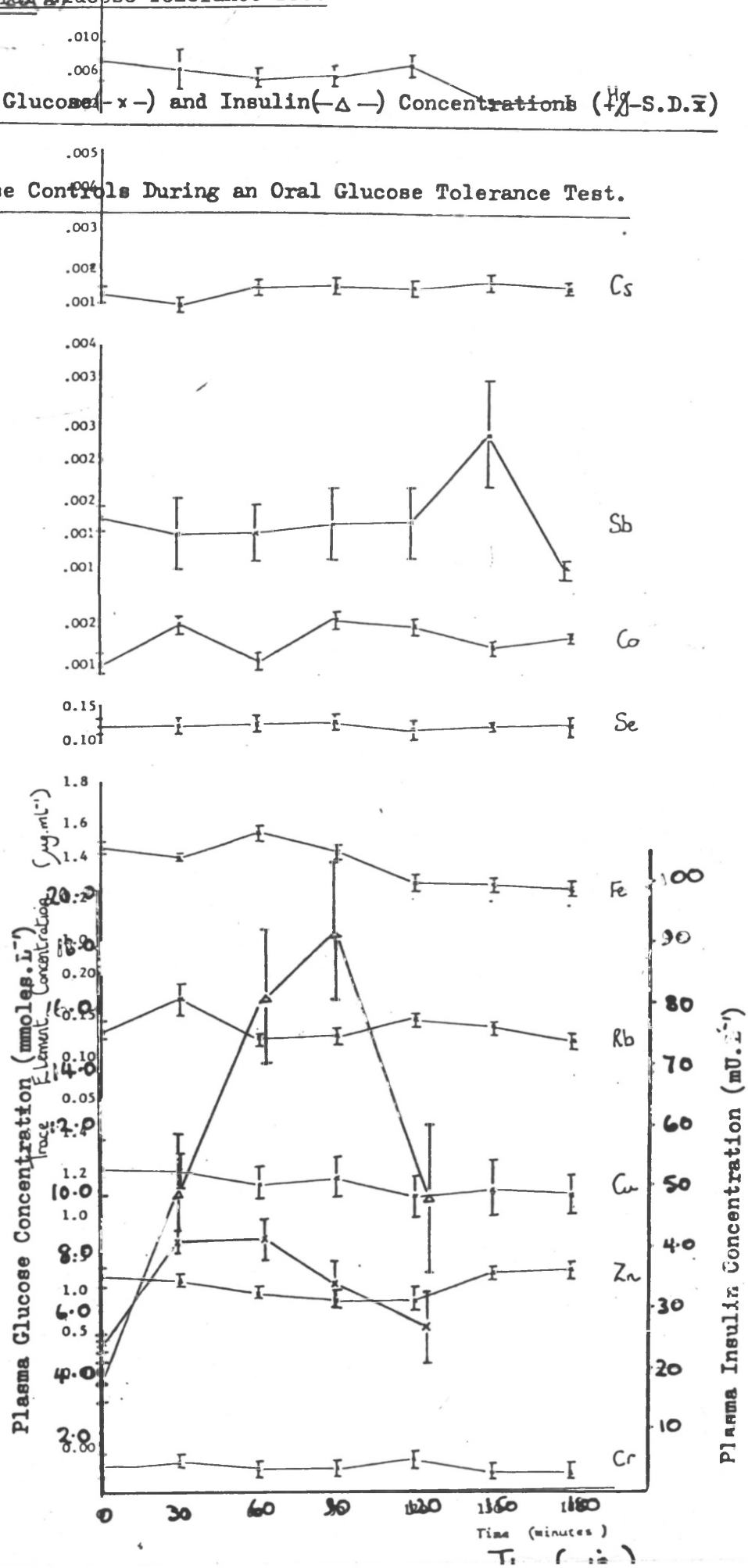


Figure 24

Trace Element Concentration in Plasma of Obese Diabetics During an Oral Glucose Tolerance Test

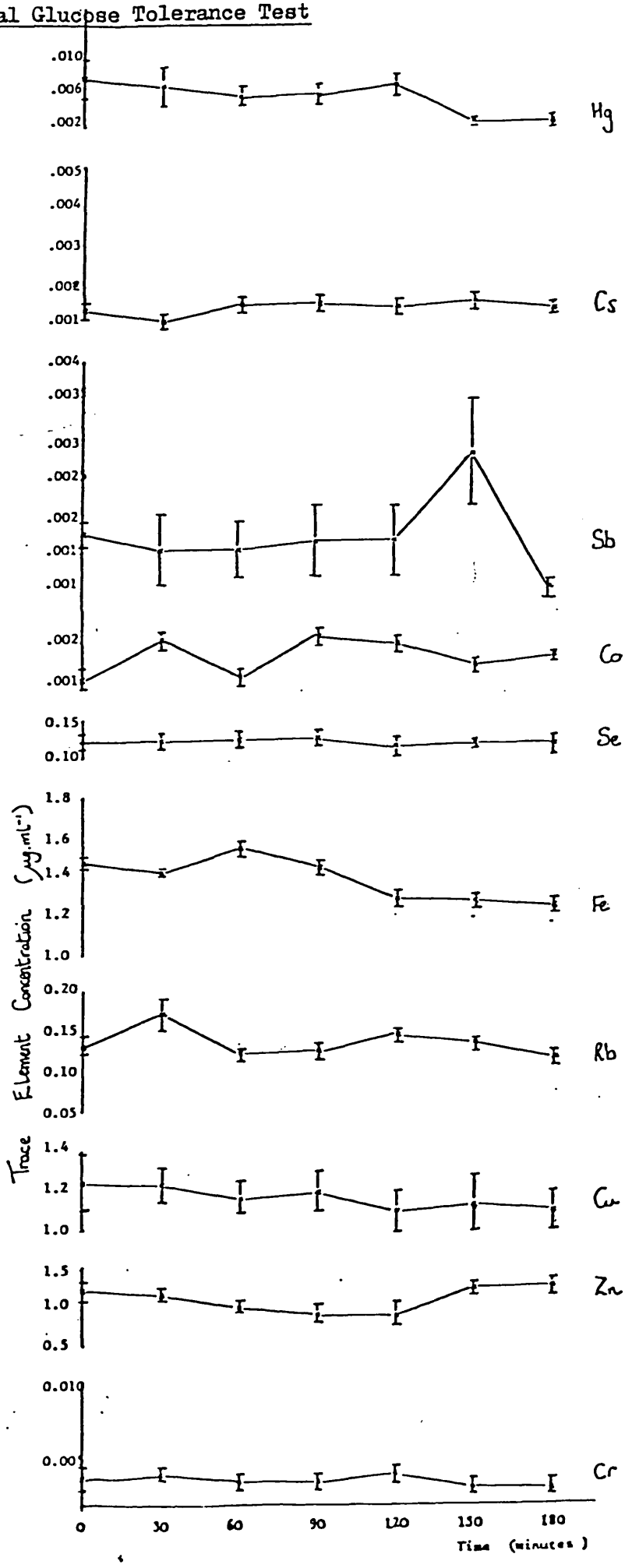


Figure 26(b)

Plasma Glucose (—x—) and Insulin(—Δ—) concentrations (+/-S.D.$\bar{x}$)

of Obese Diabetics During an Oral Glucose Tolerance test.

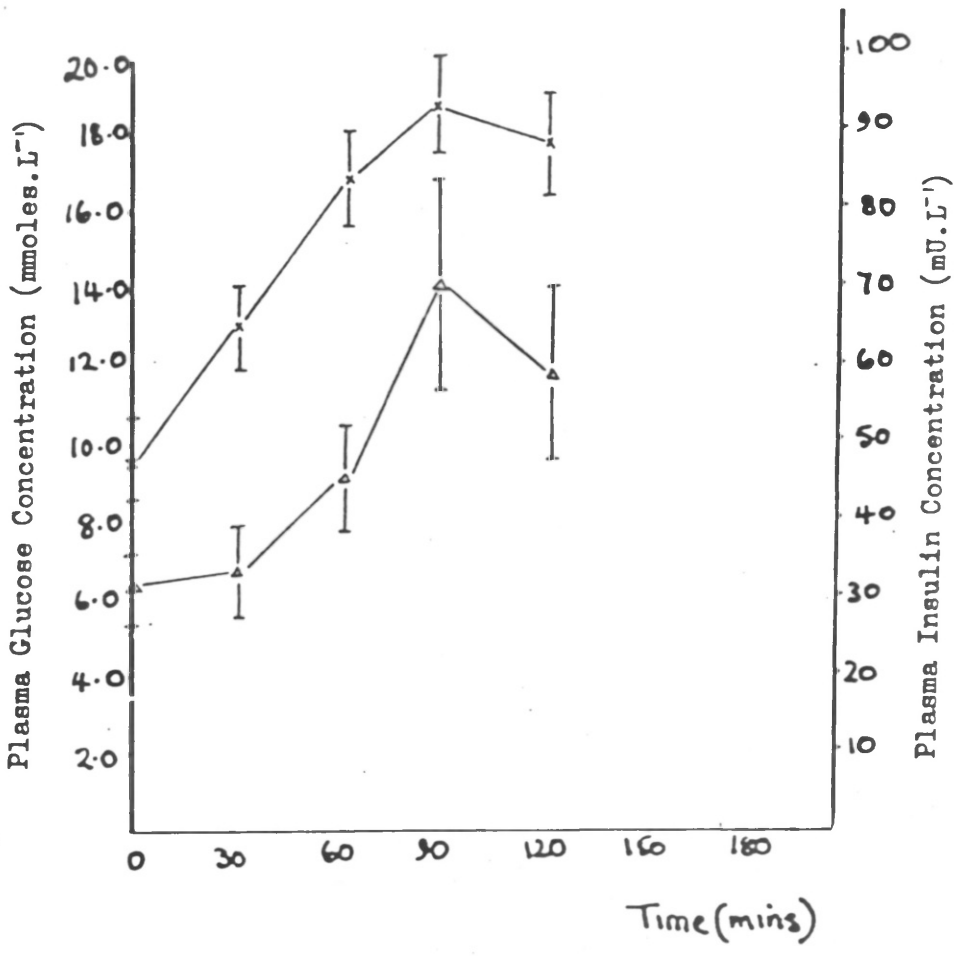


Figure 25

Figure 26(b) Trace Element Concentration in Plasma of Obese Controls During an Oral Glucose Tolerance Test

Plasma Glucose (—X—) and Insulin (—Δ—) concentrations (+/-S.D.x̄)

of Obese Diabetics During an Oral Glucose Tolerance test.

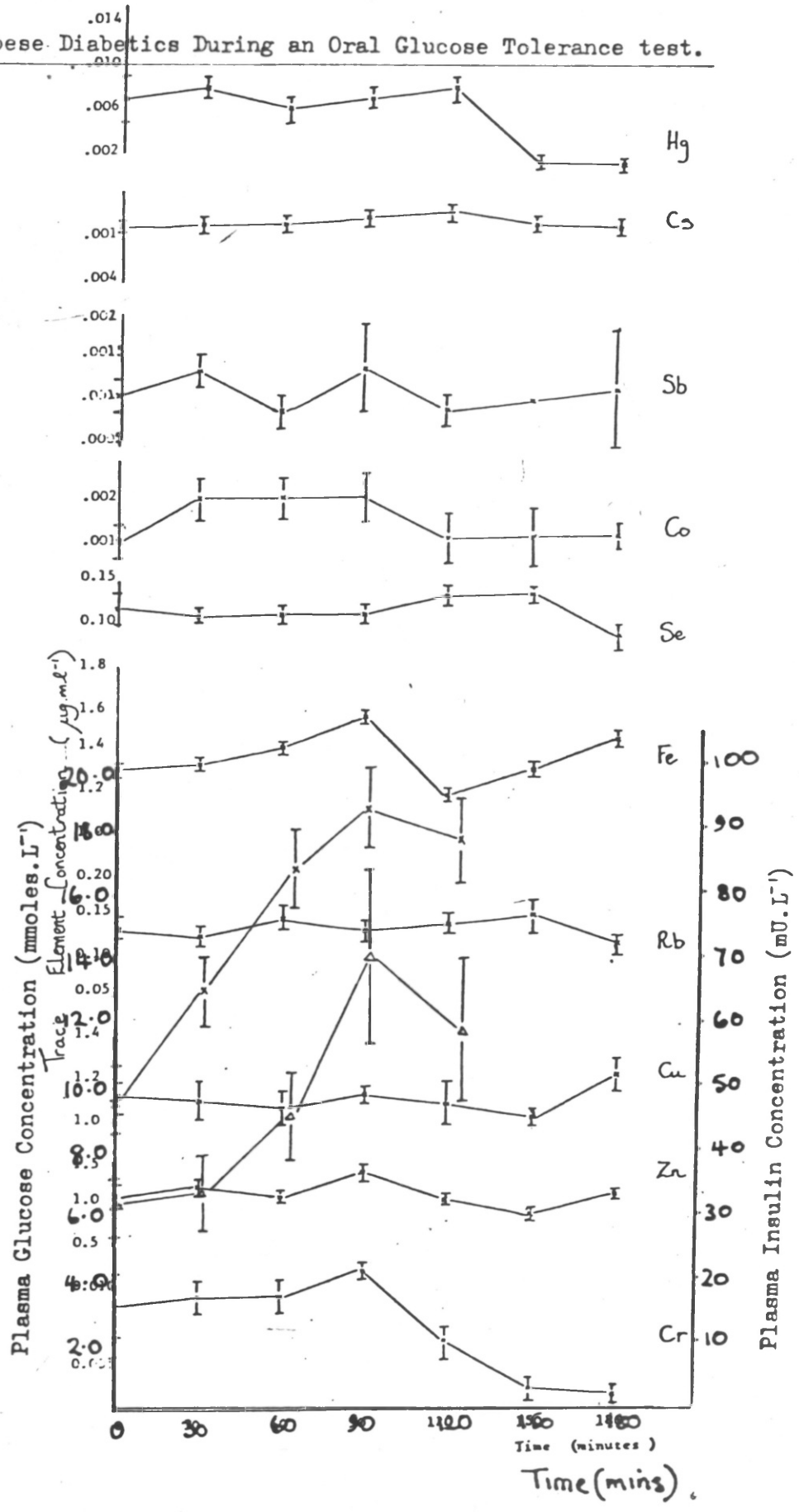
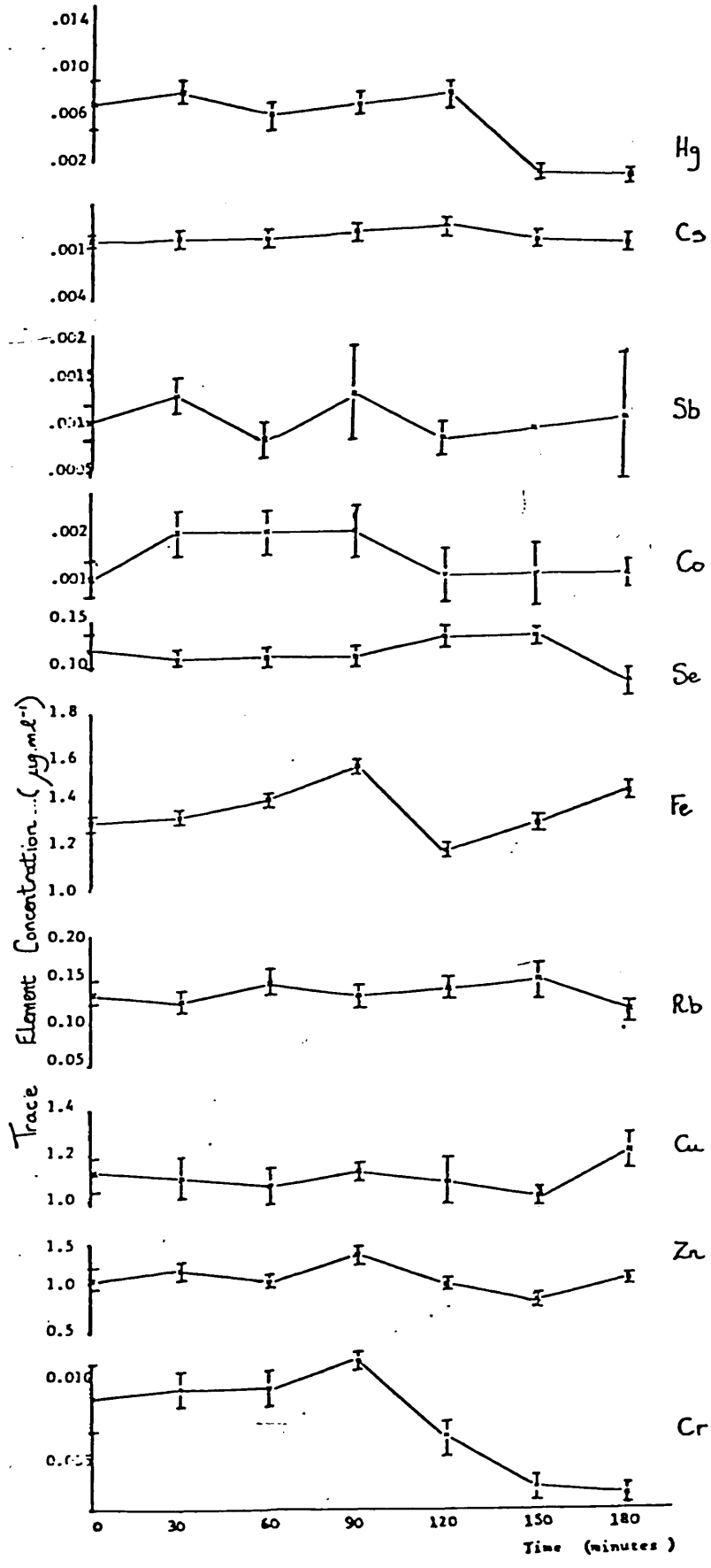


Figure 25

Trace Element Concentration in Plasma of Obese Controls During an Oral Glucose Tolerance Test



Plasma Glucose Concentration ($mmol.L^{-1}$) of Obese Controls

Table 26 (a)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	5.8	8.4	9.0	7.3	5.3	N.D.	N.D.
2	4.6	7.5	7.8	6.8	4.7	N.D.	N.D.
3	4.9	8.6	10.1	6.4	4.9	N.D.	N.D.
4	5.6	8.5	10.9	10.9	10.2	N.D.	N.D.
5	4.9	8.2	7.4	5.1	5.5	N.D.	N.D.
6	4.9	8.4	8.8	6.6	5.1	3.6	3.4
7	4.8	8.1	7.5	8.0	4.8	N.D.	N.D.
8	4.7	8.0	4.0	3.2	3.3	4.1	3.6
$\bar{x}$	5.0	8.2	8.2	6.8	5.5	N.D.	N.D.
$SD\bar{x}$	0.2	0.1	0.7	0.8	0.7	N.D.	N.D.

Plasma Glucose Concentration ($mmol.L^{-1}$) of Obese Diabetics

Table 26 (b)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	14.3	19.4	22.7	23.3	20.4	N.D.	N.D.
2	8.9	13.0	15.1	17.4	18.1	N.D.	N.D.
3	12.4	15.9	20.9	22.8	24.6	N.D.	N.D.
4	7.6	13.2	16.7	17.9	16.8	14.2	12.3
5	7.9	8.4	13.6	15.7	16.2	13.4	12.3
6	6.8	9.5	11.6	13.2	13.0	11.5	9.7
7	10.2	11.3		19.2	16.5	N.D.	N.D.
8	9.3	12.0	15.6	17.5	17.1	13.4	12.3
$\bar{x}$	9.7	12.8	16.7	18.4	17.8	N.D.	N.D.
$SD\bar{x}$	0.9	1.3	1.3	1.2	1.2	N.D.	N.D.

Plasma Insulin Concentration ($mU.L^{-1}$) of Obese Controls

Table 27 (a)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	16.0	74.0	95.0	118.0	134.0	N.D.	N.D.
2	13.0	10.0	74.0	62.0	17.0	N.D.	N.D.
3	15.0	60.0	104.0	59.0	31.0	N.D.	N.D.
4	25.0	N.D.	154.0	121.0	100.0	N.D.	N.D.
5	7.0	9.0	52.0	N.D.	34.0	N.D.	N.D.
6	31.0	80.0	60.0	179.0	35.0	N.D.	9.0
7	24.0	70.0	77.0	65.0	32.0	N.D.	N.D.
8	26.0	42.0	40.0	30.0	14.0	16.0	17.0
$\bar{x}$	19.7	49.3	82.0	90.6	49.6	N.D.	N.D.
$SD\bar{x}$	2.9	11.0	12.9	18.2	15.5	N.D.	N.D.

Plasma Insulin Concentration ($mU.L^{-1}$) of Obese Diabetics

Table 27 (b)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	13.0	26.0	72.0	N.D.	20.0	N.D.	N.D.
2	28.0	26.0	30.0	36.0	40.0	N.D.	N.D.
3	31.0	20.0	24.0	63.0	79.0	N.D.	N.D.
4	20.0	42.0	51.0	30.0	26.0	14.0	23.0
5	44.0	31.0	40.0	140.0	82.0	N.D.	N.D.
6	55.0	67.0	79.0	89.0	111.0	33.0	79.0
7	32.0	24.0	26.0	59.0	60.0	N.D.	N.D.
8	27.0	32.0	36.0	61.0	50.0	14.0	23.0
$\bar{x}$	31.3	33.5	44.8	68.3	58.5	N.D.	N.D.
$SD\bar{x}$	4.7	5.4	7.5	13.2	11.0	N.D.	N.D.

Trace Element Excretion of Obese Individuals before and after a 75g oral Glucose load

Table 28(a) : Obese Controls

Sample	Mean Elemental Concentration (µg.mmol Creat^{-1}) (SD$\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 8)	0.004 (.002)	0.39 (.13)	135.8 (30.0)	2.90 (.7)	196.2 (28.9)	173.9 (30.4)	0.53 (.10)	1.89 (.42)	0.31 (.09)	0.53 (.16)
U2 (n = 8)	0.005 (.002)	0.78 (.19)	75.1 (16.4)	2.44 (.55)	126.4 (21.4)	157.9 (38.3)	0.29 (.06)	3.55 (1.32)	0.38 (.13)	0.59 (.12)

Table 28(b) : Obese Diabetics

Sample	Mean Elemental Concentration (µg.mmol Creat^{-1}) (SD$\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 6)	0.003 (.001)	0.18 (.02)	105.1 (36.5)	6.0 (1.6)	173.8 (19.1)	195.9 (60.9)	0.41 (.07)	1.67 (.38)	0.18 (.03)	0.61 (.21)
U2 (n = 6)	0.005 (.001)	0.37 (.07)	180.5 (64.4)	2.98 (.68)	125.4 (34.0)	121.2 (22.1)	0.30 (.14)	0.38 (.08)	0.20 (.06)	0.39 (.10)

U1 - Urine collected 24 hours prior to glucose tolerance test.

U2 - Urine collected 24 hours after the glucose tolerance test.

3.4 OBSERVE THE FASTING PLASMA GLUCOSE AND INSULIN CONCENTRATIONS OF OBESE AND LEAN SUBJECTS.

NULL HYPOTHESIS 3.4(i)

Lean and obese Controls show no fasting differences in their concentration of plasma glucose.

Hypothesis accepted

NULL HYPOTHESIS 3.4(ii)

Lean and obese Maturity onset Diabetics show no fasting differences in their concentration of plasma glucose.

Hypothesis accepted

NULL HYPOTHESIS 3.4(iii)

Lean Controls and Maturity onset Diabetics show no fasting differences in their concentration of plasma glucose.

Hypothesis rejected : Lean diabetics have higher fasting glucose than lean Controls.

NULL HYPOTHESIS 3.4(iv)

Obese Controls and Maturity onset Diabetics show no fasting difference in their concentration of plasma glucose.

Hypothesis rejected : Obese Diabetics have higher fasting glucose than obese Controls.

NULL HYPOTHESIS 3.4(v)

Lean and obese Controls show no fasting difference in their concentration of plasma insulin.

Hypothesis rejected : Obese Controls have higher plasma insulin than lean Controls.

NULL HYPOTHESIS 3.4(vi)

Lean and obese Maturity onset Diabetics show no fasting difference in their concentration of plasma insulin.

Hypothesis accepted

NULL HYPOTHESIS 3.4(vii)

Lean Controls and Maturity onset Diabetics show no fasting difference in their concentration of plasma insulin.

Hypothesis accepted

NULL HYPOTHESIS 3.4(viii)

Obese Controls and Maturity onset Diabetics show no fasting difference in their concentration of plasma insulin.

Hypothesis rejected : Obese Diabetics have higher plasma insulin than obese Controls.

OBSERVE THE DIFFERENCE BETWEEN THE MAXIMUM CIRCULATING PLASMA GLUCOSE AND INSULIN CONCENTRATIONS IN LEAN AND OBESE SUBJECTS

NULL HYPOTHESIS 3.4(ix)

Lean and obese Controls show the same maximum blood glucose response to the glucose challenge.

Hypothesis accepted

NULL HYPOTHESIS 3.4(x)

Lean and obese Maturity onset Diabetics show the same maximum blood glucose response to the glucose challenge.

Hypothesis rejected : Lean Diabetics show a greater blood glucose response to the glucose challenge than the obese Diabetics.

NULL HYPOTHESIS 3.4(xi)

Lean Controls and Maturity onset Diabetics show the same maximum blood glucose response to the glucose challenge.

Hypothesis rejected : Diabetics show a greater blood glucose response to the glucose challenge than Controls.

NULL HYPOTHESIS 3.4(xii)

Obese Controls and Maturity onset Diabetics show the same maximum blood glucose response to the glucose challenge.

Hypothesis rejected : Diabetics show a greater blood glucose response to a glucose challenge than Controls.

NULL HYPOTHESIS 3.4(xiii)

Lean and obese Controls show the same maximum insulin response to the glucose challenge.

Hypothesis rejected : Obese Controls show a greater insulin response to the glucose challenge than lean Controls.

NULL HYPOTHESIS 3.4(xiv)

Lean and obese Maturity onset Diabetics show the same maximum insulin response to the glucose challenge.

Hypothesis rejected : Obese Diabetics show a greater insulin response to the glucose challenge than lean Diabetics.

NULL HYPOTHESIS 3.4(xv)

Lean Controls and Maturity onset Diabetics show the same maximum insulin reponse to the glucose challenge.

Hypothesis accepted

NULL HYPOTHESIS 3.4(xvi)

Obese Controls and Maturity onset Diabetics show the same maximum insulin response to the glucose challenge.

Hypothesis rejected : Controls show a greater insulin reponse than Diabetics to the glucose challenge.

OBSERVE THE EFFECT OF A GLUCOSE LOAD ON THE CIRCULATING AND EXCRETED TRACE ELEMENT CONCENTRATION OF LEAN AND OBESE DIABETICS AND CONTROLS.

FASTING GLUCOSE RESPONSE

NULL HYPOTHESIS 3.4(xvii)

Lean and obese Controls show no fasting differences in their concentration of trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN Obese Subjects	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN Obese Subjects
Erythrocytes	-	Cr, Cs
Plasma	Co	Cr, Cu, Zn
U 1	-	Co, Cr, Sb, Se
U 2	N.D.	N.D.

All the other trace elements show no differences between the two groups.

* indicates that the values were rejected at the t 0.99 probability level.

- indicates that their were no trace elements which fell into this category.

N.D. indicates that the trace element concentration of this medium was not determined.

NULL HYPOTHESIS 3.4(xviii)

Lean and obese Maturity onset Diabetics show no fasting differences in their concentration of trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN obese DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN obese DIABETICS
Erythrocytes	Hg, Rb, Sb	-
Plasma	Rb	-
U 1	Cr, Zn	-
U 2	-	Co, Cu, Hg, Sb, Se

All the other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.4(xix)

Lean Controls and Maturity onset Diabetics show no fasting differences in their concentration of trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	-	Fe, Rb
Plasma	Co	Cu, Rb
U 1	-	-
U 2	N.D.	N.D.

All the other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.4(xx)

Obese Controls and Maturity onset Diabetics show no fasting differences in their concentration of trace elements.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Eythrocytes	Co, Cr	Fe, Zn
Plasma	Cr	Sb
U 1	Cr, Rb, Sb	Zn
U 2	Cr, Cs, Cu, Sb	Co

All other trace elements show no differences between the two groups.

MAXIMUM GLUCOSE RESPONSE

NULL HYPOTHESIS 3.4(xxi)

Lean and obese Controls show no differences in their concentration of trace elements at the point where the circulating glucose is at its maximum.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS (obese controls)	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	Co	Cr, Cs, Fe, Rb, Zn
Plasma	Fe	Co, Cu, Cr, Zn

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.4(xxii)

Lean and obese Maturity onset Diabetics show no differences in their concentration of trace elements at the point where the circulating glucose is at its maximum.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS (close)	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	Hg, Rb	Co
Plasma	Rb	Co, Cu, Fe

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.4(xxiii)

Lean Controls and Maturity onset Diabetics show no differences in their concentration of trace elements at the point where the circulating glucose is at its maximum.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	-	Co, Fe
Plasma	-	Hg, Rb

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.4(xxiv)

Obese Controls and Maturity onset Diabetics show no differences in their concentrations of trace elements at the point where the circulating glucose is at its maximum.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	Cr, Hg, Rb	Cu, Fe
Plasma	Cr, Rb, Zn	Sb

All other trace elements show no differences between the two groups.

SECTION 3.5

Trace Element Content of the Erythrocytes of Obese Individuals after a 75g oral Glucose Challenge, following a one month period of weight loss

Table 29 : Obese Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 6									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.45 (.04)	0.002 (.0004)	10.52 (.23)	0.16 (.03)	801.3 (40.1)	3.60 (.14)	0.014 (.004)	0.016 (.005)	0.0015 (.0003)	0.0054 (.0007)
30	0.44 (.04)	0.002 (.0003)	10.85 (.77)	0.15 (.03)	845.6 (41.4)	3.82 (.32)	0.018 (.005)	0.019 (.006)	0.0012 (.00005)	0.0058 (.0011)
60	0.55 (.04)	0.002 (.0002)	10.94 (.47)	0.17 (.03)	830.6 (42.2)	3.76 (.18)	0.017 (.03)	0.018 (.004)	0.0014 (.0003)	0.006 (.0008)
90	0.48 (.03)	0.001 (.0002)	10.61 (.63)	0.15 (.02)	789.6 (21.2)	3.60 (.21)	0.018 (.005)	0.015 (.005)	0.0011 (.0009)	0.004 (.0011)
120	0.50 (.01)	0.002 (.0003)	10.43 (.66)	0.16 (.03)	796.8 (28.8)	3.64 (.24)	0.015 (.004)	0.017 (.005)	0.0012 (.0002)	0.0052 (.0010)
150*	0.60	0.005	11.50	0.13	842.8	3.62	0.019	0.0095	0.0015	0.0066
180*	0.48	0.003	10.77	0.13	860.8	3.80	0.012	0.0086	0.0013	0.0057

Table 30 : Obese Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 6									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	0.50 (.03)	0.003 (.0008)	10.49 (1.00)	0.15 (.01)	839.4 (62.1)	3.48 (.24)	0.021 (.009)	0.012 (.002)	0.0024 (.0008)	0.006 (.001)
30	0.53 (.10)	0.001 (.0003)	10.04 (.26)	0.15 (.01)	818.5 (21.4)	3.33 (.27)	0.022 (.01)	0.012 (.004)	0.0018 (.0006)	0.0042 (.0003)
60	0.47 (.07)	0.002 (.0002)	11.38 (1.01)	0.16 (.009)	896.6 (53.5)	3.84 (.34)	0.025 (.01)	0.013 (.003)	0.0026 (.0008)	0.0056 (.0006)
90	0.40 (.05)	0.002 (.0002)	8.88 (.70)	0.09 (.04)	693.4 (59.9)	2.63 (.29)	0.018 (.01)	0.012 (.005)	0.0019 (.0005)	0.0052 (.0004)
120	0.46 (.08)	0.002 (.0004)	9.01 (.72)	0.13 (.03)	708.6 (64.5)	2.76 (.28)	0.020 (.01)	0.012 (.005)	0.0018 (.0006)	0.0052 (.0003)
150*	0.46	0.001	9.87	0.14	712.1	3.23	0.0015	0.007	0.0012	0.0055
180*	0.48	0.002	10.52	0.17	747.2	3.15	0.0014	0.007	0.0010	0.0052

* indicates that only one individual gave blood at 150/180 minutes.

Trace Element Content of the Plasma of Obese Individuals after a 75g oral Glucose Challenge, following a one month period of weight loss

Table 31 : Obese Controls

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 6									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.09 (.09)	0.003 (.0005)	1.30 (.05)	0.13 (.0009)	1.60 (.11)	0.17 (.006)	0.009 (.003)	0.007 (.0008)	0.003 (.0005)	0.0015 (.0001)
30	1.14 (.06)	0.001 (.0003)	1.36 (.09)	0.12 (.02)	1.20 (.12)	0.15 (.012)	0.010 (.003)	0.008 (.002)	0.002 (.001)	0.0023 (.0009)
60	1.10 (.05)	0.001 (.0002)	1.22 (.06)	0.12 (.01)	1.48 (.18)	0.15 (.018)	0.009 (.002)	0.0074 (.002)	0.0013 (.0004)	0.0015 (.0002)
90	1.20 (.06)	0.003 (.001)	1.22 (.08)	0.12 (.016)	1.80 (.19)	0.16 (.016)	0.009 (.002)	0.007 (.001)	0.0013 (.0004)	0.0012 (.0002)
120	1.20 (.04)	0.001 (.0002)	1.15 (.09)	0.12 (.014)	1.22 (.17)	0.14 (.014)	0.012 (.003)	0.0068 (.002)	0.0008 (.00006)	0.001 (.0001)
150*	1:60	0.002	1.39	0.09	1.01	0.12	0.0005	0.0035	0.0011	0.0012
180*	1.80	0.0011	1.64	0.09	1.68	0.14	0.0005	0.0036	0.0010	0.0012

Table 32 : Obese Diabetics

Time (mins)	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$) n = 6									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
0	1.21 (.05)	0.001 (.0003)	1.32 (.12)	0.11 (.008)	1.43 (.10)	0.11 (.005)	0.015 (.009)	0.0053 (.002)	0.0018 (.0006)	0.0013 (.0002)
30	1.23 (.06)	0.002 (.0005)	1.24 (.19)	0.10 (.006)	1.66 (.39)	0.13 (.019)	0.011 (.006)	0.0056 (.002)	0.0019 (.0006)	0.0014 (.0004)
60	1.16 (.08)	0.001 (.0003)	1.16 (.17)	0.11 (.003)	1.56 (.22)	0.17 (.03)	0.009 (.004)	0.005 (.001)	0.0037 (.0002)	0.0015 (.0003)
90	1.32 (.04)	0.003 (.002)	1.68 (.15)	0.16 (.05)	1.43 (.29)	0.17 (.015)	0.005 (.003)	0.0047 (.0009)	0.0033 (.0002)	0.0016 (.0006)
120	1.16 (.05)	0.002 (.0001)	1.10 (.07)	0.09 (.009)	1.57 (.29)	0.11 (.01)	0.013 (.003)	0.012 (.0009)	0.0018 (.0006)	0.0014 (.0002)
150*	1.30	0.001	1.16	0.08	1.25	0.17	0.003	0.0025	0.0007	0.0009
180*	1.36	0.001	1.15	0.09	1.59	0.15	0.005	0.0025	0.0006	0.0009

* indicates that only one individual gave blood at 150/180 minutes.

Figure 33(a)

Plasma Glucose (—x—) and Insulin(—△—) concentrations (+/-S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test, After Weight Los

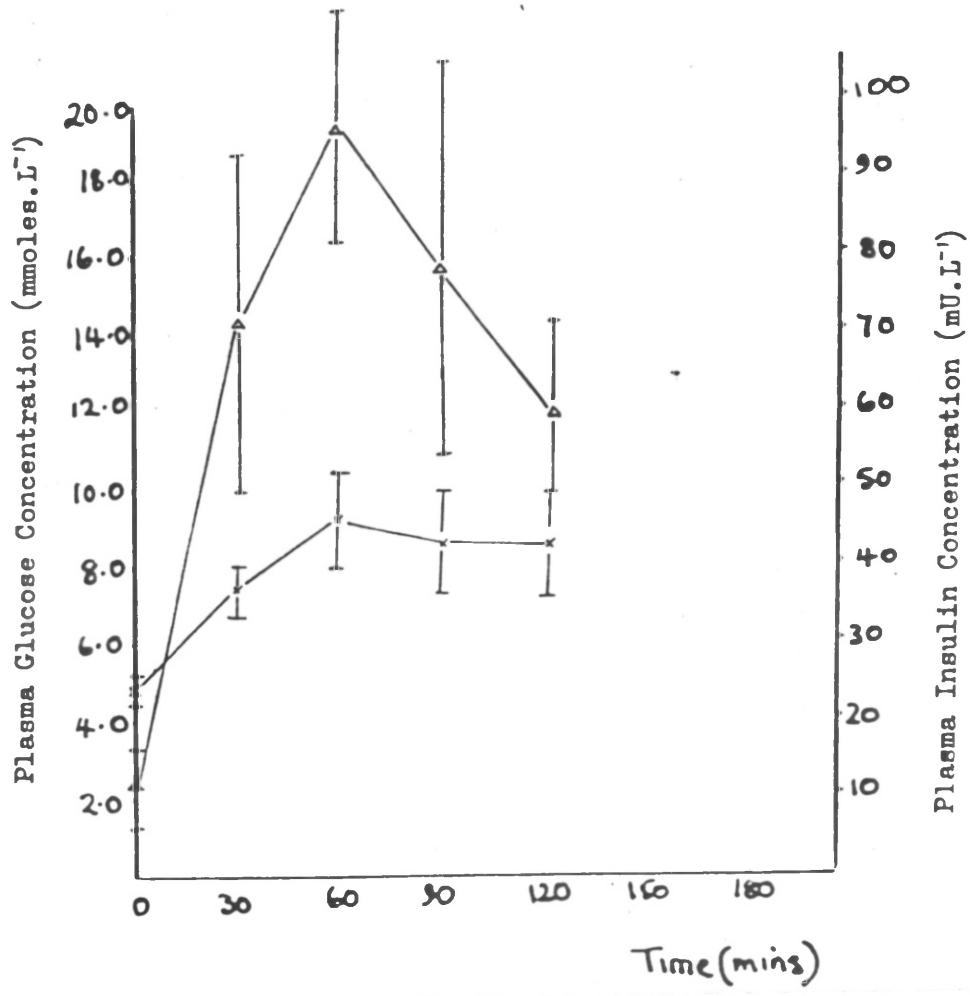


Figure 29

Trace Element Concentration in Erythrocytes of Obese Controls During an Oral Glucose tolerance Test, Following One Month's Period of Weight Loss

Figure 33(a)

Plasma Glucose (—x—) and Insulin (—Δ—) concentrations (Mean/-S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test, After Weight Los

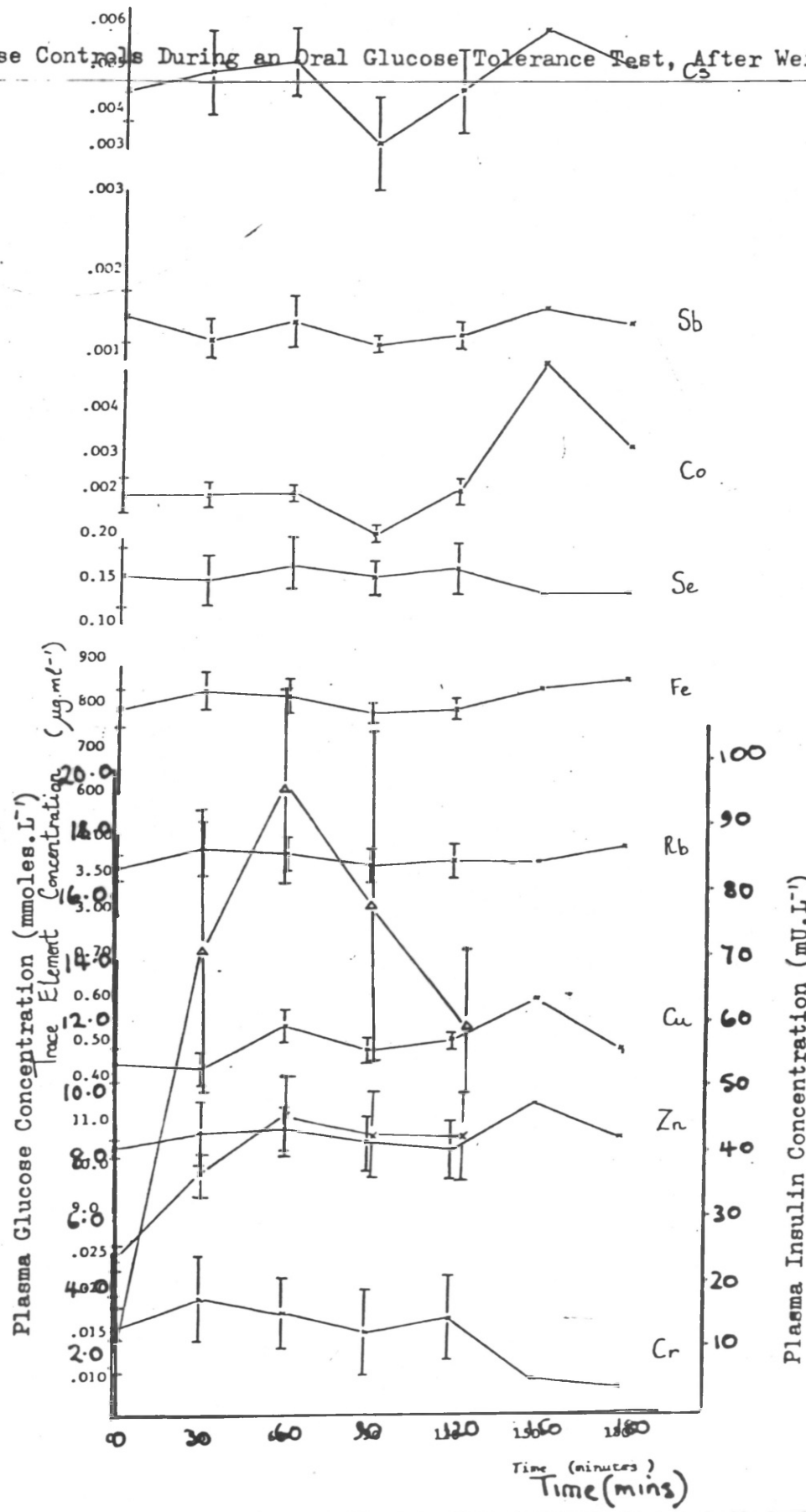


Figure 29

Trace Element Concentration inErythrocytes of Obese Controls During an Oral Glucose tolerance Test,Following One Month's Period of Weight Loss

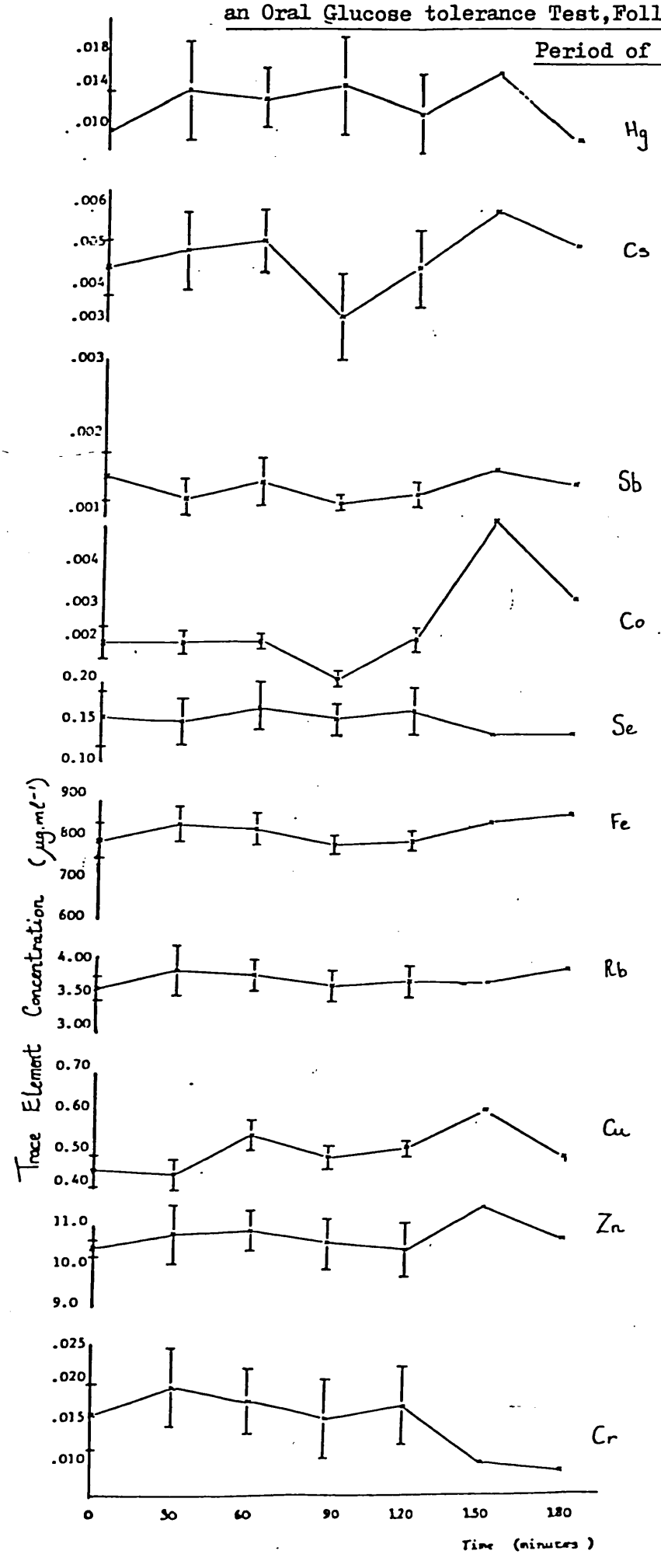


Figure 33(b)

Plasma Glucose(— X —) and Insilin (—Δ—) Concentrations (+/-S.D.$\bar{x}$)

of Obese Diabetics During an Oral GLucose Tolerance Test, After Weight l

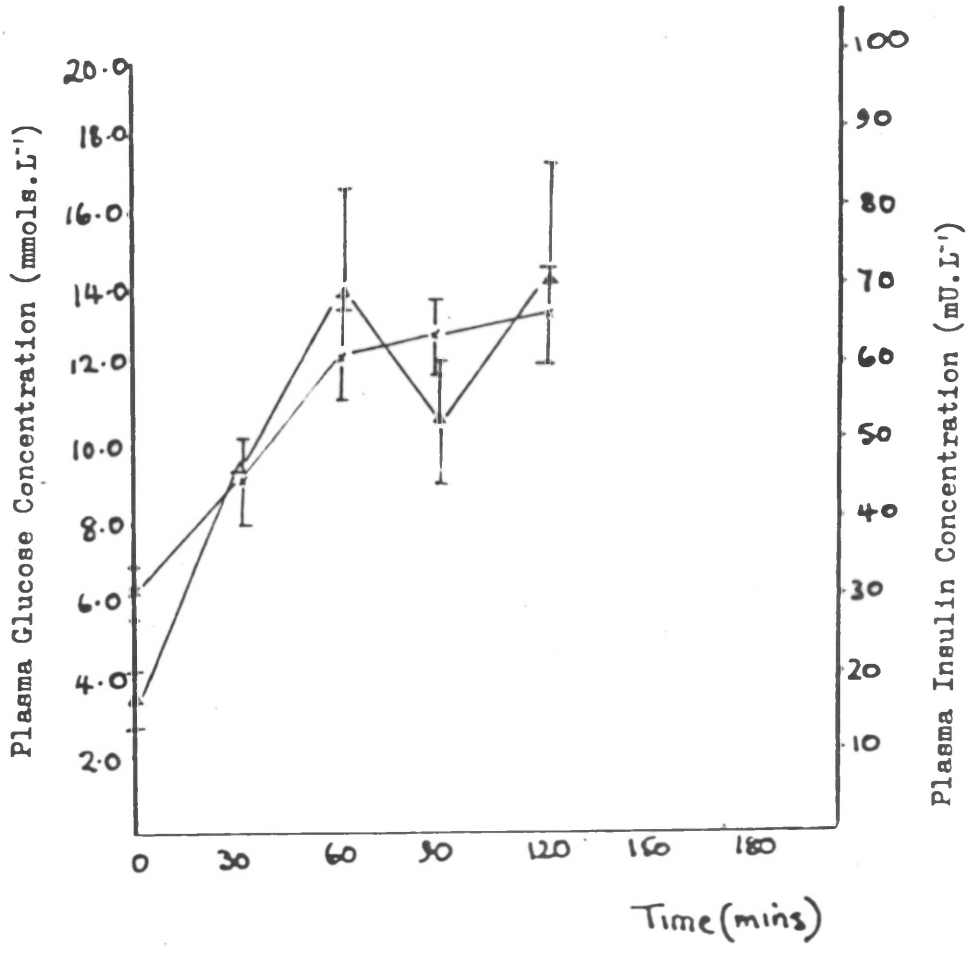
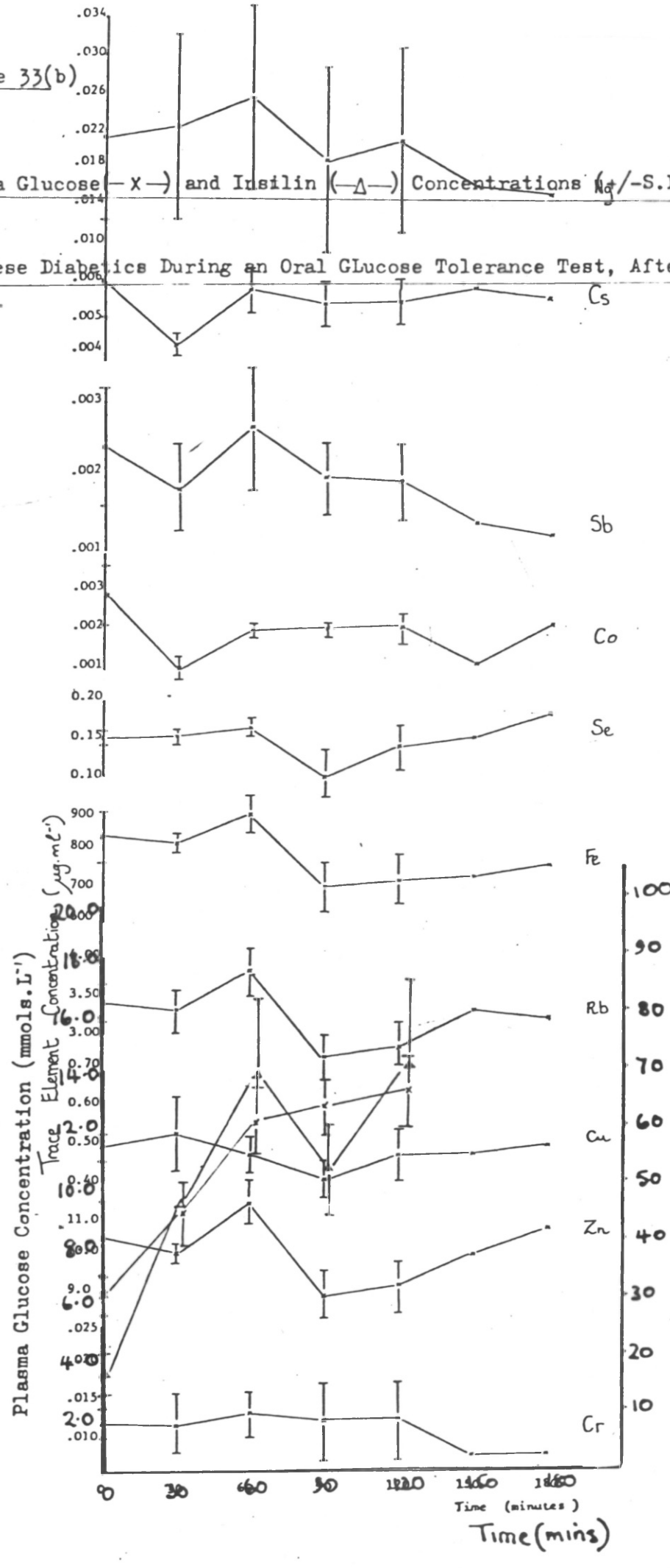


Figure 30

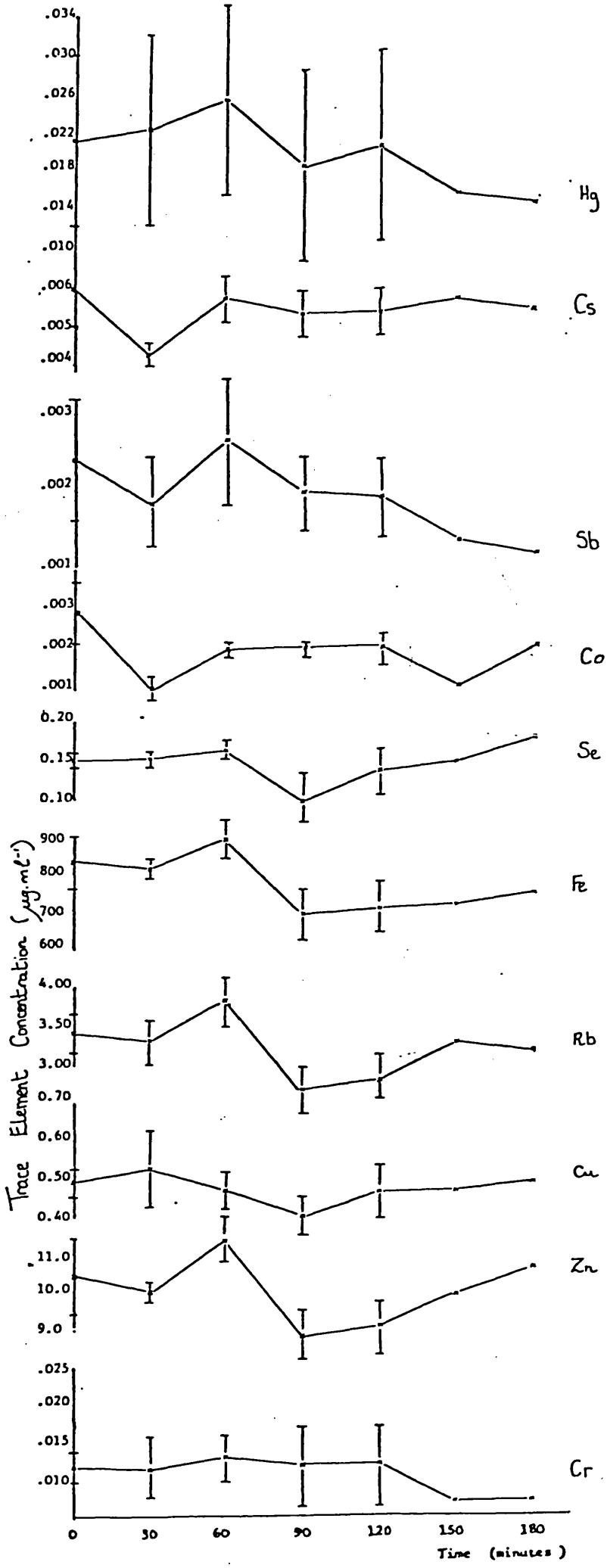
Figure 33(b)

Plasma Glucose (—X—) and Insilin (—Δ—) Concentrations (Mg/-S.D.x̄) of Obese Diabetics During an Oral GLucose Tolerance Test, After Weight Loss



Plasma Insulin Concentration in Erythrocytes of Obese Diabetics During an Oral Glucose Tolerance Test, Following One Months's Period of Weight Loss

Figure 30



Trace Element Concentration in Erythrocytes of Obese Diabetics During an Oral Glucose Tolerance Test, Following One Months's Period of Weight Loss

Figure 33(a)

Plasma Glucose (—×—) and Insulin(—△—) concentrations (+/-S.D.$\bar{x}$)

of Obese Controls During an Oral Glucose Tolerance Test, After Weight Loss.

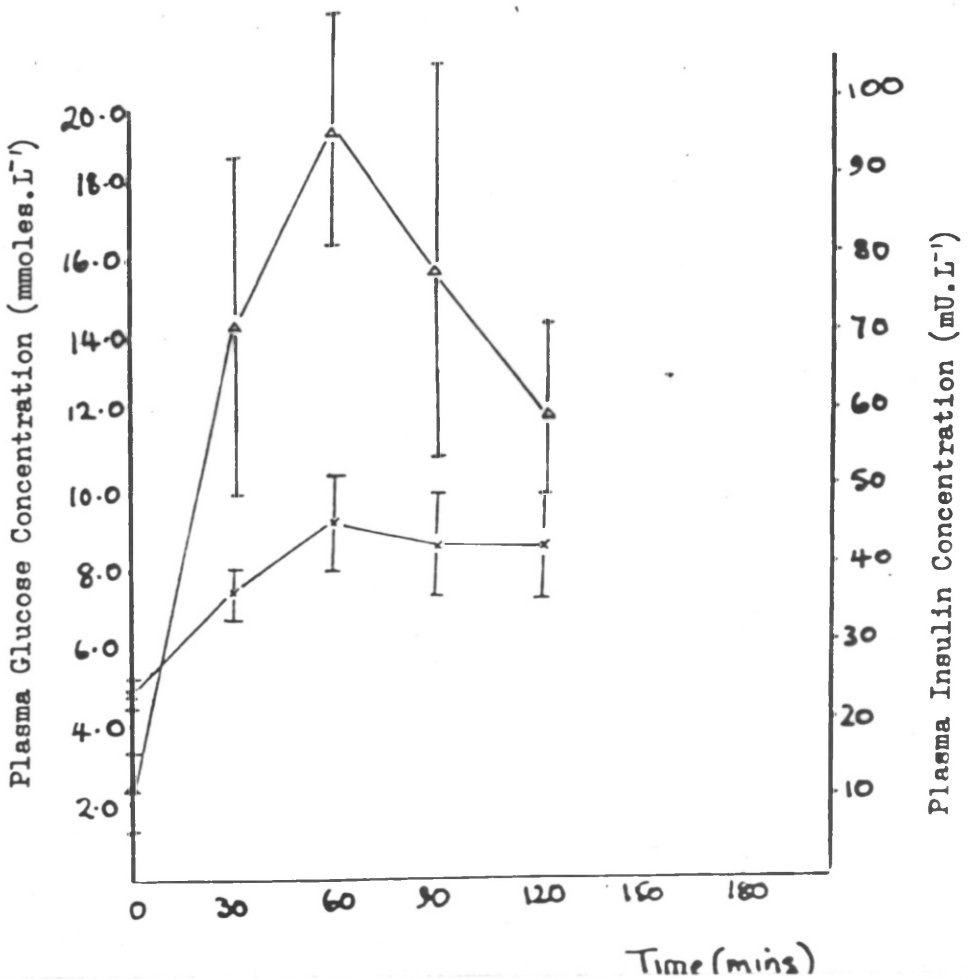


Figure 31

Figure 33(a)

Plasma Glucose (—x—) and Insulin(—Δ—) concentrations (+/-S.E.M.)
of Obese Controls During an Oral Glucose Tolerance Test, After Weight Loss.

Trace Element Concentration in Plasma of Obese Controls During an Oral Glucose Tolerance Test, Following One Month's Period of Weight Loss

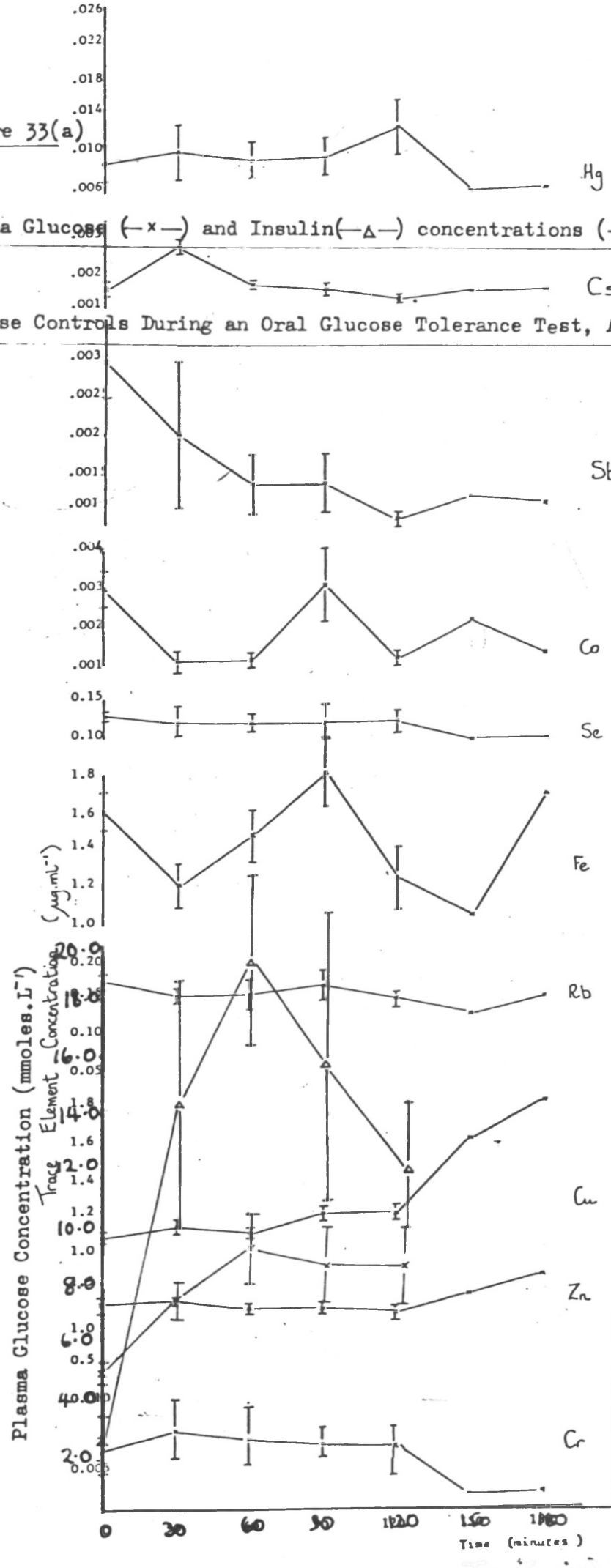
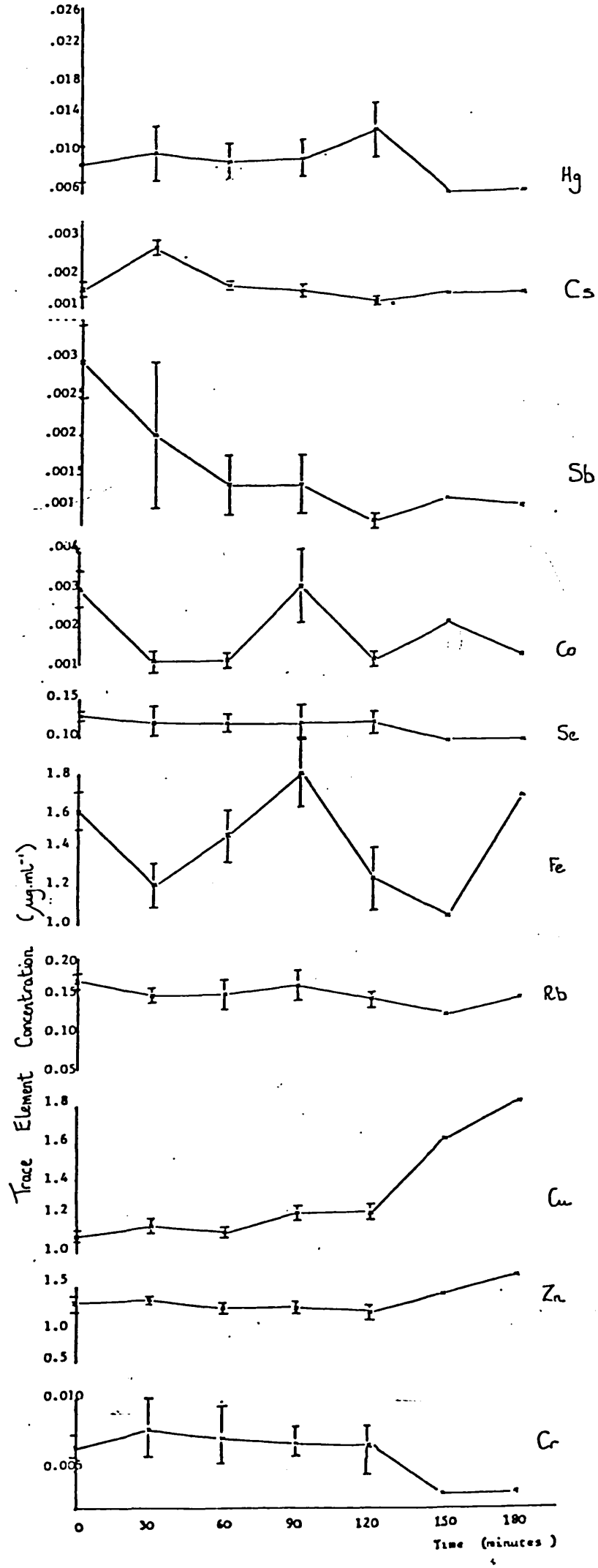


Figure 31



Trace Element Concentration in Plasma of Obese Controls During an Oral Glucose Tolerance Test, Following One Month's Period of Weight Loss

Figure 33(b)

Plasma Glucose(—X—) and Insulin (—Δ—) Concentrations (+/-S.D.$\bar{x}$)

of Obese Diabetics During an Oral GLucose Tolerance Test, After Weight Loss

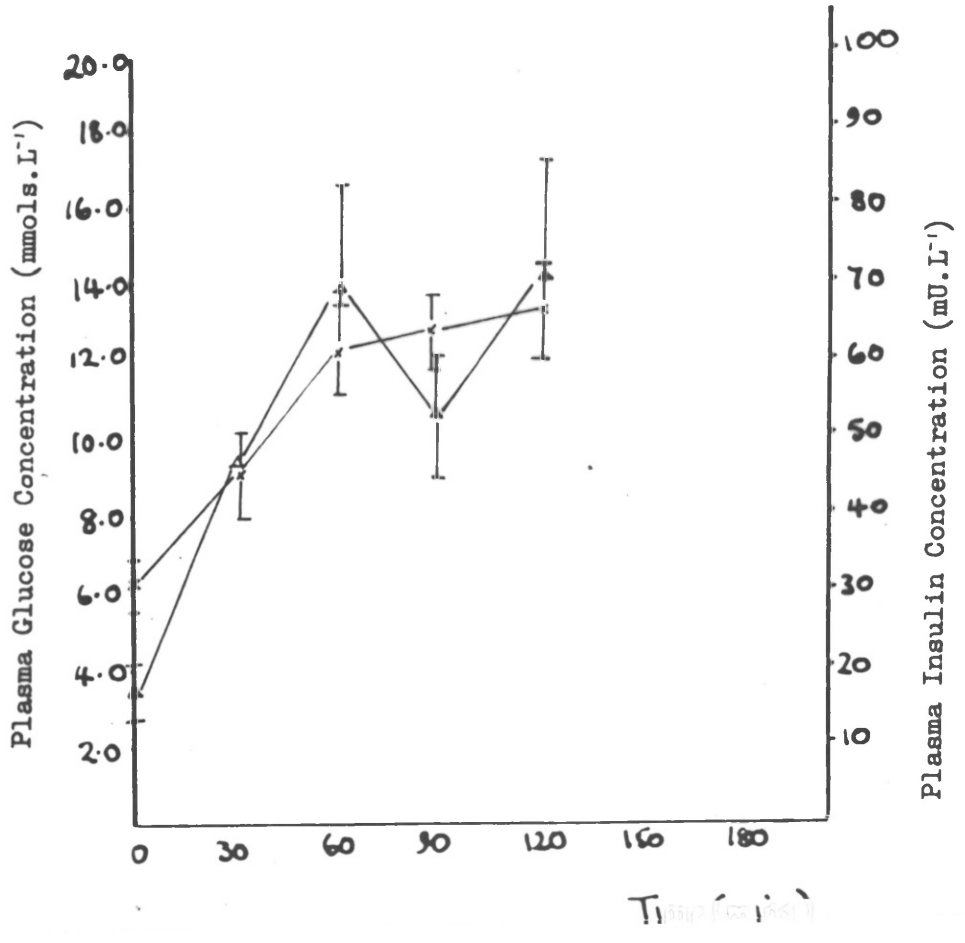
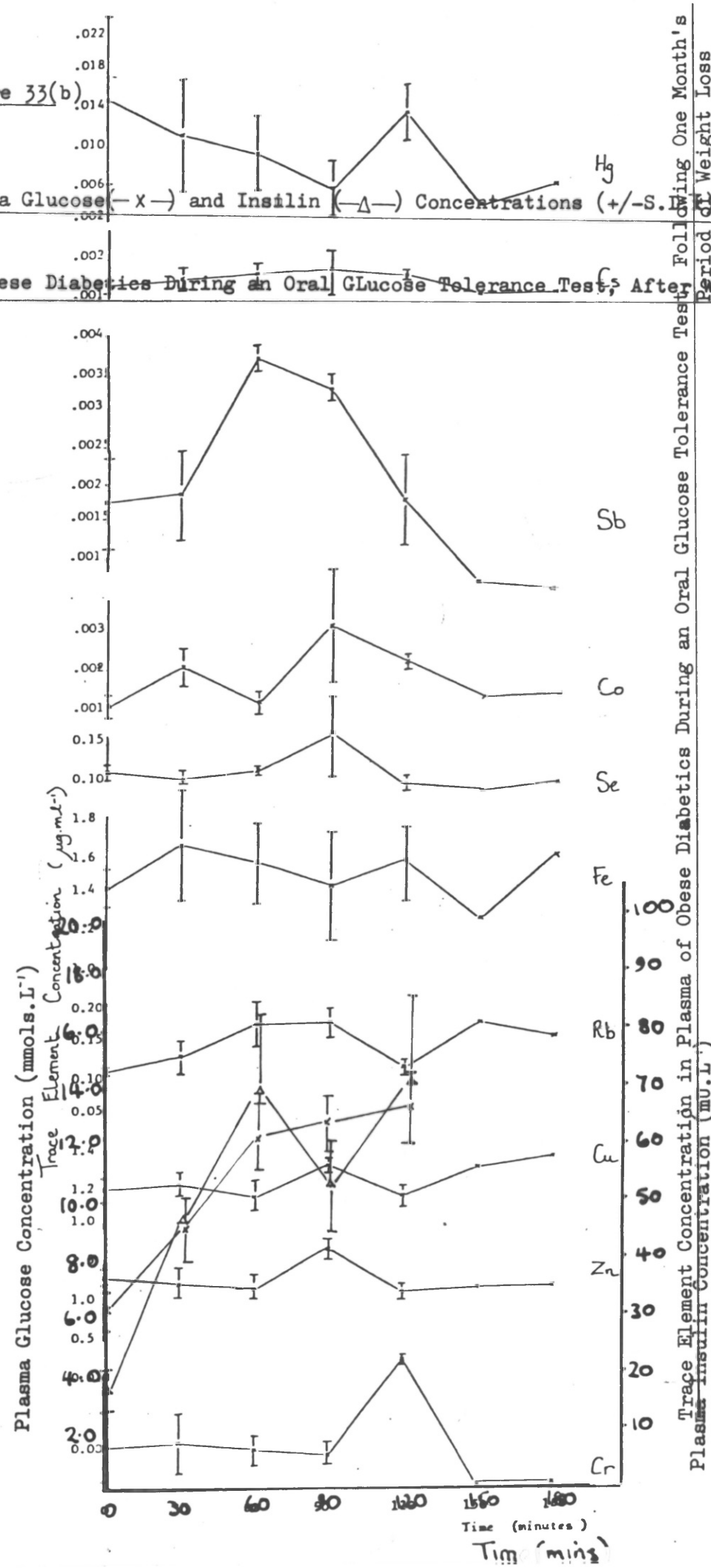
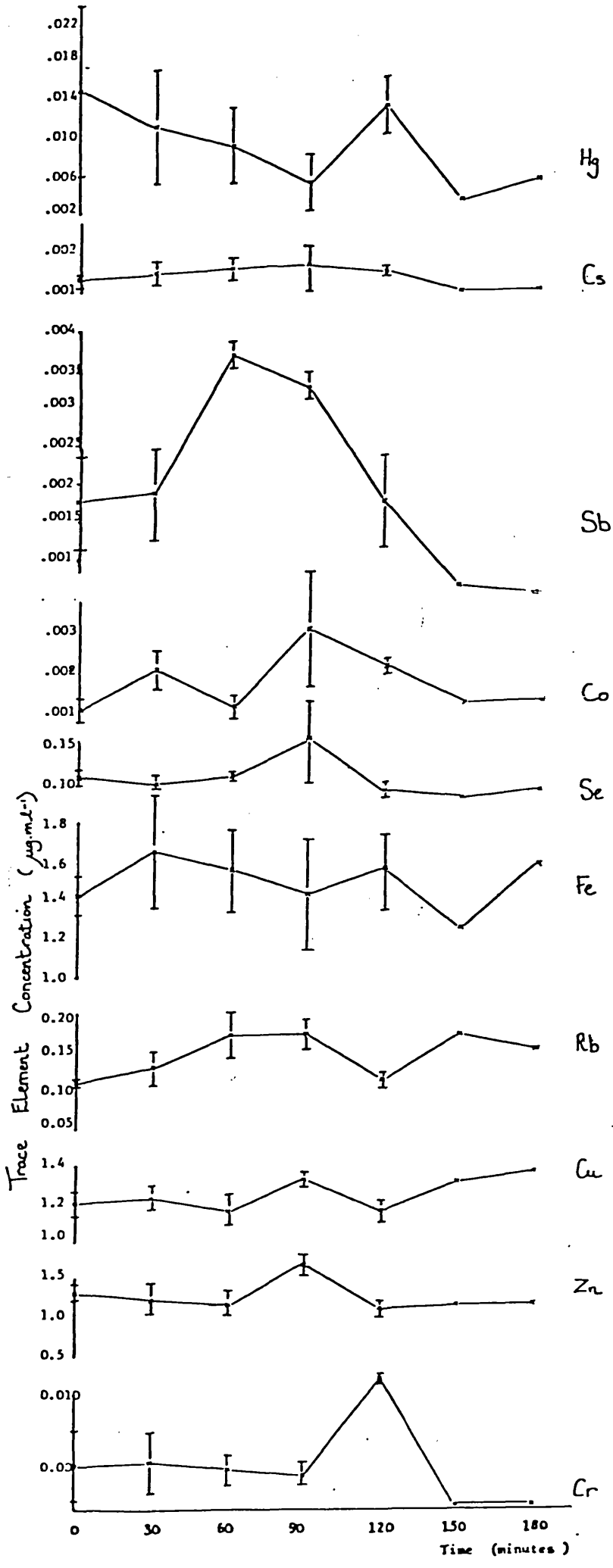


Figure 32

Figure 33(b)

Plasma Glucose (—X—) and Insilin (—Δ—) Concentrations (+/-S.E.) of Obese Diabetics During an Oral GLucose Tolerance Test, After Weight Loss Following One Month's Period of Weight Loss





Trace Element Concentration in Plasma of Obese Diabetics During an Oral Glucose Tolerance Test, Following One Month's Period of Weight Loss

Table 33 (a) Plasma Glucose Concentration (mmol.L^{-1}) of Obese Controls after weight loss

Subject Nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	5.6	7.4	9.5	9.4	7.8	**N.D.**	**N.D.**
2	4.4	6.2	8.6	6.0	5.8	**N.D.**	**N.D.**
3	4.7	7.3	12.6	12.8	12.4	**N.D.**	**N.D.**
4	4.7	7.3	8.0	6.7	5.0	**N.D.**	**N.D.**
5	4.3	8.5	8.5	8.6	8.5	**N.D.**	**N.D.**
6	4.1	7.9	8.9	9.7	9.7	9.6	7.1
$\bar{x}$	4.63	7.43	9.35	8.87	8.22	**N.D.**	**N.D.**
$SD\bar{x}$	0.22	0.31	0.68	0.99	1.10	**N.D.**	**N.D.**

Table 33 (b) Plasma Glucose Concentration (mmol.L^{-1}) of Obese Diabetics after weight loss

Subject Nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	10.4	15.0	18.1	N.D.	20.6	N.D.	N.D.
2	5.3	7.9	11.5	12.6	12.7	N.D.	N.D.
3	4.8	8.6	13.1	14.7	13.5	N.D.	N.D.
4	5.8	7.8	9.4	10.1	9.8	N.D.	N.D.
5	5.7	8.3	12.4	13.5	11.7	N.D.	N.D.
6	4.9	6.4	8.9	11.3	9.9	9.3	9.4
$\bar{x}$	6.15	9.00	12.23	12.44	13.03	N.D.	N.D.
$SD\bar{x}$	0.87	1.24	1.36	0.75	1.64	N.D.	N.D.

Plasma Insulin Concentration ($mU.L^{-1}$) of Obese Controls after Weight Loss

Table 34 (a)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	23.0	96.0	150.0	130.0	N.D.	N.D.	N.D.
2	9.0	28.0	105.0	43.0	41.0	N.D.	N.D.
3	9.0	20.0	50.0	54.0	95.0	N.D.	N.D.
4	23.0	160.0	91.0	150.0	62.0	N.D.	N.D.
5	4.0	56.0	95.0	41.0	30.0	N.D.	N.D.
6	9.0	71.0	93.0	52.0	71.0	70.0	60.0
$\bar{x}$	12.8	71.8	97.3	78.3	59.8	N.D.	N.D.
$SD\bar{x}$	3.6	22.9	14.3	21.6	11.4	N.D.	N.D.

Plasma Insulin Concentration ($mU.L^{-1}$) of Obese Diabetics after Weight Loss

Table 34 (b)

Subject nos.	Time of Sampling (mins)						
	0	30	60	90	120	150	180
1	22.0	50.0	80.0	44.0	82.0	N.D.	N.D.
2	14.0	8.0	30.0	39.0	39.0	N.D.	N.D.
3	25.0	68.0	124.0	80.0	112.0	N.D.	N.D.
4	16.0	61.0	54.0	105.0	125.0	N.D.	N.D.
5	14.0	30.0	65.0	23.0	40.0	N.D.	N.D.
6	20.0	42.0	51.0	30.0	26.0	14.0	23.0
$\bar{x}$	18.5	43.2	67.3	53.5	70.7	N.D.	N.D.
$SD\bar{x}$	2.0	9.8	14.4	14.3	18.7	N.D.	N.D.

Trace Element Excretion of Obese Individuals (following one month's period of weight loss), before and after a 75g Oral Glucose Load.

Table 35 (a) : Obese Controls

Sample	Mean Elemental Concentration (µg.mmol Creat^{-1}) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 6)	.003 (.001)	0.23 (.07)	149.9 (42.6)	4.03 (.84)	182.9 (47.2)	174.9 (41.8)	0.45 (.14)	3.07 (.86)	0.57 (.19)	0.32 (.09)
U2 (n = 6)	.006 (.002)	0.26 (.05)	143.3 (25.5)	4.24 (.66)	377.8 (87.1)	180.3 (15.8)	0.30 (.09)	2.96 (.70)	0.24 (.07)	0.76 (.13)

Table 35 (b) : Obese Diabetics

Sample	Mean Elemental Concentration (µg.mmol Creat^{-1}) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
U1 (n = 6)	.004 (.001)	0.43 (.17)	217.9 (46.4)	2.93 (1.05)	289.4 (127.9)	214.0 (24.2)	0.56 (.11)	2.14 (.54)	0.56 (.13)	0.84 (.27)
U2 (n = 6)	.004 (.001)	0.56 (.17)	577.1 (198.8)	2.36 (.45)	172.5 (34.4)	121.5 (28.6)	0.13 (.03)	0.83 (.45)	0.54 (.12)	0.28 (.08)

U1 - urine collected 24 hours prior to the glucose tolerance test.

U2 - urine collected 24 hours after the glucose tolerance test.

3.5 OBSERVE THE EFFECT OF WEIGHT CONTROL IN REDUCING HYPERGLYCAEMIA IN NON-INSULIN DEPENDENT DIABETICS AND THE SUBSEQUENT TRACE ELEMENT FLUCTUATIONS

NULL HYPOTHESIS 3.5(i)

Obese Controls, before and after a period of weight loss, show no fasting difference in plasma glucose concentration.

Hypothesis rejected : Controls had lower fasting glucose after weight loss.

NULL HYPOTHESIS 3.5(ii)

Obese Maturity onset Diabetics, before and after a period of weight loss, show no fasting difference in plasma glucose concentration.

Hypothesis rejected : Diabetics had lower fasting plasma glucose after weight loss.

NULL HYPOTHESIS 3.5(iii)

Obese Controls and obese Diabetics, after weight loss, show no fasting difference in their plasma glucose concentration.

Hypothesis rejected : Diabetics had higher fasting glucose after weight loss than the Controls.

NULL HYPOTHESIS 3.5(iv)

Obese Controls, before and after a period of weight loss, show no fasting difference in plasma insulin concentration.

Hypothesis rejected : Controls had a lower fasting plasma insulin after weight loss.

NULL HYPOTHESIS 3.5(v)

Obese Maturity onset Diabetics, before and after a period of weight loss, show no fasting difference in plasma insulin concentration.

Hypothesis rejected : Diabetics had lower fasting plasma insulin concentration after weight loss.

NULL HYPOTHESIS 3.5(vi)

Obese Controls show no fasting difference in plasma insulin concentration from obese Maturity onset Diabetics.

Hypothesis rejected : Diabetics had higher fasting plasma insulin than Controls after weight loss.

NULL HYPOTHESIS 3.5(vii)

Obese Controls, before and after a period of weight loss, show no difference in their maximum blood glucose response.

Hypothesis rejected : Controls show a greater maximum plasma glucose response after weight loss.

NULL HYPOTHESIS 3.5(viii)

Obese Diabetics, before and after a period of weight loss, show no differences in their maximum blood glucose response.

Hypothesis accepted

NULL HYPOTHESIS 3.5(ix)

Obese Controls show no difference in their maximum plasma glucose response from obese Diabetics after a period of weight loss.

Hypothesis rejected : Diabetics have a higher maximum plasma glucose response to a glucose challenge than Controls, after weight loss.

NULL HYPOTHESIS 3.5(x)

Obese Controls, before and after a period of weight loss, show no difference in their maximum insulin response.

Hypothesis accepted

NULL HYPOTHESIS 3.5 (xi)

Obese Maturity onset Diabetics, before and after a period of weight loss, show no difference in their maximum insulin response.

Hypothesis accepted

NULL HYPOTHESIS 3.5(xii)

Obese Controls show no difference from obese Diabetics in their maximum insulin response to a glucose challenge, after a period of weight loss.

Hypothesis rejected : Diabetics have a lower plasma insulin response to a glucose challenge than Controls, after a period of weight loss.

OBSERVE THE TRACE ELEMENT FLUCTUATIONS AFTER A PERIOD OF WEIGHT LOSS

FASTING GLUCOSE RESPONSE

NULL HYPOTHESIS 3.5(xiii)

Obese Controls show no fasting trace element fluctuations after a one month period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Erythrocytes	Cu	Fe, Rb
Plasma	-	Co, Cs, Fe, Rb, Zn
U 1	Co, Cs	-
U 2	Co	Fe, Se, Zn

All other trace elements show no other differences between the two groups.

* indicates that the values were rejected at the t 0.99 probability level.

- indicates that there were no trace elements which fellinto this category.

NULL HYPOTHESIS 3.5(xiv)

Obese Maturity onset Diabetics show no fasting trace element fluctuations after a one month period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Erythrocytes	-	-
Plasma	Rb	-
U 1	Se	Hg, Sb, Zn
U 2	Hg	Sb

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.5(xv)

Obese Controls and Maturity onset Diabetics show no fasting trace element differences, after a period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	-	-
Plasma	Co, Cr, Rb, Se	-
U 1	-	-
U 2	Cr, Cs, Fe, Hg, Se	Sb

All the other trace elements show no differences between the two groups.

MAXIMUM GLUCOSE RESPONSE

NULL HYPOTHESIS 3.5(xvi)

Obese Controls show no fasting differences in their trace element concentrations, at the point of maximum glucose response, after a period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Erythrocytes	Co	Cu, Rb
Plasma	Hg	Co

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.5(xvii)

Obese Maturity onset Diabetics show no differences in their trace element concentrations, at the point of maximum glucose response, after a period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Erythrocytes	Co, Fe, Zn	-
Plasma	Rb, Se	Cr, Hg

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.5(xviii)

Obese Controls and Maturity onset Diabetics show no differences in their trace element concentrations, at the point of maximum glucose response, after a period of weight loss.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Erythrocytes	Fe, Rb, Zn	-
Plasma	Cr	Sb

All other trace elements show no differences between the two groups.

Section 3.6

Trace Element Concentration of Hair Samples from Lean Individuals

Table 36 (a) : Lean Controls

Sample	Mean Elemental Concentration ($\mu g.g^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Head Hair	23.3 (21.1)	0.08 (.04)	136.1 (18.7)	0.75 (.28)	113.3 (59.8)	1.68 (.48)	0.52 (.22)	0.174 (.04)	0.160 (.11)	0.073 (.04)
Pubic Hair	25.0 (1.7)	0.03 (.009)	115.2 (23.6)	0.72 (.15)	50.9 (1.8)	2.05 (.19)	0.57 (.16)	0.046 (.007)	0.081 (.03)	0.035 (.004)

Table 36 (b) : Lean Diabetics

Sample	Mean Elemental Concentration ($\mu g.g^{-1}$) ($SD\bar{x}$) n = 5									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Head Hair	77.9 (37.8)	0.12 (.08)	185.9 (18.6)	0.66 (.09)	86.1 (30.1)	3.44 (.79)	0.38 (.12)	0.124 (.016)	0.126 (.077)	0.075 (.034)
Pubic Hair	29.9 (3.8)	0.04 (.01)	165.5 (51.7)	0.56 (.06)	67.5 (19.2)	2.53 (.50)	0.40 (.05)	0.056 (.009)	0.468 (.36)	0.040 (.008)

Trace Element Concentration of Hair Samples from Obese Individuals

Table 37 (a) : Obese Controls

Sample	Mean Elemental Concentration ($\mu g.g^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Head Hair	18.4 (3.03)	0.05 (.009)	173.1 (20.2)	0.46 (.06)	32.3 (4.71)	2.00 (.35)	0.27 (.05)	0.27 (.06)	0.043 (.012)	0.033 (.005)
Pubic Hair	90.8 (10.7)	0.18 (.11)	135.6 (15.9)	0.79 (.14)	89.9 (28.9)	5.20 (1.28)	0.29 (.11)	0.54 (.12)	0.081 (.021)	0.071 (.015)

Table 37 (b) : Obese Diabetics

Sample	Mean Elemental Concentration ($\mu g.g^{-1}$) ($SD\bar{x}$) n = 8									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Head Hair	15.9 (2.86)	0.04 (.008)	188.4 (16.1)	0.43 (.05)	25.4 (4.88)	2.04 (.46)	0.25 (.10)	0.11 (.03)	0.032 (.009)	0.032 (.0008)
Pubic Hair	197.3 (91.7)	0.39 (.33)	95.9 (36.9)	2.91 (2.14)	294.2 (244.9)	37.6 (22.8)	0.30 (.17)	0.46 (.17)	0.062 (.012)	0.055 (.009)

3.6 OBSERVE THE DIFFERENCES BETWEEN LEAN AND OBESE SUBJECTS IN THEIR TRACE ELEMENT CONCENTRATIONS OF HEAD AND PUBIC HAIR

NULL HYPOTHESIS 3.6(i)

1. Lean and obese Controls show no differences in their trace element concentration of head and pubic hair.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
Head hair	Cu	Cr, Zn
Pubic hair	Hg	Co, Cr, Cs, Cu, Fe, Rb

All other trace elements show no differences between the two groups.

* indicates that the values were rejected at the t 0.99 probability level.

- indicates that there were no trace elements which fell into this category.

NULL HYPOTHESIS 3.6(ii)

Lean and obese Maturity onset Diabetics show no differences in their trace element concentration of head and pubic hair.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
Head hair	Rb, Se	-
Pubic hair	-	Cr, Cs, Cu, Rb, Se

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.6(iii)

Lean Controls and Maturity onset Diabetics show no differences in their concentration of trace elements in head and pubic hair.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Head hair	-	Rb, Zn
Pubic hair	-	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.6(iv)

Obese Controls and Maturity onset Diabetics show no differences in their trace element concentration of head and pubic hair.

SAMPLE MEDIA	TRACE ELEMENTS SIGNIFICANTLY* DEFICIENT IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Head hair	Cr, Fe	-
Pubic hair	-	-

All other trace elements show no differences betweenthe two groups.

Section 3.7

Initial Plasma Fractionation Experiments

Table 38 Trace Elements not Protein Bound at time zero

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 8)	0.20 (.09)	0.0009 (.0002)	0.41 (.10)	0.05 (.006)	0.55 (.12)	0.12 (.02)	0.007 (.002)	0.006 (.002)	0.0008 (.0003)	0.0012 (.0001)
Obese Diabetics (n = 8)	0.32 (.12)	0.0007 (.0004)	0.58 (.09)	0.04 (.009)	0.65 (.09)	0.13 (.01)	0.003 (.0005)	0.003 (.0009)	0.0016 (.0005)	0.0012 (.0004)
Lean Controls (n = 5)	0.18 (.07)	0.0007 (.0002)	0.33 (.21)	0.04 (.02)	0.52 (.14)	0.18 (.04)	0.006 (.004)	0.002 (.0008)	0.0005 (.0002)	0.0005 (.0003)
Lean Diabetics (n = 5)	0.19 (.09)	0.0006 (.0001)	0.39 (.09)	0.05 (.008)	0.53 (.14)	0.22 (.04)	0.003 (.0009)	0.002 (.0004)	0.0007 (.0003)	0.0013 (.0001)

Table 39 Trace Elements Bound to Protein < 300,000 D at time zero

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 8)	0.26 (.08)	0.0006 (.0002)	0.79 (.12)	0.07 (.02)	0.76 (.14)	0.018 (.01)	0.0007 (.0004)	0.003 (.0009)	0.0024 (.0001)	0.00009 (.00005)
Obese Diabetics (n = 8)	0.24 (.07)	0.0007 (.0002)	0.69 (.10)	0.08 (.01)	0.82 (.16)	0.015 (.01)	0.004 (.001)	0.0017 (.0007)	0.0001 (.00007)	0.00001 (.00001)
Lean Controls (n = 5)	0.18 (.08)	0.0003 (.0002)	0.60 (.14)	0.10 (.01)	0.73 (.15)	0 0	0.008 (.0007)	0.0006 (.0001)	0.0011 (.0007)	0.0007 (.0004)
Lean Diabetics (n = 5)	0.23 (.10)	0.0007 (.0003)	0.71 (.17)	0.08 (.03)	1.45 (.40)	0.017 (.007)	0.014 (.006)	0.021 (.018)	0.0008 (.0003)	0.0002 (.0001)

Table 40 Trace Elements not Protein Bound at 60 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.21 (.10)	0.002 (.0005)	0.28 (.06)	0.04 (.006)	0.68 (.11)	0.14 (.01)	0.003 (.0007)	0.008 (.002)	0.0017 (.0008)	0.0010 (.0002)
Obese Diabetics (n = 6)	0.23 (.11)	0.0008 (.0004)	0.25 (.05)	0.03 (.008)	0.82 (.06)	0.10 (.02)	0.004 (.002)	0.003 (.0004)	0.0021 (.001)	0.0012 (.0005)

Table 41 Trace Elements not Protein Bound at 60 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.19 (.09)	0.0005 (.0003)	0.85 (.06)	0.08 (.009)	0.85 (.20)	0.01 (.01)	0.004 (.001)	0.003 (.0008)	0.0002 (.0001)	0.0002 (.00008)
Obese Diabetics (n = 6)	0.09 (.02)	0.0006 (.0005)	0.93 (.14)	0.09 (.004)	0.86 (.25)	0.03 (.02)	0.004 (.001)	0.002 (.001)	0.0002 (.0001)	0 0

Table 42 Trace Elements not Protein Bound at 90 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Lean Controls (n = 5)	0.17 (.10)	0.001 (.0003)	0.34 (.18)	0.07 (.02)	0.30 (.10)	0.17 (.01)	0.008 (.002)	0.002 (.001)	0.002 (.0007)	0.0003 (.00004)
Lean Diabetics (n = 5)	0.19 (.08)	0.0007 (.0004)	0.25 (.09)	0.03 (.004)	0.40 (.16)	0.20 (.02)	0.009 (.005)	0.002 (.0009)	0.0009 (.00001)	0.0007 (.0002)

Table 43 Trace Elements Bound to Protein ≤ 300,000 D at 90 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Lean Controls (n = 5)	0.20 (.05)	0.0003 (.0001)	0.74 (.15)	0.10 (.04)	1.64 (.40)	0.017 (.01)	0.017 (.004)	0.0003 (.0001)	0.0003 (.0001)	0.001 (.0002)
Lean Diabetics (n = 5)	0.21 (.07)	0.0005 (.0001)	0.69 (.13)	0.11 (.01)	0.92 (.21)	0.023 (.01)	0.011 (.004)	0.0024 (.0006)	0.0002 (.0001)	0.0004 (.0002)

Table 44 Trace Elements not Protein Bound at 120 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.23 (.11)	0.0007 (.00006)	0.39 (0)	0.03 (.002)	0.41 (.14)	0.13 (.003)	0.0015 (.0003)	0.002 (.0004)	0.0006 (.0003)	0.004 (.00009)
Obese Diabetics (n = 6)	0.22 (.09)	0.0007 (.0002)	0.51 (.25)	0.05 (.01)	0.65 (.32)	0.11 (.02)	0.0019 (.0007)	0.002 (.001)	0.0011 (.0006)	0.0012 (.0002)

Table 45 Trace Elements Bound to Protein $\leqslant$ 300,000 D at 120 mins.

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.15 (.04)	0.0003 (.00005)	0.81 (.08)	0.08 (.02)	0.64 (.11)	0.03 (.01)	0.0005 (0)	0.003 (.0003)	0.0003 (.0001)	0.0002 (.0001)
Obese Diabetics (n = 6)	0.13 (.08)	0.0007 (.0005)	0.51 (.21)	0.06 (.01)	0.74 (.23)	0.003 (.003)	0.0009 (.0001)	0.0002 (.0001)	0.00007 (.00001)	0 (0)

Plasma Fractionation Experiments using plasma from obese individuals after a period of weight loss

Table 46 Trace Elements not Protein Bound at time zero

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.20 (.12)	0.002 (.0005)	0.32 (.04)	0.04 (.008)	0.73 (.09)	0.14 (.009)	0.008 (.003)	0.004 (.001)	0.0013 (.0007)	0.0014 (.0002)
Obese Diabetics (n = 6)	0.31 (.10)	0.001 (.0003)	0.23 (.07)	0.04 (.02)	0.74 (.12)	0.05 (.02)	0.004 (.001)	0.004 (.0006)	0.0006 (.0001)	0.0008 (.0002)

Table 47 Trace Elements Bound to Protein $\leqslant$ 300,000 D at time zero

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.25 (.11)	0.0009 (.0008)	0.98 (.12)	0.09 (.02)	0.87 (.26)	0.02 (.008)	0.0003 (.0001)	0.0025 (.0015)	0.0013 (.0008)	0.00003 (.00002)
Obese Diabetics (n = 6)	0.27 (.08)	0.0005 (.0002)	1.09 (.07)	0.07 (.02)	0.69 (.19)	0.06 (.02)	0.011 (.01)	0.0017 (.0009)	0.0013 (.0006)	0.0005 (.0002)

Trace Elements not Protein Bound at time 60 mins.

Table 48

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.19 (.02)	0.001 (.0003)	0.31 (.08)	0.04 (.006)	0.69 (.12)	0.13 (.03)	0.003 (.0009)	0.0022 (.0004)	0.0013 (.0004)	0.0014 (.0002)
Obese Diabetics (n = 6)	0.08 (.01)	0.001 (.0003)	0.13 (.05)	0.03 (.004)	0.71 (.21)	0.15 (.01)	0.003 (.001)	0.0045 (.0018)	0.0016 (.0006)	0.0015 (.0003)

Trace Elements Bound to Protein ⩽ 300,000 D at time 60 mins.

Table 49

Subject Group	Mean Elemental Concentration ($\mu g.ml^{-1}$) ($SD\bar{x}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Obese Controls (n = 6)	0.02 (.005)	0.00004 (.0003)	0.93 (.06)	0.09 (.01)	0.87 (.22)	0.03 (.02)	0.005 (.002)	0.0053 (.0015)	0.00007 (.00004)	0.00012 (.00009)
Obese Diabetics (n = 6)	0.02 (.003)	0 (0)	1.03 (.14)	0.08 (.007)	0.85 (.05)	0.02 (.01)	0.007 (.004)	0.0007 (.0005)	0.0021 (.0017)	0 (0)

3.7 OBSERVE THE EFFECT OF THE TRACE ELEMENT CONTENT OF CERTAIN PLASMA PROTEINS DURING AN ORAL GLUCOSE TOLERANCE TEST

NULL HYPOTHESIS 3.7(i)

Lean and obese Controls show no differences in the concentration of their non bound trace elements at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
-	Cr, Cs

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(ii)

Lean and obese Controls show no differences in the concentration of trace elements bound to proteins $\leq$300,000 D, at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
Hg, Se	Cr, Rb, Sb

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(iii)

Lean and obese Diabetics show no differences in the concentration of their non bound trace elements at time zero.

TRACE ELEMETS SIGNIFICANTLY* LOWER IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
Rb	Sb, Zn

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(iv)

Lean and obese Diabetics show no differences on the concentration of trace elements bound to proteins $\leqslant$ 300,000 D at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN OBESE SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE SUBJECTS
Cs, Fe, Sb	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(v)

Lean Maturity onset Diabetics show no differences from lean Controls with respect to the concentration of non bound trace elements , at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN the Diabetics	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN The Diabetics
-	Cs

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(vi)

Lean Maturity onset Diabetics show no differences from lean Controls with respect to the concentration of trace elements bound to proteins $\leqslant$ 300,000 D, at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN OBESE (lean diabetic) SUBJECTS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN OBESE (lean diabetic) SUBJECTS
Cs	Fe, Rb

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(vii)

Obese Maturity onset Diabetics show no differences from obese Controls with respect to the concentration of non bound trace elements, at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cr, Hg, Se	Sb, Zn

All other elements show no differences between the two groups.

NULL HYPOTHESES 3.7(viii)

Obese Maturity onset Diabetics show no differences from obese Controls with respect to the concentration of trace elements bound to proteins of $\leq$300,000 D, at time zero.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cr, Cs, Sb	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(ix)

Lean Maturity onset Diabetics show no differences from lean Controls with respect to the concentration of non bound trace elements, at 90 minutes.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Se	Cs, Rb

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(x)

Lean Maturity onset Diabetics show no differences from lean Controls with respect to the concentration of trace elements bound to proteins $\leqslant$300,000 D, at 90 minutes.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cs, Fe, Hg	Co, Cr

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xi)

Obese Maturity onset Diabetics show no differences from obese Controls with respect to the concentration of non bound trace elements, at 120 minutes.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cs	Se

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xii)

Obese Maturity onset Diabetics show no differences from obese Controls with respect to the concentration of trace elements bound to proteins $\leqslant$300,000 D, at 120 minutes.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cr, Cs, Rb, Sb, Zn	Hg

All other trace elements show no differences between the two groups.

3.7 OBSERVE THE EFFECT OF THE TRACE ELEMENT CONTENT OF CERTAIN PLASMA PROTEINS DURING AN ORAL GLUCOSE LOAD, AFTER A ONE MONTH PERIOD OF WEIGHT LOSS

NULL HYPOTHESIS 3.7(xiii)

Obese Controls show no fasting differences in their non bound trace element concentration, after a period of weight loss.

TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Co, Fe	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xiv)

Obese Controls show no fasting differences in their concentration of trace elements bound to proteins $\leqslant$ 300,000 D, after a period of weight loss.

TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Co	Zn

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xv)

Obese Maturity onset Diabetics show no fasting differences in their non bound trace element concentration, after a period of weight loss.

TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
Rb, Sb, Zn	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xvi)

Obese Maturity onset Diabetics show no fasting differences in the concentration of trace elements bound to proteins $\leqslant$300,000 D, after a period of weight loss.

TRACE ELEMENTS SIGNIFICANTLY* LOWER AFTER WEIGHT LOSS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED AFTER WEIGHT LOSS
-	Cs, Rb, Sb, Zn

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xvii)

Obese Controls and Maturity onset Diabetics show no fasting differences in their non bound trace element concentrations.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Co, Cs, Hg, Rb	-

All other trace elements show no differences between the two groups.

NULL HYPOTHESIS 3.7(xviii)

Obese Controls and Maturity onset Diabetics show no fasting differences in the concentration of trace elements bound to proteins $\leqslant$300,000 D.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* HIGHER IN DIABETICS
-	Cs, Rb

All other trace elements show no fasting diferences between the two groups.

NULL HYPOTHESIS 3.7(xix)

Obese Controls and Maturity onset Diabetics show no differences in their non bound trace element concentration, at 60 minutes.

TRACE ELEMENTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Se, Zn	-

All other trace elemnts show no differences between the two groups.

NULL HYPOTHESIS 3.7(xx)

Obese Controls and Maturity onset Diabetics show no differences in the concentration of trace elements bound to proteins $\leqslant$300,000 D, at 60 minutes.

TRACE ELEMNTS SIGNIFICANTLY* LOWER IN DIABETICS	TRACE ELEMENTS SIGNIFICANTLY* ELEVATED IN DIABETICS
Cr	-

All other trace elements show no differences between the two groups.

Table 50 Trace Element Content of Thirteen Readily Available Insulin Preparations

Sample Name	Trace Element Concentration ($\mu g.ml^{-1}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Sc
Actrapid	3.10	0.007	33.8	0.12	4.93	1.04	0.028	0.067	0.022	0.014
Evan's Insulin	0.49	0.012	13.5	0.03	6.83	0.04	0.075	0.015	0.007	0.006
Hypurin	0.08	0.002	117.5	0.03	6.80	0.14	0.004	0.030	0.013	0.010
Insulatard	0.84	0.002	17.7	0.05	2.62	0.34	0.004	0.045	0.009	0.005
Isophane	0.85	0.011	17.1	0.03	1.77	0.37	0.003	0.032	0.005	0.005
Lentard	2.94	0.008	428.2	0.06	29.2	2.78	0.035	0.140	0.033	0.021
Lente	4.25	0.011	216.0	0.04	2.52	0.54	0.022	0.049	0.006	0.017
Mixtard	1.99	0.002	17.1	0.03	2.35	0.42	0.006	0.033	0.017	0.006
Monotard	2.93	0.013	339.2	0.10	23.1	2.17	0.028	0.110	0.017	0.036
Rapitard	3.55	0.008	57.4	0.05	5.14	0.99	0.018	0.060	0.050	0.007
Semitard	3.02	0.009	669.3	0.16	41.7	3.81	0.049	0.140	0.035	0.006
Ultratard	2.99	0.012	666.8	0.19	49.8	4.57	0.041	0.290	0.053	0.008
Veloinsulin	2.00	0.006	10.1	0.04	10.6	0.30	0.003	0.032	0.012	0.005

Table 51 Trace Element Content of Four Readily Available Hypoglycaemic Drugs

Sample Name	Trace Element Concentration ($\mu g.g^{-1}$)									
	Cu	Co	Zn	Se	Fe	Rb	Hg	Cr	Sb	Cs
Chlorpropamide	N.D.	0.001	0.33	0.08	6.18	3.01	0.028	0.042	0.011	0.007
Glibenclamide	6.70	0.057	2.05	0.21	190.1	2.37	0.030	0.110	0.070	0.038
Metformin	7.20	0.002	0.11	0.05	4.67	0.18	0.005	0.014	0.005	0.002
Tolbutamide	N.D.	0.001	0.19	0.05	1.56	0.13	0.005	0.025	0.003	0.002

CHAPTER IV

DISCUSSION AND CONCLUSIONS

4.1 Introduction

The previous chapter brought together all the data from a set of experiments which were designed to provide answers to several questions posed by the author after consideration of the work of earlier investigators.

Question 1:

Are circulating and excreted levels of zinc in Diabetics different from Controls?

Question 2:

Are circulating and excreted levels of copper in Diabetics different from Controls?

Question 3:

Do Diabetics exhibit low circulating concentrations of chromium?

Question 4:

Do all Diabetics have a poor chromium response to a glucose challenge? If so, is it related to the plasma glucose or insulin concentration?

Question 5:

If chromium is related to the hyperglycaemia observed in Diabetes, will the additional problem of obesity accentuate the chromium disturbances?

Question 6:

Are there any other trace elements which play a major role in the glucose uptake process?

4.2 Initial Investigation

In this initial investigation a blanket study was carried out which incorporated as many trace elements as could reasonably be measured in the maximum possible number of biological media. The three groups of individuals included Controls, Insulin requiring Diabetics and Maturity onset Diabetics; their trace element profile for one day was monitored.

(i) The fasting trace element concentrations of both groups of Diabetics were independently compared with the Controls and the results produced similar trends for certain elements- hypothesis 3.1(i)-(ii). Collectively the Diabetics had higher whole blood, serum and urine zinc concentrations together with lower plasma zinc than the Controls. This confirms the observations of Mateo et al(1975) and Ishii et al.(1980) relating to hyperzincuria in Diabetes. The copper concentrations seen in Diabetics were the same as the Controls with the exception of whole blood copper in the Maturity onset Diabetics and high serum copper in the Insulin requiring Diabetics. In the case of chromium both serum and urine levels were elevated in the Insulin requiring Diabetics and a raised whole blood chromium associated with reduced plasma concentration was observed in the Maturity onset Diabetics. The only other similarities were decreased concentration of antimony in plasma and urine along with increased whole blood and serum selenium levels, in both groups of Diabetics. Looking at the

Maturity onset Diabetics in isolation they would appear to be caesium and antimony deficient as the concentrations of these elements in whole blood, plasma and serum were depleted. The urinary excretion of antimony was also low whereas with caesium higher levels were excreted in the urine of the Maturity onset Diabetics than the Controls. Hence, the low circulating levels of caesium may be due to reduced utilisation and /or excessive excretion.

It was interesting to note that plasma and serum did not show the same results, as was expected, presumably because a large number of the trace elements were bound to the large plasma proteins which were subsequently consumed in the clotting process.

(ii) Hypotheses 3.2(i)-(iii) show the diurnal variation in the trace element concentrations observed in the three subject groups. In all the groups there was a marked increase in the number of elements excreted at the end of the day; caesium and rubidium being consistent throughout the groups. The Insulin requiring Diabetics and Controls had elevated urinary chromium concentration post dinner but in the Maturity onset Diabetic group, even though circulating chromium was elevated, chromium concentration of the urine remained unchanged. This may indicate that all the chromium accumulated during the day was needed for metabolism and hence none was excreted. Mercury stood out as an interesting element because the Controls had low circulating levels at the end of the day, whereas both groups of Diabetics had elevated circulating and excreted mercury concentrations. High levels of toxic elements like mercury were thought to be responsible,in part, for Maturity onset Diabetes.

In the Control Individuals both chromium and rubidium were higher at the end of the day whereas mercury was lower. Of the other elements caesium and copper were depleted in plasma and elevated in urine. As these elements are known to be transported attached to plasma proteins it was assumed that there was a metabolic excess which was excreted after food. The reverse may be true for zinc as the plasma and urine zinc concentrations were lowered post dinner. None of the remaining trace element alterations could be explained in a coherent way.

The Insulin requiring Diabetic individuals had low concentrations of cobalt post dinner, possibly due to cobalt deficiency, whereas mercury and selenium were in excess, unlike the Maturity onset Diabetic subjects who exhibited decreased circulating rubidium and zinc along with increased chromium, mercury and antimony.

Preliminary conclusions that could be drawn from these investigations were :

(a) The circulating and excreted concentrations of trace elements in Controls are different from Diabetics, both fasting and post dinner.

(b) Insulin requiring Diabetics do not exhibit all the same trace element anomalies as Maturity onset Diabetics and vica versa.

(c) All three groups of individuals have their own pattern of diurnal variation in trace element concentrations, which will reflect any inherent deficiencies or excess.

(d) Diabetics exhibit fasting hyperzincuria and a circulating deficiency of caesium and antimony.

(e) Insulin requiring Diabetics have decreased cobalt along with elevated mercury and selenium in all sample media post dinner.

(f) Maturity onset Diabetics have decreased rubidium and zinc along with elevated chromium, mercury and antimony post dinner.

4.3 HYPER AND HYPOGLYCAEMIA AND THEIR INFLUENCE ON CIRCULATING TRACE ELEMENT CONCENTRATIONS

Once it had been established that the trace element profile of Diabetics was fundamentally different from Controls, the next step was to find out if the trace element response to circulating glucose in Diabetics and Controls was also affected. This series of experiments was carried out using only a Control volunteer as maintaining a Diabetic patient in a state of hypoglycaemia was thought to be dangerous.

Figures 5(a) and (b) illustrate the trace element fluctuations which occurred in whole blood and plasma in response to an intravenous glucose challenge. The maximum plasma glucose concentration after ten minutes (Figure 6) coincided with a maximum positive response in the whole blood concentrations of chromium, rubidium, antimony, caesium and mercury. It would appear that the presence of glucose in the circulatory system stimulated the release of trace elements. A similar rise was noted in plasma although there was a lag period before the maximum response was recorded (30 minutes) and this increase in plasma trace elements took place during the period of glucose absorption. Three of the elements; chromium, copper and iron, gave similar responses to the presence of high plasma glucose. Any rise in whole blood concentration was complemented by a decreasing

concentration in plasma, this was especially noticable with copper and iron after the first thirty minutes. Uptake of these elements from plasma into erythrocytes would be the likely explanation.

It was also interesting to note that the responses of copper and zinc in whole blood were almost a mirror image, possibly indicating biological antagonism.

As a result of the observations during this experiment, which showed clearly how different elements respond to a glucose challenge, it was decided to pursue this work further : see section 4.5.

The experiments to investigate the circulating trace element response to an oral and intravenous glucose load, whilst in an hypoglycaemic state, were divided into three :

(i) In the first experiment the subject was given a 10g oral dose of glucose at 50 minutes.

(ii) In the second experiment the subject was given an intravenous dose of glucose at 90 minutes.

(iii) In the third experiment the subject was given a 10g dose of Guar orally at 50 minutes.

In order to successfully interpret the results of these experiments it was necessary to combine experiments (i) and (ii) rather than look at them in isolation. In each experiment the subject was stabilised for the first 50 minutes on an insulin infusion to develop a steady state of hypoglycaemia. The blood glucose concentration was maintained at 4mmoles.L^{-1} and the insulin concentration at 50mU.hr^{-1}.

Figure 8 illustrates that on introduction of glucose, orally,

there was a rise in pancreatic insulin production as glucose was being absorbed from the gastrointestinal tract. After 90 minutes the insulin concentration had started to drop back to its original level. Introduction of glucose intravenously (Figure 10) at 90 minutes resulted in a sharp increase in plasma insulin due to the rapid consumption of glucose. The presence of high levels of circulating glucose stimulated further prodution of insulin by the pancreas to allow for maximum glucose uptake, and by 150 minutes (uptake completed) the insulin concentration was declining.

In the first experiment the period of increasing plasma insulin was between 60 and 90 minutes, during this time a number of events occurred in the blood- Figures 7(a) and (b). The whole blood and plasma concentrations of chromium, rubidium, selenium, antimony, caesium and mercury increased beyond their fasting level.

However, the concentrations of cobalt and zinc decreased in whole blood and plasma due to uptake by the tissues. The samples were not collected beyond 120 minutes so it was not clearly established whether these elemental levels stabilised back to their starting concentration as the insulin concentration decreased.

The results of the intravenous glucose tolerance test provided additional information to support the data produced in experiment (i). The reduction in insulin concentration at 90 minutes, as the glucose was being absorbed, was consistent with a rise in the circulating comcentrations of whole blood and plasma chromium, zinc, rubidium, selenium, antimony, caesium and mercury. Both cobalt and iron were taken up by the erythrocytes with a corresponding

decrease in plasma concentration at this time. As the plasma insulin concentration began to rise between 100 and 120 minutes (due to glucose stimulation of the pancreas) most of these trace elements displayed a decrease in their whole blood and plasma concentrations suggesting that further uptake depleted the supply of available trace elements. The stabilisation of plasma insulin after 120 minutes, as blood glucose levelled out at 4mmoles.L , coincided with most of the trace elements returning to their fasting concentrations, the exceptions being plasma selenium, caesium and mercury along with whole blood antimony and mercury.

The conclusion drawn from these two experiments was that the stimulation for trace element mobilisation in the circulatory system was the presence of increased glucose concentrations and insulin consumption. The decreasing concentration of insulin (facilitating glucose uptake) appeared to be important as, during this period of time, the trace element concentrations displayed a clear increase in the blood. The mobilisation in the circulation of the trace elements could be due to glucose, insulin or a combination of the two. To answer this question it was necessary to observe the results of the third experiment.

In this experiment the subject was maintained in an hypoglycaemic state but after 50 minutes was given an oral dose of Guar (a glucose free carbohydrate). Allowing time for absorption of the Guar from the gastrointestinal tract the plasma insulin concentration dropped to 30mU.hr at 60 minutes, then returned to its previous steady state of 50 mU.hr . However, the trace element concentrations in whole blood changed very little, during

this period, from their resting value (Figure 11(a)). In plasma (Figure 11(b)), as the insulin concentration dropped, the concentrations of chromium, rubidium, iron, cobalt and caesium increased whereas antimony and zinc decreased. As the insulin concentration rose to 50 mU.hr most trace elements re-established their previous plasma concentration, with the exception of chromium, iron, and caesium which continued to fall. The continued decline in the level of these trace elements may have been due to either further cellular uptake or increased excretion. As there was no glucose present in the Guar the stimulation for the rise in trace element concentration in the blood must have been due to insulin utilisation, although where insulin utilisation was also coupled with glucose absorption (Figures 7 and 9) the trace element increases were much greater. The greatest increase was observed during the intravenous glucose tolerance test where glucose was rapidly absorbed and pancreatic stimulation was delayed.

The conclusion drawn from these data was that circulating concentrations of chromium, rubidium, selenium, caesium, antimony and mercury were increased in response to a glucose challenge.

GLUCOSE TOLERANCE STUDY

4.4 PLASMA GLUCOSE AND INSULIN RESPONSE TO THE GLUCOSE CHALLENGE IN OBESE AND LEAN SUBJECTS

Before investigating any trace element differences between lean / obese Controls and Diabetics it was necessary to observe whether weight had an influence on glucose disposal. Hypotheses 3.4

(i)-(viii) and Figures 19(a)-(b), 26(a)-(b) show that obesity does not appear to have an influence on fasting plasma glucose concentration as both obese and lean Diabetics have a higher glucose concentration than their Controls, whereas, obesity does influence insulin concentrations as obese Diabetics had a higher fasting insulin concentration than obese Controls. The maximum circulating plasma glucose and insulin concentrations were influenced by obesity- hypotheses 3.4(ix)- (xvi). Both obese and lean Diabetics achieve a higher circulating level of glucose than Controls, although the response of the lean Diabetics was the larger of the two. At the same time obese individuals have a greater circulating concentration of insulin with the obese Controls having higher levels than the Diabetics. In conclusion, Diabetics in general have higher plasma glucose than Controls although when coupled with obesity they also show elevated levels of plasma insulin. The Diabetic response to a glucose challenge was enhanced beyond that of a Control individual probably due to peripheral insulin resistance.

4.5 TRACE ELEMENT FLUCTUATIONS IN RESPONSE TO AN ORAL GLUCOSE CHALLENGE

Does obesity affect the concentration of circulating and excreted trace elements ?

Lean vs. Obese Controls

When comparing lean and obese Controls at time zero and at the point of maximum glucose concentration their trace element concentrations were affected by obesity, hypotheses 3.4(xvii) and (xxi); Figures 15 and 17, and Figures 22 and 24. Obese Controls

as compared with lean controls (Figures 22 and 15) had elevated levels of erythrocyte chromium and caesium also plasma chromium, copper and zinc (Figures 24 and 17).

Lean vs. Obese Diabetics

When comparing the lean and obese Diabetics, the obese Diabetics we found to be deficient in erythrocyte mercury and rubidium along with plasma rubidium (Figures 16 and 18, Figures 23 and 25). The only observed similarities between the obese Controls and Diabetics was the elevated concentration of plasma cobalt at the glucose peak.

Lean Controls vs. Lean Diabetics

In a comparison of the response of the lean Controls and Diabetics to an oral glucose challenge, lean subjects exhibited very few differences in trace element concentration during the glucose tolerance test (Figures 15 and 16, Figures 17 and 18). Only erythrocyte iron and rubidium together with plasma rubidium were found in higher concentration in the Diabetics. The increased circulating concentrations of these trace elements were not reflected in the urine (Tables 21(a)-(b)). In summary, the circulating concentrations of zinc, copper, chromium, rubidium, selenium and antimony remained fairly constant throughout the three hours in both erythrocytes and plasma of the lean Controls. A change in the erythrocyte concentration of mercury and iron was followed by the corresponding response in plasma whereas when the erythrocyte concentration of caesium rose the plasma concentration remained constant, with the opposing response in the case of cobalt. For the lean Diabetics the only elements to remain

constant in both erythrocytes and plasma were chromium and cobalt. The vast majority of the trace elements (mercury, antimony, iron and rubidium) showed a decline in erythrocyte concentration closely followed by a drop in plasma concentration. Finally, the concentrations of copper, caesium and selenium rose steadily in the erythrocytes whilst remaining constant in the plasma.

Obese Controls vs. Obese Diabetics

The obese individuals displayed a greater number of trace element anomalies than the lean subjects (Figures 22 and 23, Figures 24 and 25). Obese Diabetics were found to be consistently chromium deficient. Figure 22 illustrates the copper and zinc interaction seen in the earlier experiments on hyperglycaemia. The decline in the concentration of erythrocyte chromium, mercury and zinc, without a corresponding increase in plasma or urine concentration, must indicate that cellular uptake was responsible for their disposal. Cobalt showed an interesting response in both erythrocytes and plasma, where a peak of concentration in the former was associated with a concentration trough in the latter at both 60 and 150 minutes. Glucose oxidation is known to be aided by the addition of cobalt and as erythrocytes are insulin independent, this effect may be accounted for by increased glucose metabolism of this tissue. For obese Diabetics both erythrocytes and plasma chromium concentrations declined over the period of three hours and this may indicate that chromium is important in glucose uptake of the obese Diabetics. Mobilisation of copper, iron and zinc appeared to take place during this experiment as both erythrocyte and plasma concentrations of these elements rose

as the glucose concentration declined. In the case of mercury, rubidium and selenium an elevation of erythrocyte concentration was accompanied by a decline in plasma concentration.

Although the above account gives an element by element statement of the trace element changes during the oral glucose tolerance test the salient points are listed below :

(a) Obesity, alone, appears to lead to an elevation of cobalt, chromium, copper, caesium and zinc in the obese Controls along with the elevation of cobalt and the decline of mercury and rubidium in obese Diabetics.

(b) Lean Controls, during the course of the glucose tolerance test, mobilised cellular cobalt and caesium, whilst mercury and iron were utilised by the tissues.

(c) Lean Diabetics, during the course of the glucose tolerance test, appeared to mobilise copper, caesium and selenium whereas iron, mercury, antimony and rubidium were subject to cellular uptake.

(d) Obese Controls, during the course of the glucose tolerance test, mobilised cobalt into their erythrocytes from the tissues whereas chromium, mercury and zinc were taken up by the tissues.

(e) Obese Diabetics are chromium deficient.

(f) Obese Diabetics, during the course of the glucose tolerance test, mobilised cellular copper, iron and zinc.

Unlike the results in section 4.3 these glucose tolerance experiments do not show clear cut similarities between the different groups. However, the peaks of trace element activity

did occur at the point of glucose absorption as observed earlier. It may be that using an intravenous glucose tolerance test would have provided more answers, as then gastrointestinal absorption would not have been a limiting factor. Unfortunately this facility was not available to the author. In retrospect these experiments may have yielded more accurate information if larger sample groups of mixed sexes had been available as participants. The lean individuals, in this study, were all men whereas the obese individuals were all women, this probably adversely affected the results.

4.6 THE CHANGING PLASMA GLUCOSE AND INSULIN RESPONSE IN OBESE SUBJECTS AFTER WEIGHT LOSS

A number of obese individuals underwent a one month period of dietary restriction in order to reduce their weight and hopefully their hyperglycaemia. The Cambridge diet (a nutritionally balanced dietary supplement which contains trace elements) provided only 330 Calories per day during this period and at the end of the four weeks the mean weight loss of the obese Controls was 9.72 kg and with the obese Diabetics was 6.83 kg (Table 14). The weight loss resulted in a reduction in fasting hyperglycaemia and circulating insulin concentration of the obese Diabetics- hypotheses 3.5(i)-(vi) [Figures 33(a) and (b)]. However, the Diabetics still displayed higher fasting plasma glucose and insulin levels than the Controls.

The maximum glucose response in the obese Controls was greater after the period of weight loss although the plasma insulin concentration remained unchanged. The obese Diabetics displayed

no differences in circulating plasma levels of glucose and insulin after the period of weight loss- hypotheses 3.5(vii) - (xii). The overall effect was that the Diabetics had higher glucose and lower insulin levels than their corresponding Controls after weight loss.

4.7 THE ALTERED TRACE ELEMENT FLUCTUATIONS IN OBESE SUBJECTS (AFTER WEIGHT LOSS) IN RESPONSE TO AN ORAL GLUCOSE CHALLENGE

The process of weight control not only reduces the hyperglycaemia observed in obese individuals but also alters their trace element metabolism with regard to the way they respond to an oral glucose challenge.

Controls

Comparing obese Controls before and after weight loss the most striking difference was the increase in the number of trace elements excreted in the urine (Table 28(a) and 35(a)). The elevated circulating concentration of iron was excreted along with excess selenium and zinc. Whereas cobalt appeared to be deficient because its concentation in urine, both before and after the glucose challenge, was low. At the point of maximum glucose response the erythrocytes were depleted of cobalt and the plasma was showing an excess, which would indicate mobilisation from the tissues was taking place, especially as the excretion of cobalt was lower than before the period of weight loss- hypotheses 3.5(xiii) and (xvi). Comparing Figures 22 and 24 with 29 and 31 it can be seen that the zinc/copper interaction observed in the erythrocytes initially has now disappeared and the actions of these two elements are now synchronised . The biphasic response

of cobalt has also ceased to ex ist after weight reduction, possibly because there was no excess glucose. The circulating concentrations of selenium, rubidium, caesium and zinc were fairly constant in these individuals whereas iron and cobalt appeared to be mobilised from the tissues.

Diabetics

A comparison of the obese Diabetics before and after weight loss revealed that the excretion of antimony was increased during the period of the glucose tolerance test along with the conservation of mercury by reducing its excretion (Tables 28(b) and 35(b)). Several elements were significantly lower in the erythrocytes (cobalt, iron and zinc) and plasma (selenium and rubidium) at the point of maximum glucose response whereas plasma chromium, at this point, was elevated- hypotheses 3.5(xiv) and (xvii). Figures 30 and 32 illustrate the trace element fluctuations observed in obese Diabetics after weight reduction. The circulating concentrations of copper, rubidium, selenium, iron and cobalt all show a maximum response in plasma at 90 minutes (glucose maximum) and return to their original concentrations by 150 minutes. Although the circulating level of chromium declines in the course of the test a large increase in chromium concentration in plasma was recorded at 120 minutes which coincided with the maximum insulin concentration. As described earlier, the reduction in circulating chromium concentration was probably due to facilitation of glucose uptake by insulin. After their period of weight loss the Diabetics mobilised chromium from their tissues rather than depleting their erythrocyte concentration. The remaining trace

elements, mercury and caesium, had only one small peak of activity during the three hours where their concentration changed.

Controls vs Diabetics

Obese Controls and Maturity onset Diabetics, both having been on weight reducing diets, show different responses to a glucose challenge. Both fasting and at a maximum glucose concentration the Diabetics were deficient in chromium and rubidium, in order to alleviate this a number of elements were conserved by excreting lower levels in the urine (chromium, caesium, iron, mercury and selenium) and increasing their plasma concentrations between 90 and 120 minutes. In conclusion, it would appear that the obese Controls mobilised tissue cobalt and iron (as before their period of weight loss) but they no longer released chromuim, mercury and zinc to their tissues. As for the obese Diabetics, they now appeared to be chromium and rubidium deficient but were able to release these two trace elements from the tissues when necessary. Although the weight loss relieved the hyperglycaemia it did not reduce sufficiently the circulating concentrations of insulin. High circulating insulin in the Diabetics was in part responsible for the increase in circulating trace element concentrations which were then excreted in the urine. Unfortunately this led to the deficiencies observed in these individuals. In conclusion, the hyperinsulinaemia observed in Diabetics whether before or after weight loss appeared to be responsible for the trace element abnormalities observed in these experiments.

4.8 TRACE ELEMENT DIFFERENCES BETWEEN LEAN AND OBESE INDIVIDUALS IN HAIR SAMPLES

The use of hair as an indicator of disease has been disputed by many investigators but as this medium was easily obtained from the patients the trace element concentration of head and pubic hair was measured.

Both types of hair collected from obese individuals had higher concentrations of the elements chromium, caesium, copper and rubidium, than the lean individuals, in both Controls and Diabetics (Tables 36(a) and (b); 37(a) and (b)). Why this should be so was not known except that it reflected the increases in plasma trace elements, and as the obese patients tended to have less hair than the lean individuals the elements may have been more concentrated. A comparison of the differences in the trace element content of hair from lean and obese subjects revealed no striking trends. In fact the only significant differences registered were elevated levels of rubidium and zinc in head hair of the lean Diabetics and low levels of chromium and iron in the head hair of the obese Diabetics.

The data provided by these experiments would suggest that no significant correlations exsist between trace elements in hair and disease, except perhaps in the case of obese Diabetics who showed a persistent tendency to be chromium deficient and this was upheld by recording a low value for hair chromium.

4.9 TRACE ELEMENT INTERACTION WITH CIRCULATING PLASMA PROTEINS

All the investigations led to one general conclusion that plasma chromium and rubidium were important trace elements in the process of glucose disposal. Hence, samples of plasma were separated into two major fractions and the trace element concentration of these was measured.

Once again lean individuals did not have the same pattern of bound and non bound trace elements as obese subjects. Obese Controls had higher fasting levels of total chromium and bound rubidium than the lean Controls. Whereas obese Diabetics had only elevated levels of non bound rubidium- hypotheses 3.7(i)-(iv) and Tables 38 and 39- as compared to lean Diabetics.

On comparing lean Controls with lean Diabetics the only trace element difference was that of bound rubidium which was significantly higher in the Diabetics- hypotheses 3.7(v) and (vi). However, obese Diabetics displayed a deficiency of trace elements, both bound and non bound, one of which was chromium- hypotheses 3.7(vii) and (viii).

Although the above data gave an insight into the possible disturbance of chromium and rubidium metabolism in Diabetics, the biggest discrepancy should have occured at the point where these trace elements were in greatest demand i.e. at the point of maximum glucose uptake. A comparison was made of the trace element concentration of these plasma fractions over a time span covering the period of glucose absorption.

Both the lean Controls and Diabetics had a decrease in the concentration of chromium bound to protein and a decrease in the

amount of rubidium bound to protein during this period (Tables 40 - 45). From these results it was assumed that the bound chromium was being used in the glucose absorption process, possibly taken into the tissues, whereas the non bound chromium concentration remained stable during this period. These data would support the ideas of previous investigators who have suggested that chromium was part of a Glucose Tolerance Factor (Mertz 1974), needed for the cellular uptake of glucose, interacting with plasma insulin.

Looking at the data for the obese individuals, the Controls showed an increasing concentration in the amount of rubidium protein-bound and a drop in the level of non bound chromium whereas the bound levels of chromium remained constant. Over the same period of time the obese Diabetics exhibited a decline in the amount of chromium and rubidium that was protein bound. The delayed response to an oral glucose challenge in obese individuals coupled with hyperglycaemia and hyperinsulinaemia may have been partly due to the inability of these individuals to make use of their protein bound chromium. As these individuals have already been established as chromium deficient not only in total plasma but now also in protein bound chromium, this may explain their inability to adequately absorb glucose.

After a period of weight loss the fasting concentrations were unaffected, however the chromium concentration at the glucose peak was lower in the protein bound fraction- hypotheses 3.7(xiii)-(xx) [Tables 46-49].

Further evidence would be needed to confirm whether there is the possibility of a chromium-protein complex present in the blood. However, as chromium can also be classified (in higher concentration) as a toxic element it would be correct to assume this deficiency may be due not to a lack of the chromium in the plasma but possibly a lack of the protein to which it becomes bound. Diabetics are known to have a disturbed protein metabolism and therefore this factor may be more important in the adequate disposal of glucose than was previously thought. Further investigations into the intravascular mobilisation of trace elements should include a full study of the larger metal binding proteins to provide a clearer picture of the involvement of chromium (and other trace elements) in glucose absorption.

4.10 TRACE ELEMENT CONCENTRATION OF HYPOGLYCAEMIC DRUGS

Although all individuals participating in this study were deprived of their medication twenty four hours prior to sample collection, the trace element concentration of the commonly prescribed hypoglycaemic drugs were measured.

In general the insulin preparations and the hypoglycaemic tablets contained high levels of most of the trace elements, with the exception of zinc which was much higher in the insulins (Tables 50 and 51). When looking at these data it was also clear how important deprivation of medication was to the investigation. The high concentrations of many of the elements such as chromium and rubidium, which were thought to be important in this investigation, would possibly have masked any fluctuations taking place had medication been continued. It may be true to say that

24 hours was not sufficient time to reduce the masking effect of the drugs, however, patients were not subjected to conditions thought to be detrimental to their well being.

4.11 CONCLUSIONS

At the begining of this chapter several questions were posed which can be answered in the light of the data produced during the total study.

Question 1 :

Are the circulating and excreted levels of zinc in Diabetics different from Controls ?

Yes. All Diabetics exhibit hyperzincuria and their circulating levels of whole blood and serum were higher than those found in Control individuals. Both of these observations would confirm the idea that a reduction in insulin production in Diabetes leads to an excess of circulating zinc, which can be excreted via the urine.

Question 2 :

Are circulating and excreted levels of copper in Diabetics different from Controls ?

No. Diabetics did not show any significant differences in circulating or excreted levels of copper. However, this does not necessarily imply that copper metabolism in the tissues was not affected because copper may have been adequately controlled by homeostasis in blood as it, like zinc, is a vitally important element in the human body.

Question 3 :

Do Diabetics exhibit low circulating concentrations of chromium ?

Yes. Maturity onset Diabetics were seen to be chromium deficient, however, the few Insulin requiring Diabetics taking part in this study had elevated serum and urine concentrations of chromium. The increased excretion of chromium by these individuals would indicate that although serum levels were raised the excess chromium was excreted i.e. not available for glucose absorption. It was shown that protein bound chromium was required in this process of glucose uptake and this would not be easily filtered at the glomerulus.

Question 4 :

Do Diabetics have a poor Cr response to a glucose challenge ? If so, is it related to the plasma glucose or insulin concentration ?

Yes. All the Diabetics (studied in these experiments) had a poor response to a glucose challenge. Intravascular mobilisation of trace elements was related to both insulin and glucose concentrations. Although the plasma concentrations of these elements increased with insulin action on the tissues, the effect was enhanced by the presence of glucose.

Question 5 :

If chromium is related to the hyperglycaemia observed in Diabetes, will the additional problem of obesity accentuate the chromium disturbances ?

Yes. Obesity leads to the elevation of circulating levels of chromium, copper, caesium and zinc in the Controls in order to deal with the excess glucose whereas these increases were not measured in the Diabetics. Hence, the chromium deficiency was greatest in the obese Maturity onset Diabetics.

Question 6 :

Are there any other trace elements which play a major role in the glucose uptake process ?

The following elements were all elevated in the plasma of all individuals during a glucose challenge, at the same time as chromium ;

Antimony

Caesium

Mercury

Rubidium

Selenium

The uptake of cobalt and iron into the erythrocytes also appeared to be important for oxidation of glucose within this tissue.

The involvement of plasma caesium and rubidium may be in association with the membrane ATPase for uptake of glucose into cells especially as Diabetics are known to be hypokalemic i.e. rubidium may substitute for potassium in this pump mechanism. What roles antimony, selenium and mercury play in this process are unknown.

One of the main problems with this type of work was whether the trace element fluctuations were a true reflection of the disturbed metabolism. As the work developed it became apparent that the isolation of the protein carriers for the trace elements of interest was of vital importance, because these proteins would yield more reliable results of the altered metabolism than total plasma concentration. As yet noone has isolated the Glucose Tolerance Factor although there appears to be data substantiating

its ex istence. However, the experiments carried out in this work were designed to achieve the maximum effect possible without endangering the participants with the hope that any discrepancies would come to light.

Analytically, the techniques of Neutron Activation Analysis and Atomic Absorption Spectrometry were the most sensitive techniques for measuring the low concentrations seen in the human body. Care was taken in educating medical staff on the proce dures of sample collection for trace element studies to reduce the possibility of contamination. However, the detection of certain elements was approaching the lower limits of the instrumentation and so large standard deviations were inevitable e.g. cobalt, chromium, antimony and mercury.

4.12 FUTURE WORK

These investigations into the role of trace elements in Diabetes mellitus, as well as answering several pertinent questions, put forward new ideas for future investigators to pursue. These include :

(a) Isolation of the protein carrier responsible for the increase in circulating levels of chromium in response to a glucose challenge.

(b) Do Diabetics show a deficiency of chromium in their insulin dependent tissues of the body which leads to reduced peripheral disposal of glucose ?

(c) Observe the trace element concentrations of the insulin independent tissues i.e. brain. How do they differ from those of insulin dependent tissues ? Or is this insufficiency, in the insulin dependent tissues of Diabetics, purely a membrane phenomenon.

(d) What is the role of rubidium in glucose tolerance, is it related to the membrane ATP pump ?

(e) Repeat the experiments with Insulin requiring Diabetics to observe whether their lack of insulin leads to a disturbed chromium response in the blood. They may have excess concentrations of circulating protein bound chromium if deprived of their insulin stimulus.

The field of trace element research has only just begun to break the surface in the vast world of medical research and until investigators are made fully aware of the drawbacks the progress will be slow. Hopefully, this work has awakened ideas which in the future may lead to improved metabolic control for patients with Diabetes mellitus.

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