TRACE METAL BIOCHEMISTRY : INFLUENCE OF INTRAVENOUS INFUSION OF A MULTIELEMENT MIXTURE

Mrs Shailja Vijay Nigdikar, B.Sc (Hons)

University Department of Pathological Biochemistry

Glasgow Royal Infirmary

(Medical Science)

A thesis submitted for the degree of Master of Science, to the

Faculty of Medicine, University of Glasgow,

Glasgow, Scotland, U.K.

August 1982

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I would like to express my thanks and gratitude to Dr G.S.Fell, for his encouragement, excellent guidance and personal interest in my subject during the past two years.

Also, I would like to thank Professor H.G.Morgan, for giving me the opportunity to work in his department, where the laboratory facilities are excellent and the staff members are very helpful.

Financial assistance from Kabi-Vitrum, Sweden, is gratefully acknowledged.

I am more than grateful to Dr A.Shenkin, for his guidance, and also to Dr D.J.Halls, who gave me continuing help and advice. Also, Mrs P.Dunbar, for her valued co-operation.

Last, but not least, I wish to thank Dr A.D.S.Smith, for his help in obtaining the samples which were essential to the success of my research.

SUMMARY

Trace element biochemistry following a multielement intravenous infusion in man was studied. A new trace-element additive mixture was prepared by Kabi-Vitrum, Sweden (code 4854) to meet the requirements of moderately ill patients. This mixture provided a daily supply of Fe 20 μmol (1.12 mg); Zn 100 μmol (6.54 mg); Cu 20 μmol (1.27 mg); Mn 15 μmol (0.82 mg); Cr 0.4 μmol (20.8 μg); Se 0.4 μmol (31.58 ug); Mo 0.2 μmol (19.19 ug); I 1.0 μmol (0.13 mg); F 50 μmol (0.95 mg). To evaluate the efficacy of this mixture in meeting the trace-element requirements in patients, three different studies were undertaken. Serum and urine samples were collected with precautions to avoid contamination. Levels of zinc, copper, manganese, chromium and selenium, in serum and urine were determined by proven atomic absorption techniques. Results were compared statistically within and between the groups and to appropriate reference values. This thesis deals with the determination of zinc, copper, manganese, chromium and selenium in serum and urine. Methods for Mo, I and F were not readily available. Studies were as follows:

Crystalloid Study: In this study, twenty patients undergoing elective abdominal surgery were randomly allocated into two groups. Group A received saline and glucose (5%) only for four post-operative days. Group B received an additional daily infusion of trace-element mixture (code 4854). In Group A serum zinc fell sharply, but returned to normal by day five. Serum copper, manganese, chromium and selenium were unchanged and urinary excretions did not alter. In Group B, serum zinc followed the same pattern as Group A, but serum copper and selenium were mostly unaffected. Serum and urinary chromium and manganese were markedly increased during infusion, whereas

urinary zinc, copper and selenium were not significantly increased.

Surgical I.V.N.: In this study, sixteen patients undergoing elective surgery were studied for more than seven days. They received 14 g N and 3000 KCal/day (2000 KCal as glucose, and 1000 KCal as Intralipid) and trace element additive mixture. These patients were divided into two groups. Group A as anabolic patients with urine N less than 12 g/day. Group B as catabolic patients with urine N greater than 14 g/day. In both groups, serum values of zinc, copper and selenium remained normal, while manganese and chromium increased. Urinary excretion of copper was unaffected, while that of zinc, manganese, chromium and selenium was increased.

Medical I.V.N.: Four patients were studied for more than fourteen days. The infusion regimen was similar to that used in surgical IVN. Serum zinc and copper were maintained during infusion and manganese and chromium were elevated, while selenium was at the lower level of normal range. Urinary excretion was increased for chromium, zinc and manganese, while copper and selenium were unaffected.

The supplement of zinc and copper was sufficient to correct any pre-existing abnormality and to maintain most of the patients. However, for patients with excessive intestinal fluid loss higher amounts may be desirable. The provision of manganese and chromium may have been excessive, only half of the amount provided may be enough for most patients.

No clinical or biochemical abnormality suggestive of deficiency or toxicity for any of the infused elements was found.

CHAPTER 1

INTRODUCTION

INTRODUCTION

The human body mostly consists of water, protein, carbohydrate and fat. Inorganic elements in tissue could be classified as major elements such as the electrolytes (Na, K, Cl) or minerals such as (Ca, Mg, P). These are present at levels of several hundred parts per million, that is milligrams per litre. There are also about fifty inorganic elements present at lower concentrations which have been described as "trace elements", since by earlier analytical techniques their levels could not be reliably determined. Bioscientists define trace elements as a group of elements that occur in animal and plant tissues at concentrations ranging from several hundred μg/g to barely detectable levels of the order of pico gram per gram (pg/g). By widely accepted custom, elements that do not exceed the tissue concentrations of iron are included in this category:

An element is defined as "essential element" if:

A) The amount present in the tissue of a particular species is constant.

B) Removal from diet produces reproducible abnormalities.

C) Addition back to diet of that element alone, reverses the changes.

D) Clinical reversible changes are accompanied by biochemical effects.

Major Elements and Electrolytes

These are the eleven elements present in substantial amounts forming 99.5% of tissue. They are carbon, hydrogen, oxygen, nitrogen, sulphur, calcium, magnesium, phosphorous, sodium, potassium and chloride.

1.2 Essential Trace Elements

There are fourteen trace elements which are present in the human body, at concentrations less than 50 mg/kg of tissue and in body fluids at less than 1 mg/litre (in micromolar or nanomolar concentrations). Less than half of these essential elements are of recognised importance in human medicine even although their biological requirement has been demonstrated in one or more animal species (Underwood 1979). The elements are F, Cr, Fe, Co, Cu, I, Mn, Mo, Ni, Se, Si, V, Sn, Zn. Exclusion of each of these trace elements from the diet of experimental animals produces a reduction in growth, failure of normal development, or other adverse effects on health, which are reversible uniquely upon re-introduction of that element to the diet .

The first discovery of an essential trace metal (Iron) was in the 17th century. Two centuries later, iodine was also added in this category, but since 1970 with the development of analytical techniques such as polarography, neutron activation and spectrophotometry, more and more essential trace elements have been discovered. Table 1 gives a list of essential trace elements and their year of discovery. The experience of the past two decades suggests that the number of trace elements of biological interest, may continue to increase in the future.

TABLE 1

Discovery of Trace Elements

Element	Year	Effect of deficiency
Iron (Fe)	17th century	Anaemia
Iodine (I)	1850-19th century	Goitre
Copper (Cu)	1928	Anaemia and bone problems
Manganese (Mn)	1931	Growth impairment, reproductive disorders, neuromuscular diseases
Zinc (Zn)	1934	Growth defect, skin disorders for both man and animals
Cobalt (Co)	1935	Growth and anaemia
Molybdenum (Mo)	1953	Essential for animal growth
Selenium (Se)	1959	Growth and fertility in animals
Chromium (Cr)	1959	Growth, reduce life span, disturbances of glucose, lipid and protein metabolism
Tin (Sn)	1970	Animal growth
Vanadium (V)	1971	Animal growth
Fluoride (F)	1971	Dentalcaries in animal and man
Silicon (Si)	1972	Connective tissue
Nickel (Ni)	1974	Animals only

Other elements such as Arsenic (As), Cadmium (Cd) and Lead (Pb), are considered as border-line cases.

Many of these elements are of fundamental biochemical importance. For example, zinc is a key factor in nucleic acid and protein synthesis, selenium is a part of the cellular anti-oxidant defence. The increased sensitivity and specificity of modern analytical methods, such as atomic absorption spectrophotometry when carefully applied, can reliably determine most of the essential elements

at the levels normally occurring in human tissue and body fluids.

The ultimate objective of trace element research is the understanding of the mode of action of an element at the molecular and organ level, of its metabolism and requirement by different species and of the consequences of inadequate intake. This knowledge serves as the basis for assessment of the nutritional status of individuals and population groups and if necessary, as justification for intervention prgrammes to assure adequate intakes.

Patients with massive injuries, after extensive surgical procedures, or critically ill patients, can be stabilised and maintained for long periods by the use of supplementary nutrition such as intravenous feeding. In giving the parenteral nutrition regimen, most consideration is usually given to the volume of fluid which should be infused, the amount of amino acid nitrogen necessary and the amount and chemical form of energy substrate. Although these are the major nutrients, it is important to remember that the objective of complete parenteral nutrition is to provide by the intravenous route, all nutrients in the same amount and chemical form as would normally be absorbed via the gastro-intestinal tract from an adequate oral diet. Thus, an appropriate infusion of major and minor elements in their appropriate chemical form, together with all vitamins, should ideally be given.

To have complete intravenous nutrition theoretically, fourteen essential elements should be added to the regimen. But this is not practicable for three reasons:

A) The amounts and chemical form in normal diet are not known for all trace elements, especially those at the lowest level. (Silicon, Nickel, Arsenic, Vanadium, Molybdenum).

B) The amount and chemical form needed intravenously is unknown since the various factors in regulating normal gastro-intestinal

uptake are far from understood.

C) The altered intravenous requirement of various categories of ill patients are not known.

With those uncertainties, it is considered wise to only propose addition of the better established elements of known metabolic importance. Elements in this category are zinc, copper, iron, chromium, selenium, manganese, molydenum, fluoride and iodide. The elements in this group can be considered in three main categories:

1) Those where clinical deficiency or incidence of biochemical depletion is well established during intravenous nutrition. For example, Iron, Zinc and Copper.
2) Those where clinical deficiency or depletion although reported, is much rarer, for example, chromium, selenium and molybdenum.
3) Those where although the metabolic and biochemical importance of the element is undoubted, there are few or equivocal reports of deficiency or depletion during intravenous feeding. For example, manganese, fluoride and iodide.

This thesis deals with the experimental use of a trace element additive mixture given to hospital patients.

A mixture of trace elements has been available for some time in this country. (Addamel^{R}, Kabi-Vitrum, Sweden). Studies have suggested that the amount of zinc, in particular, may be too low for most patients and the level of manganese may be too high. Moreover, chromium and selenium are not present. Therefore, a new solution (code 4854) has been formulated by Kabi-Vitrum, Sweden, the composition of which is being designed to meet the requirement of most patients receiving parenteral nutrition. This is based on the recommendations Shenkin & Wretlind (1974),together with levels of intake suggested by an expert panel of American Medical Association

and Recommended Dietary Allowance of the United States National Academy of Sciences (1980).

The new essential trace element solution (code 4854) contains nine essential trace elements, the composition of which is shown in Table 2. This thesis deals with the determination of zinc, copper, manganese, chromium and selenium in urine and serum of all patient groups studied.

Methods for molybdenum, fluoride and iodide were not readily available, so no supplemental studies are reported.

TABLE 2

Composition of New Trace Element Solution (4854) and Addamel^{R}

Element present in solution	New trace element solution (code 4854)	Addamel^{R}
Zinc (Zn)	100 µmol : 6.54 mg	20 µmol : 1.31 mg
Iron (Fe)	20 µmol : 1.12 mg	50 µmol : 2.79 mg
Copper (Cu)	20 µmol : 1.37 mg	5 µmol : 0.32 mg
Manganese (Mn)	15 µmol : 0.82 mg	60 µmol : 3.30 mg
Selenium (Se)	0.4 µmol : 31.58 ug	-
Chromium (Cr)	0.4 µmol : 20.8 ug	-
Molybdenum (Mo)	0.2 µmol : 19.19 ug	-
Fluoride (F)	50 µmol : 0.95 mg	50 µmol : 0.95 mg
Iodide (I)	1.0 µmol : 0.13 mg	1.0 µmol : 0.13 mg
Magnesium (Mg)	-	1.5 µmol : 36.47 ug
Calcium (Ca)	-	5.0 µmol : 0.20 mg

(The preparations comes as solution containing 3 gram Sorbitol and water to 10 ml).

1.3 Different Studies Undertaken

Three different studies were undertaken to evaluate the new trace element additive (code 4854).

1) Evaluation of new trace element solution (4854) as an additive to standard crystalloid infusions in post-surgical adult patients:

As a part of the evaluation it was necessary to assess the effects of infusion of essential trace metals over a short period of time. The possibility of abnormal serum levels being associated with infusion of this solution would therefore be detected. This was a double blind study of twenty surgical patients randomly divided into two groups.

Group A: Ten patients received saline and glucose (5%) for four days post-operation.

Group B: Ten patients received 4854 additive, together with saline and glucose (5%).

The aim of this study was to investigate the compatibility of trace metal additive, with saline and glucose, and to see the efficacy of this solution in meeting trace element requirements of post-operative patients receiving only crystalloid infusions. Furthermore, the control patients who did not receive the trace metal mixture supplied information as to the changes in serum and urine trace metals following surgical procedures.

2) Evaluation of trace metal solution (4854) as an additive to parenteral nutrition solution in post-surgical adult patients:

The aim of this study was to investigate the compatibility of solution 4854; and to investigate the efficacy in meeting the trace element requirements of patients being intravenously fed. Sixteen surgical patients were studied for more than seven days.

3) Evaluation of trace element solution (4854) as an additive to parenteral nutrition solution in medical adult patients:

The aim of this study was to evaluate the 4854 solution when added in parenteral nutrition of more stable patients. Four patients were selected for this study, who were on intravenous feeding for more than fourteen days.

CHAPTER 2

METHODS OF TRACE METAL ANALYSIS

CHAPTER 2

2.1 METHODS FOR TRACE METAL ANALYSIS

Zinc

Serum zinc and urine were analysed by flame atomic absorption spectrophotometry (FAAS) (Peaston 1973).

0.5 ml of serum was diluted with 2.5 ml of 10% isopropanol and measured against 10% isopropanol diluted 3.9, 7.8, 15.3 and 31.2 µmol/l standards.

1 ml of urine was diluted with 5 ml of 10% isopropanol and measured against 10% isopropanol diluted 3.9, 7.8, 15.3 and 31.2 µmol/l standards. All zinc analysis was done by routine Glasgow Royal Infirmary staff.

Copper

Serum copper was analysed by flame atomic absorption spectrophotometry (Peaston 1973). 0.5 ml of serum was diluted with 2.5 ml of isopropanol and measured against 0.25, 0.5, 1.0 and 2.0 mg/l aqueous standards.

Urine copper was analysed by carbon furnace atomic absorption spectrophotometry (Halls et al 1981). All samples were acidified before the analysis. 200 µl of urine diluted with the same amount of distilled deionised water was measured against aqueous 20, 40, 60, 80 and 100 µg/l Cu standards.

Manganese

Serum and urine manganese were analysed by carbon furnace atomic absorption spectrophotometry (Halls 1980). (Perkin Elmer model number 2280).

Standards: Standards were made 0, 2, 4 and 6 µg/l of manganese in 0.1 molar nitric acid (Aristar).

Samples: 200 µl of sample + 200 µl of distilled deionised water in acid-washed auto sample cups.

Instrument Conditions

Wavelength : 279.5 nm

Slit : 0.7 nm

Normal graphite tube was used.

Background correction : ON

Scale expansion : 10 times

Injection volume : 20 µl

Stage	Temp	Ramp rate	Hold time (seconds)
Dry	120 ^{o}	7	13
Ash	1100^{o}	7	23
Atomise	2700^{o}	2	10
	(Gas flow 10 ml/min, Az -2, Rec -2)		
Clear	2700^{o}	2	5

Urine Manganese

Standards: Standards were made 0, 2, 4 and 6 µg/l of manganese in 0.1 molar nitric acid (Aristar).

Samples: 200 µl of sample + 200 µl of distilled deionised water in acid-washed auto-sampler cups.

Instrument Conditions: Wavelength : 279.5 nm

Slit : 0.7 nm

Background correction : ON

Scale expansion : 8 times

Injection volume : 20 µl

Normal graphite tube

Stage	Temp	Ramp rate	Hold time (seconds)
Dry	100^{o}	7	13
Ash	1100^{o}	7	23
Atomise	2700^{o}	2	10
	(Gas flow 30 ml/min, Az-2, Rec -2 sec)		
Clear	2700^{o}	2	5

Chromium

Urine chromium

Urine chromium was analysed by carbon furnace atomic absorption spectrophotometry (Perkin Elmer model number 2280). The method was developed by Dr Halls and was as follows:

Standards: Standards were made 0, 2, 4 and 6 µg/l of chromium in 0.1 molar nitric acid (Aristar).

Samples: All urine samples were acidified (1 ml conc. H SO (Aristar) to 25 ml urine) on collection. 200 µl of sample + 200 µl of distilled deionised water in acid washed auto-sampler cups.

Instrument Conditions

Wavelength : 357.9 nm

Slit : 0.7 nm

Injection volume : 20 µl

Background correction : ON

Scale expansion : 20 times

Normal graphite tube

Stage	Temp	Ramp rate	Hold time (seconds)
Dry	110°	7	20
Ash	1200°	7	20
Atomise	2400°	0*	5
		(Gas flow 30 ml/min, Az -2, Rec -2)	
Clear	2700°	1	5

(* Temperature sensor attached to furnace was set at 0 ramp rate)

Serum Chromium

Standards: Standards of 0, 2, 4 and 6 µg/l of chromium in 0.1 molar nitric acid (Aristar) were prepared. Each standard was diluted 1:1 with serum sample low in Cr conc.

Samples: 200 µl of sample + 200 ul of distilled deionised water in auto-sampler cups. Water blank was run.

Instrument Conditions: Wavelength : 357.9 nm

Slit : 0.7 nm

Injection volume : 20 µl

Background correction : OFF

Scale expansion : 20 times

Stage	Temp	Ramp rate	Hold time (seconds)
Dry	110°	7	20
Ash	1200°	7	20
Atomise	2200°	0	5
	(Interior flow 10 ml/min, Az -2, Rec 0)		
Clear	2700°	1	5

All glassware was acid-washed in Aristazr 20% nitric acid, as were all micropipette tips and auto-sampler cups. They were then rinsed in deionised water and air dried.

Selenium

Analysis of selenium in urine and serum was done by hydride generation atomic absorption spectrophotometry. Perkin Elmer model number 370 was used along with MHS 10.

Principle

All samples were digested with a mixture of perchloric and nitric acids to remove organic material. Hydrochloric acid was added to the digest which was then diluted to about 25 ml. Selenium was then determined by hydride-generation atomic absorption spectrophotometry. The selenium was reduced by sodium borohydride to give hydrogen selenide, which was carried by a stream of nitrogen to a heated silica-absorption cell in the light path of the spectrophotometer. Thermal breakdown of the hydrogen selenide gave selenium atoms which gave rise to an atomic absorption signal.

Reagents

Perchloric acid (72% W/V) (Aristar); Nitric acid (Aristar),

Hydrochloric Acid (Analar), Selenium standard solution 1 mg/ml (BDH), Sodium borohydride (BDH), Sodium hydroxide (Analar). Hydrochloric acid was diluted 2:5 with distilled water to form 4 molar HCl. 3% sodium borohydride in 1% sodium hydroxide was prepared in distilled deionised water, which was used as the stock solution.

Precautions against contamination

Digestion tubes, glass beads, pipette tips, sample cups, were acid-washed with 20% nitric acid before use.

Digestion Procedure

2 ml of urine or 0.5 ml of serum was added to digestion tubes. 5 ml of conc. nitric acid, 2 ml of perchloric acid and 2 glass beads were added. Then the tubes were placed in digestion block and heated at 110^{o}C for one hour, 150^{o} C for one hour, and 200^{o} C for one hour. Tubes were cooled, 7 ml of 4 molar hydrochloric acid was added. The solution was mixed vigorously. They were heated again at 80^{o} C for thirty minutes. Tubes were cooled and the solution was transferred into plastic containers by washing the digestion tubes three times with distilled deionised water, to give a final volume of 25 ml.

Calibration Solutions

0, 20, 40 and 60 ng of selenium were added in plastic 25 ml containers. To it, perchloric acid and 2 ml of hydrochloric acid were added and finally diluted to 25 ml with distilled deionised water.

Instrumental Conditions

Selenium EDL lamp with power supply (6 watts maintained during the analysis).

Wavelength : 196 nm

Slit : 2 nm (alternative)

Time constant : TC_2

Polypropylene bottle on MHS 10 filled with sodium borohydride (3%) up to mark.

2.2 Sample Collection and Storage

Sample collection was the most important factors in getting good analytical results, since contamination could give misleading results. Contamination could occur by two different ways:

A) Collection, B) Storage. However, the contamination problem was reduced by using the appropriate techniques in collecting and storing the samples. The disposable papier-mache urine bottles were the main source of contamination while collecting urine (Fell et al 1980). The easily contaminated elements were zinc and manganese. Figures of contamination are shown in Table 3.

TABLE 3

The effect of leaving urine in papier-mache containers on the concentration of minerals in urine

	Zn	Cu	Mn
	----------	(μmol/l)	------
Urine before it was put in container	37	0.27	0.01
After 5 minutes in container	45	0.22	11.6
After 10 minutes in container	52	0.21	17.0
After 15 minutes in container	53	0.26	17.9
After 30 minutes in container	56	0.28	20.1

Manganese increased rapidly after only five minutes in the paper-mache urine bottles. This unphysiological increase of manganese concentration was used as an index of such contamination. To avoid this, plastic urinals were used. For twenty-four hour collections, urine was stored in acid-washed plastic bottles. Smaller portions of urine were kept in a number of plastic containers, which were stored at 4-10^{o}C while awaiting analysis.

It is known that when blood is taken through stainless steel needles it becomes contaminated (Behne 1980), partic ularly for manganese. This was also shown by Fell et al (1980). Levels of contamination are shown in Table 4. To reduce this problem, a "Venflon" plastic cannula was used to take the blood sample. First, 10 ml of blood was discarded and used for routine analytical work. The last 20 ml of blood was taken into acid-washed plastic centrifuge tubes. After centrifuging, the serum was stored in plastic washed 5 ml tubes (Z5) and stored at 4-10^{o} C while awaiting analysis.

TABLE 4

Sequential blood samples via "Venflon" cannula

Blood samples taken	Mn conc in serum µg/litre (Mean of results from three different subjects)
First 10 ml of blood	0.55
Second 10 ml of blood	0.35
Third 10 ml of blood	0.35
Fourth 10 ml of blood	0.35

2.3 Clinical Experiments

The trace metal mixture (code 4854) is an aqueous preparation suitable for intravenous injection, the composition of which is shown in Table 2. One ampoule (10 ml) per day was added to the intravenous nutrition regimen of patients. Three studies were undertaken.

A) As an additive to standard crystalloid infusions

Twenty patients undergoing elective abdominal surgery were selected. Informed consent was obtained and they were divided randomly into two groups. One group of ten patients received only saline and glucose (5%) intravenously for four days after operation. The other group received in addition, one ampoule of 4854 solution per day. Blood and twenty-four hour urine samples were collected prior to surgery, and blood after operation, on days 1, 3, 5 and 6, and urine (24 hour) daily for four days after operation. All samples were collected by procedures described previously (Section 2.2) to minimise contamination, and analysed for zinc, copper, manganese, chromium and selenium, by atomic absorption spectrophotometry methods (Section 2.1). The study was "double blind" and the code broken to identify the additive (4854) group only after

all analyses were complete.

B) As an additive in patients receiving total parenteral nutrition after surgery (Surgical I.V.N)

Sixteen patients undergoing elective surgery were selected, informed consent was obtained. All the patients received a three litre bag of aqueous nutrients containing amino-acids (Vamin 9.5%), Glucose (50%), Saline (0.9%), Magnesium (6 mmol), Phosphate (15 mmol), plus one ampoule of (4854) trace metal solution. Also, 0.5 litres fat emulsion (Intralipid 20%) and fat water-soluble vitamins (Solvito, Vitlipid), were supplied. This regimen supplies 3000 KCals/day and 14 grams of nitrogen per day. Patients were classified as either:

1) Stable anabolic patients (urine N less than 12 g/day) or :
2) Catabolic patients (urine N greater than 14 g/day).

Each patient was studied for at least one week. Most of them for two weeks or more. The regimen was infused through a central venous catheter.

1) Pre-infusion measurements were made for serum samples and for twenty-four hour urine samples.
2) Twice a week twenty-four hour urine samples and serum samples were analysed.
3) Final samples were obtained on the day of the last infusion. In some patients, samples were collected twice a week after stopping the infusion.

C) As an additive to parenteral nutrition in medical patients (Medical I.V.N)

This study was to investigate more stable patients likely to require feeding for longer periods. Four patients were selected, and informed consent was obtained. Patients were

studied for more than fourteen days. The infusion regimen was similar to that used in the surgical I.V.N. groups.

1) Pre-infusion measurements were made on serum and twenty-four hour urine samples.
2) Twice a week, twenty-four hour urine samples, and serum samples, were analysed for a total of fourteen days, or more in some cases.
3) Final samples were obtained on the day of the last infusion. In some patients, samples were obtained twice a week after cessation of the infusion of the 4854 mixture.

2.4 Statistical Techniques

Statistical significance was evaluated by the "Mann-Whitney" test, because results could not be shown to have a Gaussian distribution in all groups, and some were clearly skewed.

The most obvious method of comparing two groups, the "T-test", was discarded in favour of the non-parametric equivalent, the Mann-Whitney test. This compares the medians of the two test groups, rather than their means and thus eliminates the bias in group means caused by either very high or very low outliers. This test was applied to three different studies as follows:

1) Crystalloid study

   Each post-operative day was compared with the day prior to the operation for both groups (with additive and without additive). Also, both groups were compared on each particular day throughout the study for urine and serum concentrations.

2) Surgical I.V.N

   Both groups (anabolic and catabolic) were compared on each day. Also, for each group, each post-infusion day

was compared with the pre-infusion day to see the significance for urine and serum trace element concentration.

3) Medical I.V.N

Each post-infusion day was compared with the pre-infusion day to see the significance, for urine and serum, of trace element concentration.

CHAPTER 3

REFERENCE VALUES

CHAPTER 3

3.1 REFERENCE VALUES

Introduction

Each study of trace metals in biological material such as blood serum and urine, when present at low concentrations (< μmol/litre) requires confirmation that the methods of sample collection, storage and analysis are valid and in reasonable agreement with the lowest published values for healthy subjects (Versieck et al 1980). Therefore, in addition to using the established reference ranges of Glasgow Royal Infirmary Biochemistry Department, it was decided to measure the levels of zinc, copper, manganese, chromium and selenium in serum and urine for fifty healthy laboratory staff.

Out of fifty volunteers, twenty-six were males and twenty-four females. Twenty-four hour urine was collected for two consecutive days, and one blood sample was taken at 10.00 a.m. The subjects had fasted overnight. All samples were collected by the procedures described previously (Section 2.2) to minimise contamination. Samples were analysed by atomic absorption spectrophotometric methods (Section 2.1). Tables 5 and 6 show the normal ranges for each element.

TABLE 5

Normal Range in Urine for Five Trace Metals

Element	Mean $\bar{x}$	S.D.	$\bar{x} \pm 2$ S.D. (A)	Observed range 95% limit (B)
Zinc (μmol/vol)	9.70	4.68	0.3–19.0	3.3 - 21.4
Copper " "	0.33	4.95	0–2.23	0.20– 0.58
Manganese (nmol/vol)	26.5	14.1	0–54.6	5.0 - 60.0
Chromium " "	20.0	10.0	0–40	6.0 - 43.0
Selenium " "	388.0	162.0	63–1036	176.0 - 804.0

TABLE 6

Normal Range in Serum for Four Trace Metals

Element	Mean $\bar{x}$	S.D.	$\bar{x} \pm 2$ S.D. (A)	Observed range 95% limit (B)
Zinc (μmol/vol)	14.5	1.8	10.9-18.1	12.5-17.5
Copper " "	15.8	2.9	10.0-21.7	11.5-23.5
Manganese (nmol/vol)	14.4	5.2	4.0-25.0	7.0-27.0
Selenium " "	1130.9	312.0	507.0-1755.0	583.0-1646.0

From these results, two "normal ranges" can be calculated:

A) By statistical calculations

That is, the mean $\pm$ 2 standard deviation, assuming Gaussian distribution.

B) Observed range

This was calculated by examining the frequency distribution for each element and then excluding the two outliers for urine, and one outlier for serum. This is equivalent to the 95% observed range. It is felt that these values are more representative of the result actually found in the population studied and that the statistically derived results are less useful, although the convention of $\bar{x} \pm 2$ S.D. is commonly employed.

The reference values may differ according to the age and sex of the population. To obtain a close match with the patient group was virtually impossible. Therefore, the "normal range" was used as an initial guideline to evaluate results from the study.

A comparison of "normal values" with some published results are shown in Tables 7 and 8 for serum and urine, which clearly indicates that the "normal range" found is similar to other published results.

TABLE 7

Comparison of "normal range" in serum with selected published results

	Subjects	Zinc	Copper	Manganese	Selenium
		µmol/l		nmol/l	
This study	26 male 24 female	12.5–17.5	11.5–23.5	7–27	583–1646
Previous GRI results		12.0–18.0	15.0–25.0	-	-
Parr et al (1964)	6	12.5–15.6	10.9–18.3	-	-
Davies et al (1968)	36 male 31 female	11.6–19.1	-	-	-
Versieck et al (1974)	46	10.6–18.5	11.5–29.9	7–19	-
Prasad et al (1976)	70	13.1–21.5	-	-	-
Behne et al (1978)	25	8.5–17.3	-	-	1114–1621
Koch et al (1956)	58	-	10.2–21.3	-	-
Olatunbosun et al (1976)	37	-	11.0–23.6	-	-
Stump et al (1977)	32 male	-	14.2–24.9	-	-

cont ...

cont

	Subjects	Zinc	Copper	Manganese	Selenium
		-------µmol/l---------		----------nmol/l--------	
Damsgaard et al (1973)	11	-	-	7.0 -14.0	874-1342
D'Amico et al (1976)	19	-	-	14.0-23.0	-
Morss et al (1972)	11	-	-	-	697-2140
Maxia et al (1972)	-	-	-	-	583-785
Rhead (1972)	7	-	-	-	899-1621
Fell et al (1980)	-	-	-	-	1013-1520

Table 8

Comparison of "normal range" in urine with selected published results (mainly as quoted by Versieck et al)

	Subjects	Zinc	Copper	Manganese	Chromium	Selenium
		-----µmol/vol----		----------nmol/vol----------		
This study	26 male 24 female	3.3-21.4	0.20-0.58	5.0-60.0	5.0-43.0	176-804
Previous GRI results	-	4.6-10.6	-	-	-	-
Revusova et al (1973)	17 male 4 female	0.6-21.8	-	-	-	-
Vandon et al (1970)	32	4.0-11.0	-	-	-	-
Meret et al (1971)	45 female	2.2-11.9	0.16-1.8	-	-	-
Meret et al (1979)	37 male	4.6-7.8	0.25-0.99	-	-	-
Dawson et al (1969)	10 male	4.0-12.5	-	-	-	-
Dawson et al (1969)	10 female	4.2-10.8	-	-	-	-
Wilson et al (1966)	10 female	-	0.13-0.35	-	-	-
Wilson et al (1966)	10 male	-	0.17-0.35	-	-	-
Vanomer et al (1973)	-	-	-	40.0-70.0/1	-	-
Watanable et al (1978)	-	-	-	1.0-49.01/	-	-

CONT ...

cont

	Subjects	Zinc	Copper	Manganese	Chromium	Selenium
		-----µmol/vol----		----------nmol/vol----------		
Halls et al (1980)	-	-	-	2.0-27.0/1	-	-
Fell et al (1980)	-	-	-	-	-	380-887
Griffiths et al (1973)	-	-	-	-	-	101-241
Davidson et al (1972)	12	-	-	-	76.0-308.0	-
Routh (1980)	6	-	-	-	9.0-45.0	-
Guthrie (1978)	12	-	-	-	8.0-35.0	-

3.3. Results

Urine Zinc

The distribution of results appears skewed (Figure 1) with a normal range of 3.3-21.4 μmol/volume. A problem in measuring zinc is contamination during collection. Female volunteers were asked to avoid possible contamination of the urine by faecal material or menstrual blood. Results are similar to other published estimates (Table 8).

Serum Zinc

Serum zinc has a Gaussian distribution (Figure 2). The normal range 12.5-17.5 μmol/l which is in agreement with published results. Since most literature results are in reasonable agreement (Table 7), this suggests that there are no major methodological problems in the determination of serum zinc, assuming control over sampling technique.

Urine Copper

The distribution of results appears Gaussian (Figure 3) and the normal range is 0.20-0.58 μmol/volume. Values are similar to other published data (Table 8).

Serum Copper

Results show a skewed distribution (Figure 4). The normal range is 11.5-23.5 μmol/litre. This agrees with the published results (Table 7). In this study, twenty-four female subjects who were not taking contraceptive pills, were chosen, since this drug increases serum copper concentrations. large numbers of studies have been published on serum copper concentrations and most are remarkably consistent even when obtained by different techniques; this shows that there are no serious methodological problems in serum copper determination.

URINE ZINC EXCRETION

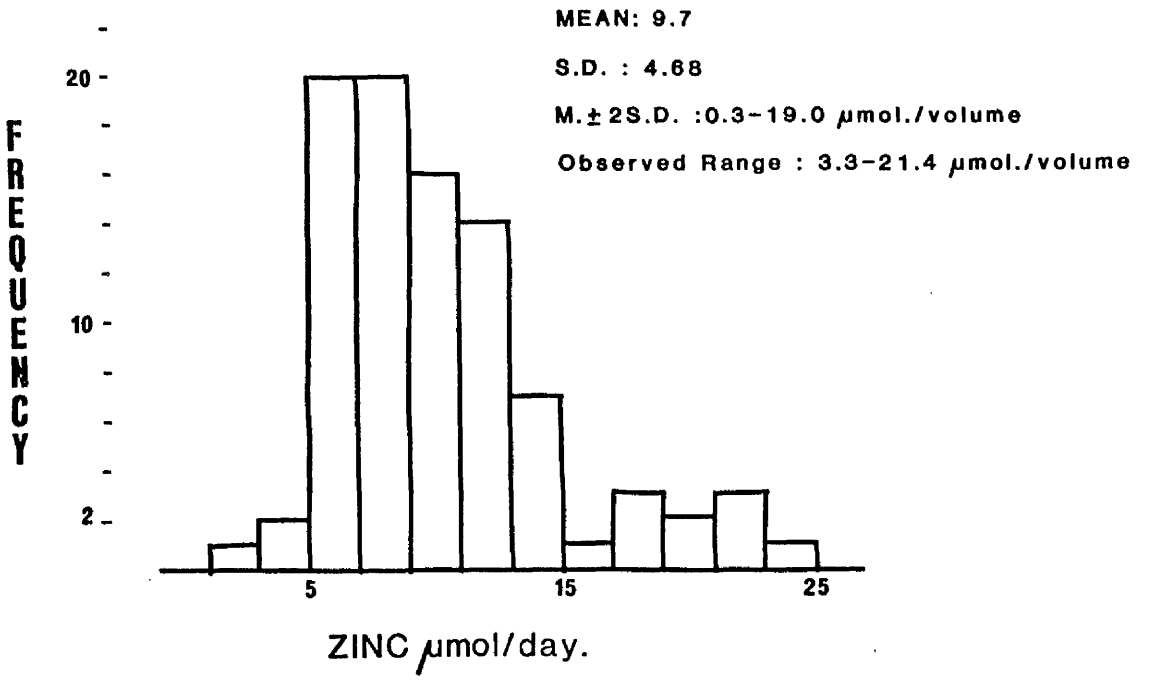


Figure 1

Urine zinc excretion in fifty persons, 26 male and 24 female.

SERUM ZINC CONCENTRATION

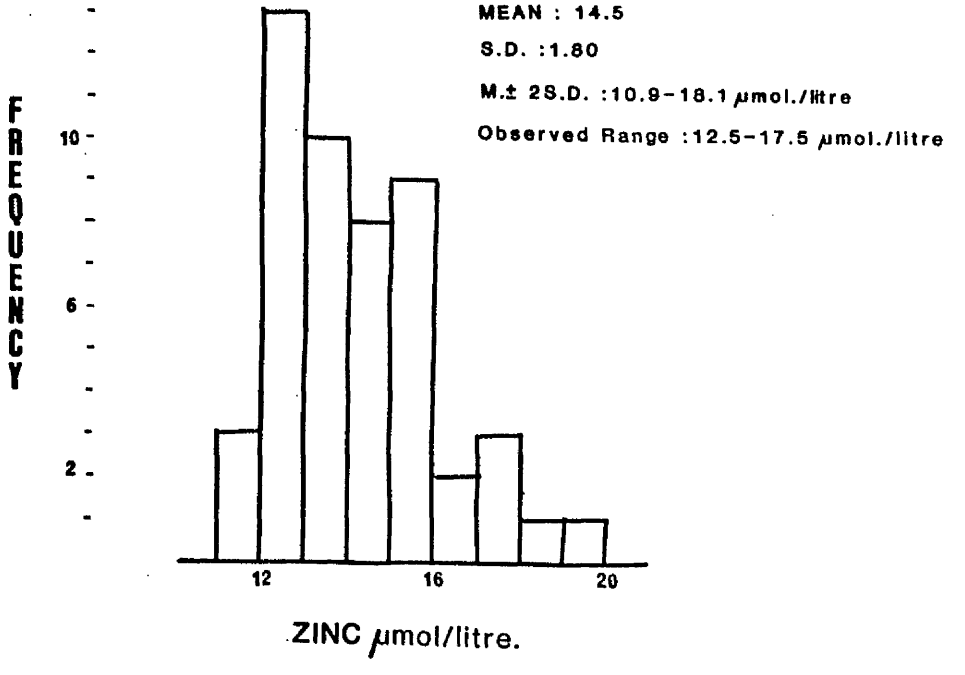


Figure 2

Serum zinc concentrations in fifty persons, 26 male and 24 female.

URINE COPPER EXCRETION

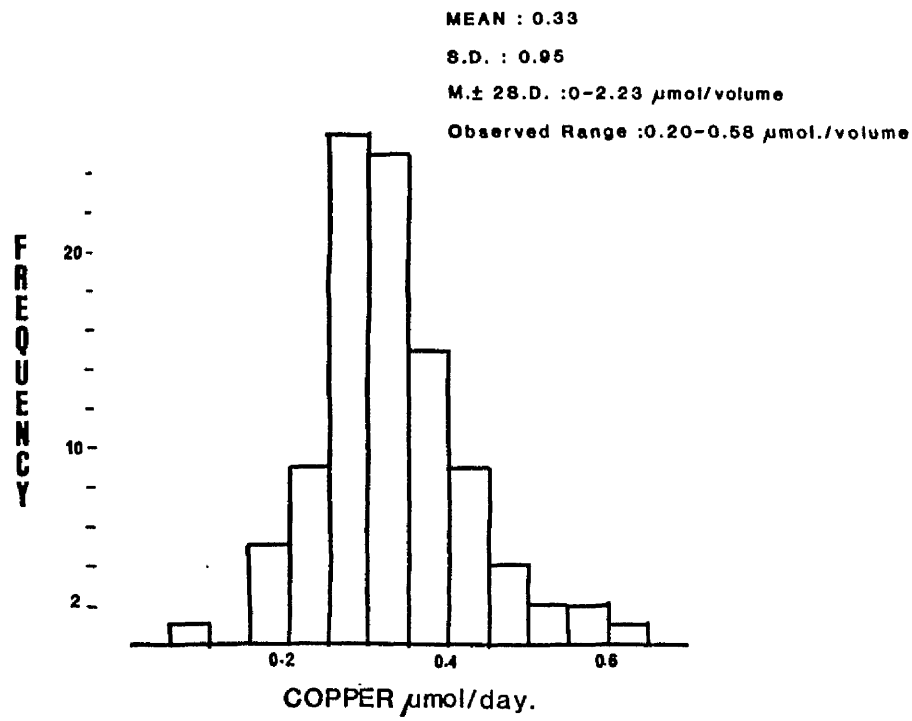


Figure 3

Urine copper excretion in fifty persons, 26 male and 24 female.

REFERENCE RANGE
SERUM COPPER CONCENTRATION

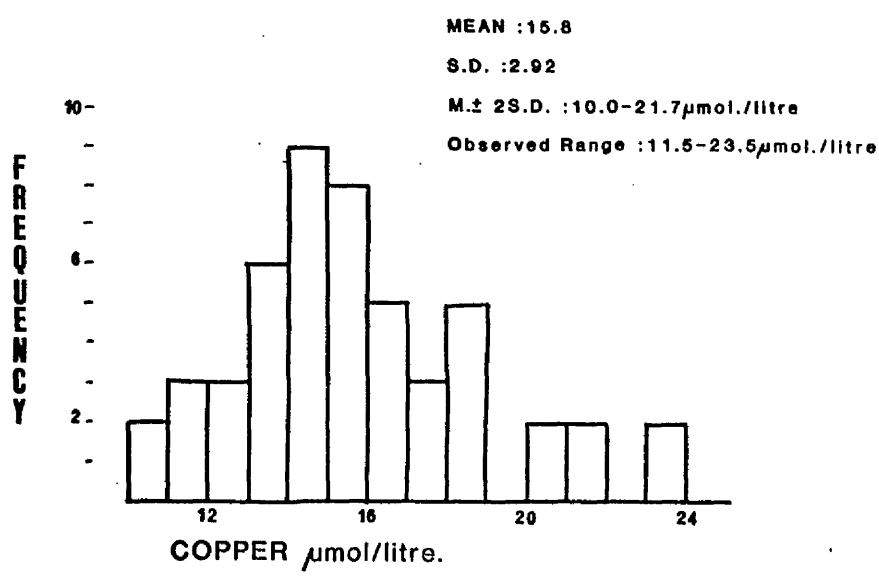


Figure 4

Serum copper concentrations in fifty persons, 26 male and 24 female.

Urine Manganese

The distribution of results appears Gaussian (Figure 5) and the normal range is 5.0-60.0 nmol/volume. This metal is difficult because of the risk of contamination and problems of accurate analysis at the low concentrations found in urine. These problems are illustrated by disagreement between the published results (Table 8).

Serum Manganese

Serum manganese has a skewed distribution (Figure 6). The normal range is 7-27 nmol/volume, which is in agreement with a number of recently published results (Table 7). The main problem in determining serum manganese is in making measurements close to the detection limit of current CFAAS techniques without interference from the matrix, and in collecting and preparing samples without introducing contamination.

Urine Chromium

The distribution of results appears Gaussian (Figure 7), with a normal range 6.0-43.0 nmol/volume. "Pyro-coated" furnace tubes were used in analysing urine chromium by CFAAS technique, since these tubes give more sensitivity than normal graphite tubes. Also, by reducing the atomisation temperature for chromium, an anomalous interference from the urine matrix was eliminated. This element is very difficult to measure, since it is present in small quantities. Also, the quality of sample and sample preparation is decisive for final results. There are a number of discrepancies in the literature, partly due to contamination and partly due to the choice of analytical techniques (Table 8).

Serum Chromium

Normal values for serum chromium could not be determined,

REFERENCE RANGE
URINE MANGANESE EXCRETION

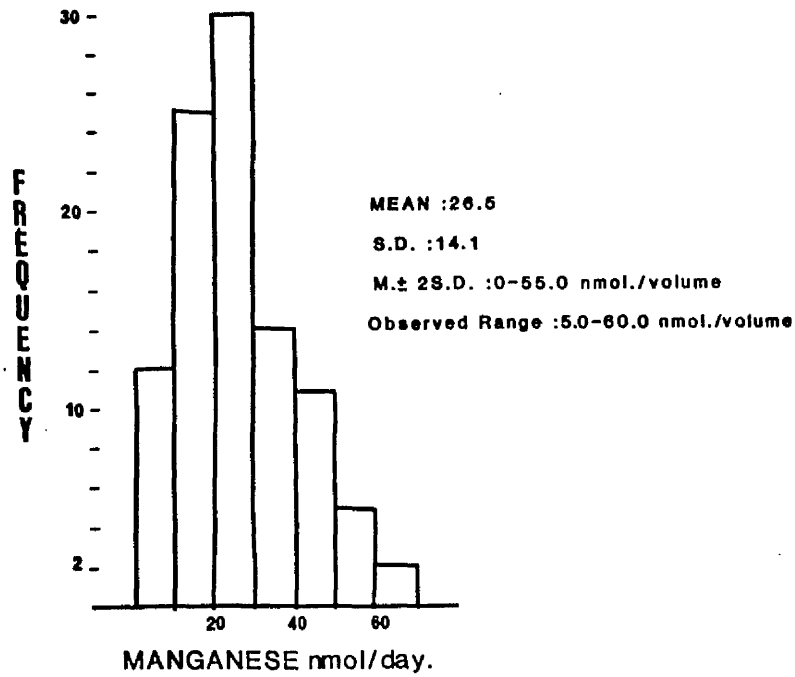


Figure 5

Urine manganese in fifty persons, 26 male and 24 female.

REFERENCE RANGE
SERUM MANGANESE CONCENTRATION

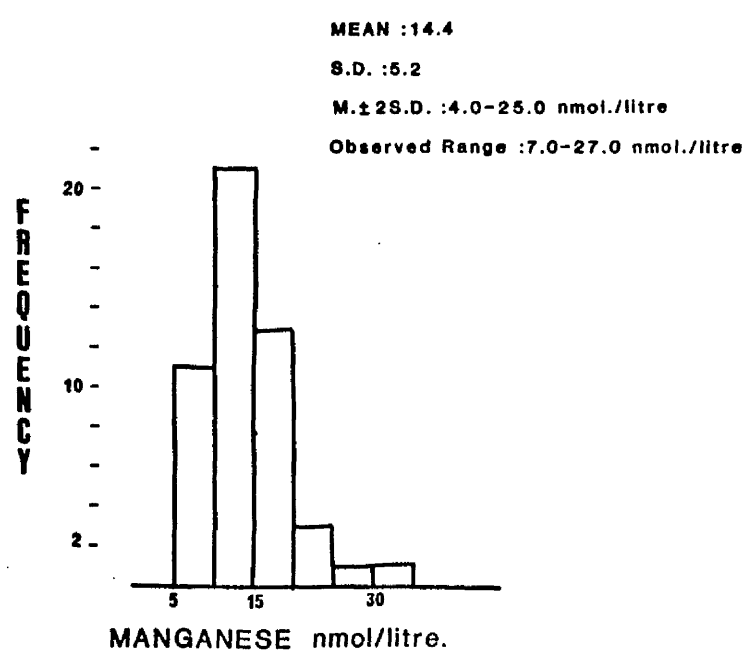


Figure 6

Serum manganese concentrations in fifty persons, 26 male and 24 female.

REFERENCE RANGE
URINE CHROMIUM EXCRETION

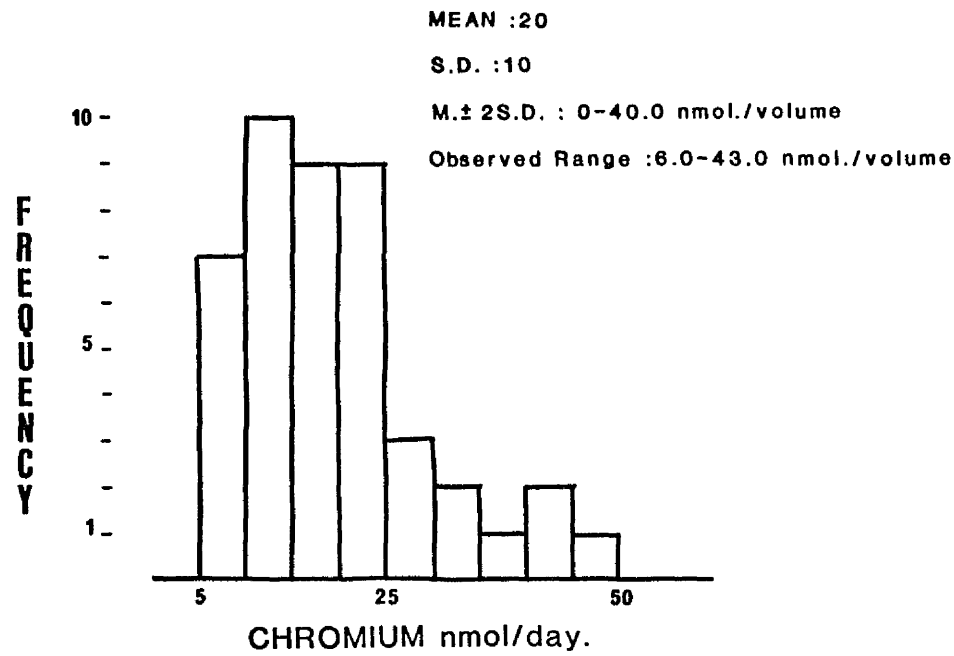


Figure 7

Chromium excretion in twenty-three persons, 13 male and 10 female.

REFERENCE RANGE
URINE SELENIUM EXCRETION

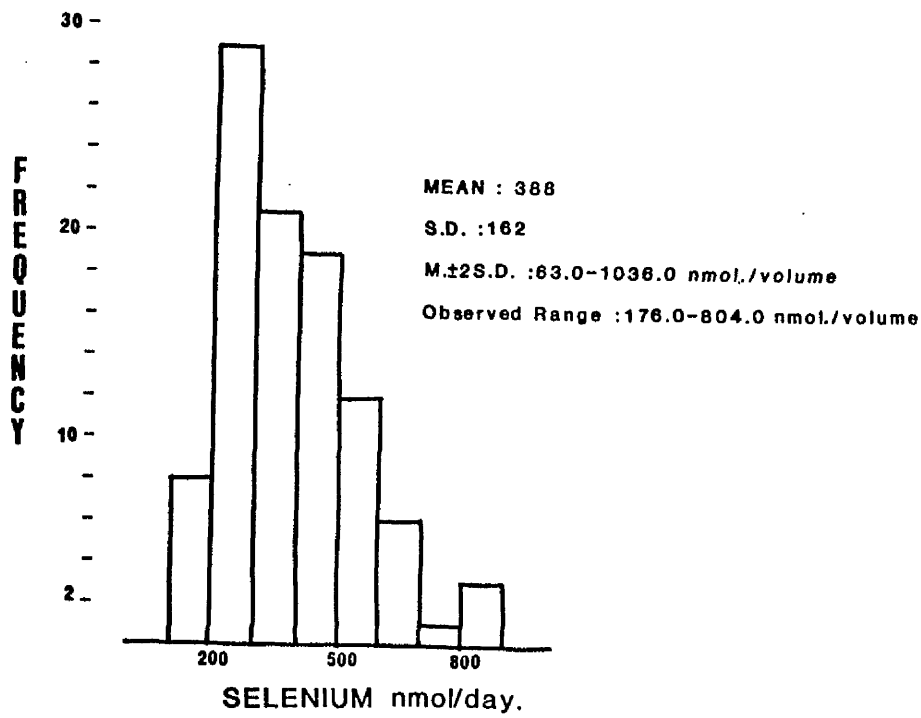


Figure 8

Selenium excretion in fifty persons, 26 male and 24 female.

since the estimated levels are less than 5 nmol/litre, which is close to the detection limit of the CFAAS method. Therefore, only increases above the "normal range" can be considered in the study.

Urine Selenium

The distribution of results appears skewed (Figure 8) with a normal range 176-804 nmol/volume. Urinary selenium excretion is dietary-dependent and obviously will vary with recent selenium intake. Furthermore, since selenium is a volatile metal, possible losses during the digestion period prior to analysis, must be avoided. Variations in published results are therefore to be expected (Table 8).

Serum Selenium

Serum selenium has a Gaussian distribution (Figure 9), with the normal range 583-1646 nmol/litre, which is similar to some of the published results (Table 7). It is difficult to define a "normal range" for selenium as the geographical differences in selenium intake exist.

REFERENCE RANGE
SERUM SELENIUM CONCENTRATION

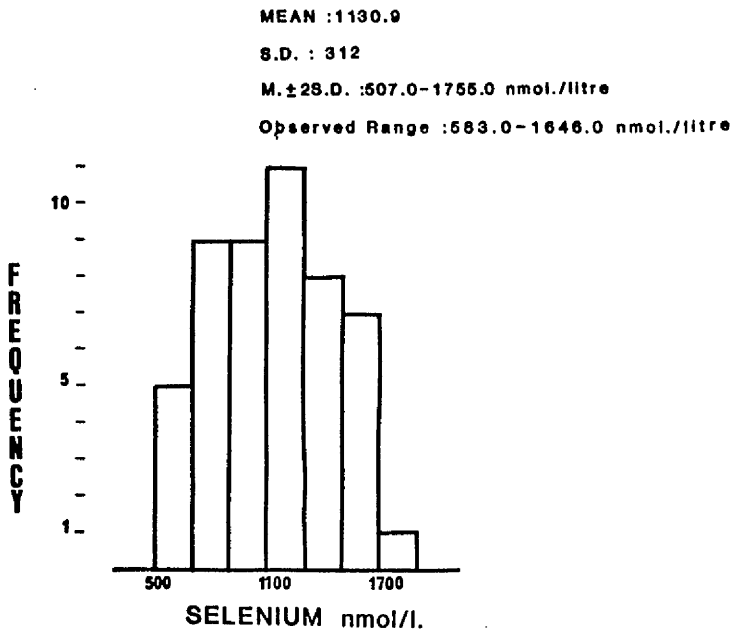


Figure 9

Serum selenium concentrations in fifty persons, 26 male and 24 female.

CHAPTER 4

INDIVIDUAL TRACE METALS : ZINC, COPPER, MANGANESE, CHROMIUM AND SELENIUM

CHAPTER 4

ZINC

4.1.1 Introduction

Zinc has long been known to be an essential element for the growth and development of living things. The first reports demonstrating zinc deprivation in vivo appeared more than one hundred years ago. The universal importance of this metal to growth in all phyla was not appreciated until much more recently. An important discovery considered zinc an essential component of erythrocyte carbonic anhydrase. However, more than seventy zinc metalloenzymes have been identified within the past decade. Many of these are concerned with various stages of nucleic acid and protein synthesis. Therefore, zinc is necessary for tissue growth and regeneration, and the consequences of severe deficiences are serious (Riordan 1976).

Clinical interest in zinc has increased with the description of symptomatic deficiency, rapidly reversible with zinc therapy. This occurs in children with a congenital defect of zinc absorption (acrodermatitis enteropathica) and also in children and adults fed intravenously with regimens deficient in zinc. Malabsorption of zinc has also been found in malnourished populations and linked to a complex syndrome of hypogonodal dwarfism. Undue delay in the healing of surgical wounds and leg ulcers is also linked to zinc deficiency (Prasad 1978).

4.1.2 Body Composition

The whole body content of zinc is around two grams. In terms of percentage content of the body zinc, a significant amount, about 20%, is in skin, especially in epidermal layers, 20%

in skeletal bone and 60% in muscle mass. All metabolically active tissue contains zinc, but certain tissues such as the choroid and retina of the eye, and body fluids such as prostatic and seminal fluid, have especially high zinc concentrations. Zinc is also present in small quantities in nail and hair.

Zinc is unevenly distributed in whole blood, 75-88% of the total zinc of normal human blood being contained in erythrocytes (red cells); 12-22% in plasma and 3% in leucocytes (white cells).

4.1.3 Dietary Intake : Absorption and Excretion

The recommended dietary intake for zinc is 15-25 mg/day, the higher intake being suggested for pregnant and lactating women. The best sources of zinc are good quality animal or plant protein. Several factors influence the absorption and retention of zinc and thus its availability from diet. Phytate which is present in cereal grains, markedly impairs the absorption of zinc (Reinhold et al 1976; Prasad et al (1979). Recent studies indicate that higher fibre intake, which is common in subjects consuming high cereal diets, is detrimental to zinc availability. The binding of zinc to fibre of wheat is particularly important, because in contrast to other components, fibre is not degraded by digestive secretions. As a result, zinc remains attached to fibre and is transported to the large intestine where absorption does not occur. Ultimately, it is lost in faeces. The effect of chelating agents on zinc absorption has been studied in experimental animals. Chelating agents form complexes with zinc from dietary sources and make it available for absorption, for example, E.D.T.A. and penicillamine act in this way.

Normally, only a small percentage (about 8-20%) of ingested dietary zinc is absorbed. Absorption of zinc is difficult to ascertain, since excretion of zinc is nearly all via gut. Becker et al (1971), concluded that zinc absorption is variable with body size, levels of zinc in diet and presence in the diet of potentially interfering substances such as calcium, phytate, other chelating agents and vitamin D.

Once zinc is absorbed, it binds to plasma albumin in the portal blood and is concentrated initially in the liver. In peripheral blood plasma, total zinc levels are 12-18 μmol zinc/litre; of this, 40% is strongly bound to alpha 2-macroglobulin , (this zinc is not available for exchange); the remainder is bound to albumin (here zinc is available for exchange) and some is in equilibrium with a smaller amount of zinc amino-acid complexes (1% of the total).

The main route of excretion of zinc is in faeces. The faecal output consists of both unabsorbed dietary zinc and endogenous zinc re-excreted into the gut. Using an oral dose of Zn^{65} , Spencer et al (1965) showed that after twenty-one days, 70% of the dose is excreted in faeces, 2% in urine, with 28% tissue retention. Urinary zinc excretion is about 10 μmol (650 μg/day) and is independent of dietary intake. Significant loss of zinc in sweat also occurs. Prasad et al (1963) reported 1.5 mg/l zinc for whole sweat and 0.9 mg/l for cell-free sweat.

4.1.4 Biochemical Importance

Various studies have shown that zinc is a constituent of more than seventy metalloenzymes. Many of these are concerned in various stages of nucleic acid and protein synthesis.

In some cases these enzymes have a catalytical function, while some cases metal has a role for conformation or stabilisation. The first demonstration of a specific biological function which was critically dependent on the presence of zinc was carbonic-anhydrase. Many enzymes involved in nucleic acid metabolism such as thymidine kinase, DNA polymerase, RNA polymerase and reverse transcriptase, require zinc for their activities. Most stages of both nucleic acid and protein synthesis are zinc-dependent, therefore cellular growth and tissue regeneration are affected by zinc deficiency. Recent studies (Prasad et al 1974), indicate that thymidine kinase is very sensitive to lack of zinc.

In the past few years, zinc has been found in both DNA and RNA polymerase. When the tissue of zinc-deficient animals was examined, it was found to be lower in zinc, RNA, DNA and total protein whereas ribonuclease activity and the concentration of free amino-acids was increased (Underwood 1977). The activity of R.N.Ase is also regulated by exogenous zinc and thus, zinc appears to play a very important role in RNA and DNA metabolism.

4.1.5 Clinical Effects of Deficiency

For many years, it was considered virtually impossible for human beings to become zinc deficient. The naturally occurring deficiences were not due to a simple inadequacy of zinc intake, but rather were the result of several conditions that decreased the availability of dietary zinc and accelerated its loss from the body. Malabsorption, cirrhosis of the liver, chronic renal disease and other chronically debilitating diseases may induce zinc deficiency. In 1961, a group of eleven Iranian adult males were reported to show clinical features, such

as anaemia, hepatosplenomegaly, short stature and marked hypogonadism. They all gave the history of geophagia, and there was no evidence of blood loss or hookworm infestation. Oral zinc therapy reversed many of these effects.

Symptoms of acute zinc deficiency include severe but localised skin rash with loss of head and body hair. There may be abdominal pain with diarrhoea, and also altered mood. These signs and symptoms improve rapidly, that is within two weeks, of adequate zinc provision. In chronic zinc deficiency effects noted are failure of normal growth in children and young adults, poor appetite and depression of the senses of tase and smell (Prasad 1979). A functional depression of immune competence has been noted in malnourished children, which could be reversed by zinc therapy (Golden et al 1978). Reduction in the rate of normal wound healing is also often associated with zinc deficiency (Prasad 1978).

4.1.6 Biochemical Assessment of Zinc Status

Ideally, techniques such as metabolic balance studies, should be performed. This will involve the measurement of total input from all sources, that is, dietary and intravenous, and total excretion by all routes, that is, urine, faeces and even sweat. Such studies are difficult in a clinical situation; although demonstration of negative zinc balance over a period of several days or weeks is strong evidence of impending zinc deficiency. This procedure is not practicable, on a wide scale.

In addition, the dynamic aspects of zinc turnover and distribution should be studied using radioactive or stable isotopes. Again, it is not practicable in clinical patients on a wide scale. Therefore, direct analysis of zinc concen-

trations in serum, red cells, leucocytes, urine and hair, has to be used. Analysis of zinc in hair has ben suggested as a guide to body status, but this is subject to difficulties of interpretation. Serum zinc analysis is by far the most widely used inex. "Normal values" are 12.5-17.5 μmol/l (Table 6) but a large variety of non-specific factors lower serum zinc without the presence of tissue zinc deficiency. any form of stress, injury or surgery, infection disease or steroid drugs, will lower serum zinc (Fell et al 1978). Only when values are persistently less than 10 μmol Zn/l, can deficiency be postulated and even then serum albumin should also be considered.

Urinary zinc excretion, which increases as a part of the metabolic response to injury and infection, can also be a useful guide. High urine zinc excretion (greater than 50 μmol/24 h) indicates a continuing catabolic state (or influence of chelating substances in intravenous fluids). As the patient gains weight, serum and urinary zinc will tend to fall, signalling the need for increased supply, to allow tissue regeneration.

Various factors, such as the time of sampling, techniques of sampling (haemolysis increases zinc levels) and of analysis, must be considered carefully. Indirect means of zinc assessment, based upon the biochemical functions of zinc, have been proposed. Low levels of the zinc enzyme, alkaline phosphatase, during deficiency, increase to within the normal range with successful zinc therapy. This is less useful when there is pre-existing liver disease, since alkaline phosphatase is already high in the serum of this type of patient.

4.1.7 Zinc in Intravenous Nutrition

The low levels of zinc present in most intravenous solutions, coupled with the tendency towards increased urinary losses, suggest that the patients requiring intravenous nutrition may develop zinc deficiency. In addition, serum levels of zinc in patients beginning intravenous nutrition are often less than normal. A number of recent reports have illustrated the relationship between serum zinc levels and treatment with intravenous nutrition (Kay et al 1976; Arakawa et al 1976), which suggests that additional zinc should be provided to patients receiving intravenous nutrition. It is clear that the amount required to maintain zinc equilibrium varies in individual patients, dependent upon several factors.

A) Body content of zinc at the time of beginning of total parenteral nutrition.

B) Rate of loss of zinc in urine and faeces or by other routes.

An average intravenous supply of 100 μmol/day of zinc was used during the study.

4.1.8 Results of this Study

Urine Zinc

A) Crystalloid Study

Urine excretion in both groups was similar at the start and on the post-operative days 1, 2, 3 and 4 (Tables 9 and 10). There was no significant difference between the groups on these days. Also when each post-operative day was compared with the pre-operative day, there was no significant difference (Figure 11). At the end of the study, in the group given additive out of eight patients, six were within the normal range (3.3-21.4 μmol/volume) and two were above, while in the group without the additive, out of nine patients eight

TABLE 9

Zinc in Urine, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (μmol/vol)			
			1	2	3	4
1	J.B	11.9	6.7	7.5	11.8	15.1
2	J.M	9.7	7.7	7.2	12.5	10.2
3	S.M	9.3	6.9	10.8	14.1	25.5
4	W.W.	3.2	9.0	5.5	8.7	9.5
5	C.M	*	*	*	*	*
6	W.A	17.7	12.3	14.5	23.1	28.2
7	J.S	*	6.9	6.2	8.3	9.2
8	B.S	3.3	10.7	10.2	10.5	10.0
9	W.R	7.5	16.2	*	*	*
10	A.P	4.6	1.6	5.9	5.7	6.8
Mean		8.4	8.7	8.5	11.8	14.3
S.D		4.9	4.1	3.1	5.3	8.1

TABLE 10

Zinc in Urine, Patients without 4854 Infusion

No.	Name	Pre-op	Post-op (μmol/vol)			
			1	2	3	4
1	M.M	6.3	9.6	7.1	10.6	13.5
2	A.C	3.9	3.5	3.3	4.6	8.5
3	W.L	10.5	14.9	13.7	15.3	16.5
4	J.M	4.3	6.1	12.9	15.0	14.3
5	M.M	8.1	8.0	17.7	9.7	13.8
6	H.S	12.4	8.8	6.8	15.5	16.5
7	J.M	34.5	8.8	16.9	13.3	9.0
8	T.C	33.7	*	*	*	*
9	R.M	14.8	28.7	21.8	*	25.6
10	H.S	6.4	9.2	13.1	6.9	9.5
Mean		13.5	10.5	12.6	11.4	14.1
S.D		11.4	7.5	5.9	4.1	5.3

* Urine contaminated

were within the normal range and one was above.

B) Surgical I.V.N

Both anabolic and catabolic surgical patients started with a high zinc excretion (35.0 ± 22.2 μmol/volume for anabolic patients; 68.3 ± 7.6 μmol/volume for catabolic patients) (Table 11 and 12). There was no significant difference between the groups on starting day, but there was a significant difference (P less than 0.05) throughout the study (Figure 12). At the end of the study zinc excretion was 54.6 ± 22.0 μmol/volume for anabolic patients and 115.7 ± 32.7 μmol/volume for catabolic patients. All eight patients from the anabolic group and all three patients from the catabolic group, were above the normal range for urine zinc excretion.

C) Medical I.V.N

In this group, zinc excretion starts above the normal range (Table 13) and remains the same throughout the study (Figure 13). On days 1-4, zinc excretion of all four patients was above the normal range; on days 9-12, three patients were above and one was within the range. At the end of the study, one patient was above, and one was within the normal range.

Serum Zinc

A) Crystalloid Study

Both groups, prior to operation, started within the normal range (with additive 13.5 ± 2.7 μmol/l and without additive 12.9 ± 2.9 μmol/l). On post-operative day 1, serum zinc dropped considerable in both groups, and was below the normal range (Tables 14 and 15). When this day was compared with the day prior to the operation, there was a significant difference (P less than 0.05). Serum zinc started to increase from day 4 (Figure 14). At the end of the study the mean zinc concentration in the group without additive was 12.0

TABLE 11

Zinc in Urine, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (μmol/vol)	Post-Infusion Days Mean 1-3	8-10	15-17	Stopping Value
1	W.M	-	30.3	38.1	-	18.8
2	J.W	-	40.3	53.5	61.8	-
3	J.T	-	28.0	50.3	-	-
4	I.P	34.1	19.7	21.3	37.3	-
5	D.M	64.1	55.9	78.1	56.9	-
6	J.N	-	34.3	39.1	54.7	-
7	M.K	-	-	54.2	101.1	-
8	M.G	10.5	-	-	47.0	-
9	W.W	-	71.3	50.2	55.3	-
10	A.G	32.4	-	-	26.1	-
Mean		35.0	39.6	47.9	54.6	18.8
S.D		22.2	17.8	16.4	22.0	-

TABLE 12

CATABOLIC GROUP

No.	Name	Pre-Infusion (μmol/vol)	Post-Infusion Days Mean 1-3	8-10	15-17	Stopping Value
1	A.G	75.1	66.6	61.4	-	-
2	A.M	73.2	72.6	61.0	-	-
3	D.M	58.1	-	66.4	-	-
4	H.W	-	91.8	41.9	78.2	-
5	I.C	67.4	109.0	115.4	132.0	61.1
6	G.R	-	112.7	105.0	137.4	-
Mean		68.3	90.0	74.8	115.7	61.1
S.D		7.6	20.9	28.7	32.7	-

TABLE 13

Zinc in Urine, Medical I.V.N. Study

No.	Name	Pre-Infusion (μmol/vol)	Post-Infusion Days			
			Mean 1-4	9-12	17-20	25-28
1	R.M	-	38.6	49.2	-	-
2	P.P	-	24.8	18.4	16.6	19.2
3	E.I	58.8	50.8	45.8	59.9	48.1
4	J.C	24.2	21.7	28.6	49.6	-
Mean		41.5	34.0	35.5	42.0	33.7
S.D		17.3	11.6	12.6	18.5	14.5

TABLE 14

Zinc in Serum, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (μmol/l)			
			1	3	5	6
1	J.B	14.5	11.5	12.0	16.0	14.0
2	J.M	13.5	8.0	11.5	11.0	11.0
3	S.M	14.0	5.0	10.0	15.0	15.5
4	W.W	11.5	8.5	10.0	12.5	11.5
5	C.M	20.3	13.5	18.3	25.0	21.0
6	W.A	14.3	9.5	10.0	13.0	17.3
7	J.S	13.5	8.5	8.5	11.0	11.5
8	P.S	10.0	9.0	10.0	14.5	13.0
9	W.R	12.5	11.0	11.0	15.0	13.0
10	A.P	12.0	8.5	11.0	14.5	12.5
Mean		13.6	9.3	11.2	14.8	14.0
S.D		2.7	2.3	2.7	3.9	3.1

TABLE 15

Zinc in Serum, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (μmol/l)			
			1	3	5	6
1	M.M	10.0	9.5	10.0	10.5	10.7
2	A.C	11.5	7.5	8.0	10.5	12.5
3	W.L	12.0	9.5	11.0	12.5	11.5
4	J.M	13.0	8.5	10.0	13.5	14.0
5	M.M	12.0	7.5	10.5	12.0	12.5
6	H.S	15.0	7.0	9.5	13.5	13.5
7	J.M	8.5	8.5	8.0	8.0	9.5
8	T.C	14.5	12.5	12.0	14.0	13.5
9	R.M	13.0	9.0	14.0	12.5	13.5
10	H.S	19.5	14.0	10.0	15.0	13.0
Mean		12.9	9.4	10.3	11.3	11.9
S.D		2.9	2.2	1.8	4.1	1.9

± 1.6 µmol/l, which is below the normal range, while the group given additive was 14.0 ± 3.1 µmol/l, within the range.

In the group given the additive, at the beginning of the study, out of ten patients six were within the normal range, three were sub-normal and one was above. On post-operative day 1, eight patients were sub-normal and two were normal. On post-operative day 3, nine patients were sub-normal and one was normal. On day 5, seven patients were normal, two were sub-normal and one was above. On the last day, six patients were normal, three sub-normal and one above. In the group without the additive, at the beginning of the study, out of ten patients five were sub-normal, four normal, and one was above. On post-operative day 1, eight patients were sub-normal and two were normal. On day 3, nine patients were sub-normal and one was normal. On post-operative day 5, six patients were normal four were sub-normal. On the last day, seven patients were normal and three were sub-normal.

Serum albumin also dropped considerably on post-operative day 3 (Figure 17). There was no significant difference between the groups throughout the study (Tables 19 and 20).

B) Surgical I.V.N

The anabolic group started with a normal zinc concentration (Table 16), while the catabolic group was below the normal range (Table 17). In the anabolic patients, zinc remained the same throughout the study, and at the end of the study the mean zinc concentration was 14.8 ± 4.0 µmol/l, while in the catabolic group, it started to increase from days 7-11 and at the end of the study it was 17.0 ± 5.6 µmol/l. There was no significant difference between the groups throughout the study. At the end of the study, in the anabolic group, out of five patients

TABLE 16

Zinc in Serum, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (μmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	9.0	11.8	-	13.7
2	J.W	21.5	21.3	18.1	16.9	19.7
3	J.T	11.0	-	10.5	-	-
4	I.P	12.0	9.3	-	15.5	-
5	D.M	16.5	10.0	12.0	-	13.5
6	J.N	8.5	12.5	13.5	13.8	-
7	M.K	13.0	-	20.0	17.8	-
8	M.G	7.5	11.5	8.5	7.0	-
9	W.W	15.5	14.7	15.5	16.9	19.7
10	A.G	12.0	-	11.0	14.0	10.5
Mean		12.8	12.3	13.1	14.0	14.8
S.D		4.2	4.2	3.9	3.4	4.0

TABLE 17

CATABOLIC GROUP

No.•	Name	Pre-Infusion (μmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	13.0	11.5	13.5	-	-
2	A.M	-	11.2	9.3	10.5	-
3	D.M	7.5	11.0	11.5	-	-
4	H.W	14.0	10.0	12.0	22.5	13.5
5	I.C	10.5	12.5	14.5	15.0	-
6	G.R	-	10.0	16.5	16.3	21.5
Mean		11.0	10.8	12.5	15.8	17.0
S.D		3.2	0.8	2.4	4.9	5.6

three were within the normal range and two were above. In the catabolic group, both patients were above the normal range (Figure 15).

C) Medical I.V.N

In this group, patients started below the normal range (7.8 ± 0.9 μmol/l) and rose steadily up to days 7-11, then remained the same until the end of the study (Table 18 and Figure 16). At the end of the study, zinc concentration was 10.5 ± 1.6 μmol/l. Of three patients, two were sub-normal and one was within the normal range.

TABLE 18

Zinc in Serum, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (umol/l)						Stopping Value
			Mean	1-4	7-11	14-18	21-24	28-32	
1	R.M	6.8		6.0	-	8.5	-	-	12.5
2	P.P	-		6.0	8.3	9.0	9.0	5.3	10.5
3	E.I	9.0		15.3	11.9	12.6	12.6	15.3	8.5
4	J.C	7.5		-	11.0	11.5	-	-	-
Mean		7.8		9.1	10.4	10.4	10.8	10.3	10.5
S.D		0.9		4.4	1.5	1.7	1.8	5.0	1.6

TABLE 19

Serum Albumin, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (g/l)			
			1	3	5	6
1	J.B	41.0	40.0	35.0	38.0	39.0
2	J.M	43.0	38.0	36.0	35.0	33.0
3	S.M	46.0	38.0	33.0	36.0	35.0
4	W.W	38.0	33.0	32.0	33.0	33.0
5	C.M	39.0	38.0	32.0	36.0	35.0
6	W.A	39.0	31.0	29.0	27.0	35.0
7	J.S	38.0	33.0	29.0	28.0	31.0
8	P.S	30.0	33.0	30.0	28.0	31.0
9	W.R	37.0	35.0	33.0	32.0	35.0
10	A.P	38.0	37.0	37.0	34.0	34.0
Mean		38.9	35.6	32.6	32.7	34.1
S.D		4.2	3.0	2.8	3.9	2.3

TABLE 20

Serum Albumin, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (g/l)			
			1	3	5	6
1	M.M	31.0	30.0	29.0	28.0	30.0
2	A.C	40.0	38.0	37.0	29.0	31.0
3	W.L	36.0	35.0	32.0	31.0	32.0
4	J.M	42.0	35.0	33.0	34.0	36.0
5	M.M	37.0	35.0	30.0	31.0	31.0
6	H.S	43.0	38.0	34.0	36.0	37.0
7	J.M	36.0	32.0	30.0	29.0	30.0
8	T.C	36.0	32.0	28.0	30.0	29.0
9	R.M	34.0	33.0	30.0	33.0	32.0
10	H.S	43.0	39.0	31.0	34.0	34.0
Mean		37.8	34.7	31.4	31.5	32.2
S.D		4.0	3.0	2.7	2.6	2.7

TABLE 21

Serum Albumin, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (g/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	25.0	29.0	-	32.0
2	J.W	38.0	39.0	36.0	35.0	36.0
3	J.T	30.0	-	27.0	-	-
4	I.P	6.0	33.0	-	33.0	39.0
5	D.M	33.0	35.0	31.0	-	34.0
6	J.N	33.0	28.0	31.0	-	31.0
7	M.K	25.0	29.0	34.0	32.0	-
8	M.G	24.0	23.0	25.0	23.0	27.0
9	W.W	26.0	26.0	26.0	30.0	33.0
10	A.G	25.0	-	29.0	31.0	26.0
Mean		26.7	29.8	29.8	30.7	32.3
S.D		9.1	5.5	3.6	4.1	4.3

TABLE 22

CATABOLIC GROUP

No.	Name	Pre-Infusion (g/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	26.0	24.0	31.0	-	-
2	A.M	-	24.0	25.0	25.0	-
3	D.M	22.0	26.0	27.0	-	-
4	H.W	26.0	23.0	30.0	36.0	37.0
5	I.C	25.0	23.0	25.0	28.0	26.0
6	G.R	-	22.0	35.0	38.0	42.0
Mean		24.8	23.7	28.8	31.8	35.0
S.D		1.9	1.4	3.9	6.2	8.2

TABLE 23

Serum Albumin, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (g/l)					Stopping Value
			Mean	1-4	7-11	14-18	21-24	28-32	
1	R.M	20.0		21.0	-	32.0	-	-	28.0
2	P.P	-		23.0	25.0	31.0	32.0	27.0	26.0
3	E.I	31.0		32.0	32.0	35.0	34.0	36.0	34.0
4	J.C	22.0		-	31.0	32.0	-	-	-
Mean		24.3		25.3	29.3	32.5	33.0	31.5	29.3
S.D		5.9		5.9	3.8	1.7	1.4	6.4	4.2

4.1.9 Discussion

Zinc excretion

The main route of zinc excretion is normally via the gastro-intestinal tract, consisting of both unabsorbed dietary zinc and zinc re-excreted into the lumen of the gut in various secretions. However, in the patient studies, all zinc was given intravenously and little or no faecal material is passed during total intravenous nutrition. Therefore, for practical reasons, no attempt was made to collect the faecal output and it is therefore assumed that the principal route of loss of zinc and other metals is in urine.

For the purpose of comparison, the total urinary output over the period of the study in each patient group was calculated, and then expressed as a percentage of the known intravenous dosage given during the same period. This gives an approximate idea of the relative retention and loss for each metal.

1) Crystalloid Study

The calculated percentage of urinary excretion was 9.9 $\pm$ 4.7% of the total infused dose of 400 µmol (26.16 mg) zinc. Thus, about 90% of the infused zinc is retained by the patient. Since the "crystalloid" group receive no energy or nitrogen input, they are mildly catabolic. They are in negative nitrogen balance excreting about 10.5 $\pm$ 4.3 g/l of nitrogen. This could explain the increasing excretion of zinc at the end of the four day period (Figure 11), since zinc is lost in urine, together with other products of tissue catabolism (Fell et al 1977).

2) Surgical and Medical I.V.N

In the surgical group, the anabolic patients had an average zinc output of 46.7 $\pm$ 16.7%, retaining therefore, about 53% of the infused dose. Whereas the more catabolic surgical patients excreted some 84.2 $\pm$ 26.8% of the infused zinc, retaining about 15% only of the zinc infusion. The medical I.V.N. group excreted 37.1 $\pm$ 13.6% of their infused zinc and retained about 63%.

The surgical anabolic group and the medical I.V.N. group therefore behave similarly and retain more than half of the zinc infusion. The more ill catabolic surgical patients are possibly in negative overall balance due to increased tissue breakdown.

A similar tendency was reported by Askari et al (1979) in fourteen trauma patients. Thirteen patients had skeletal injuries and one had gunshot wounds. The number of days studied per patient varied from nine to thirty days. The zinc excretion in these patients was three to four times more than normals. (9 normal subjects excreted 504 µg zinc/day and fourteen trauma patients 2534 µg zinc/day). These results are similar to the above study results. Also, Fell et al (1977) have shown that in severely catabolic patients, some three to five weeks after a 55% burn injury, there is a greatly increased urinary zinc excretion.

All these results suggest that there is a response to injury or trauma whereby urinary zinc excretion increases according to the degree of stress.

Serum Zinc

1) Crystalloid Study

The patients from both groups prior to operation started within the normal range of serum zinc, but the level drops rapidly after the operation, as a response to the stress of surgery. The serum zinc concentration then increases steadily (Figure 14). By the end of the study (day 6), the treated group had serum zinc values within the normal range. However, the untreated group had values just below the lower limit of normal. This suggests that the infused zinc was being utilised by these patients.

Hallbook et al (1978) studied forty-five patients subjected to surgical trauma. The patients were randomly distributed into two groups. One group received 20 mg zinc (307 μmol zinc) and 1.2 mg copper (19 μmol) per day intravenously, and the other did not. There was a fall in serum zinc concentration in both groups after operation. The fall was more pronounced in the patients not receiving zinc. Serum zinc concentrations then increased steadily. These results are similar to the results reported in this study.

Tengrup et al (1977) studied forty-nine patients. They were divided into five different groups. One group of thirteen patients subjected to cholecystectomy was similar to this study. He also found that after operation, there was a fall in serum zinc concentration, and then it increased steadily.

Total serum zinc values are affected by the level of zinc binding proteins present in circulating blood. The main effective transport protein appears to be albumin. Therefore, levels of this protein should be considered when discussing serum zinc results. Serum albumin in both groups starts at the same concentration, drops considerably and

then increases steadily, parallel to the serum zinc changes (Figure 17).

Hallbrook et al (1978) found serum albumin to be slightly higher in patients given extra zinc and copper. Serum albumin also fell and increased later. There was a correlation between serum zinc and albumin. These findings are similar to the results reported in this thesis.

After injury or stress, protein synthesis in the liver switches off or decreases, also albumin is redistributed into extravascular spaces. Therefore, a fall in serum zinc levels is observed, as 60% of the zinc present in serum is bound to albumin. Hence the provision of intravenous zinc during the post-operative period is unlikely to influence such effects, as zinc supply will not be limiting at this stage. Although in the "crystalloid group" extra zinc provision does increase the serum zinc concentration, it is unlikely to be of clinical benefit. Zinc supply is of more use during long-term treatments.

2) Surgical and Medical I.V.N

The anabolic and catabolic surgical patients started within the normal range, and remained the same throughout the study. Although in the catabolic patients, serum zinc concentration increases at the end of the study, it is not a significant rise. Serum albumin results are parallel to serum zinc results in both groups (Figure 18). In medical patients, serum zinc starts at the lower limit of normal and then it rises to normal, and remains the same throughout the study. This suggests that the zinc infused was being utilised by these patients.,

Conclusions

Various authors have reported zinc deficiency during intravenous nutrition (Strobel et al 1978; Gordon et al 1978; Suita et al 1978; Latimer et al 1980, and Sorouji et al 1978). Oral therapy with zinc sulphate is widely used. A dosage of 50 mg zinc per day should correct most deficiencies. Higher dosage, 150 mg zinc per day, was originally prescribed but this could be excessive and may cause malabsorption of other elements, such as copper.

The intravenous requirmement varies with the type of patient under treatment, and the extent of their losses.

For the majority of the patients, 100 μmol (6.54 mg) zinc per day provided, is quite enough to avoid depletion or deficiency, and to maintain serum zinc concentrations within the normal range. However, for the patients with extensive fistula and faecal losses, more zinc may be necessary. The American Medical Association suggested the intravenous supply of zinc 2.5-4.0 mg (38.2-61.2 μmol) for stable patients and an additional 2 mg for adult catabolic patients. Persistent hypoproteinaemia is reported by Fodor et al (1972) in a severely zinc deficient patient, and is only improved when substantial zinc replacement therapy is given.

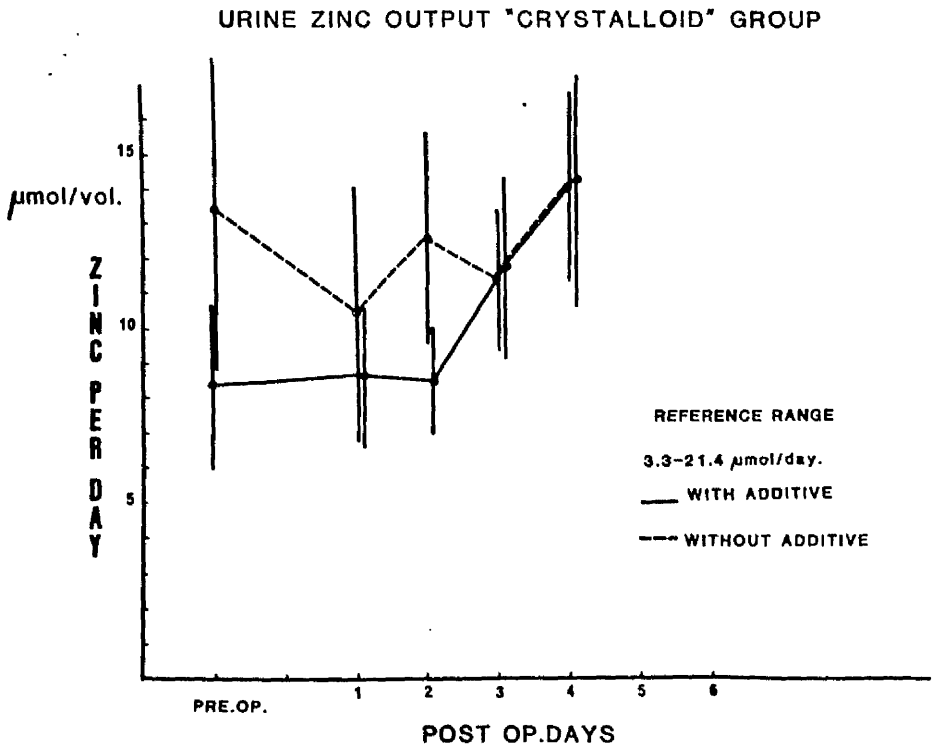


Figure 11

Comparison of zinc excretion in two groups of ten patients, with 4854 and without 4854 infusion.

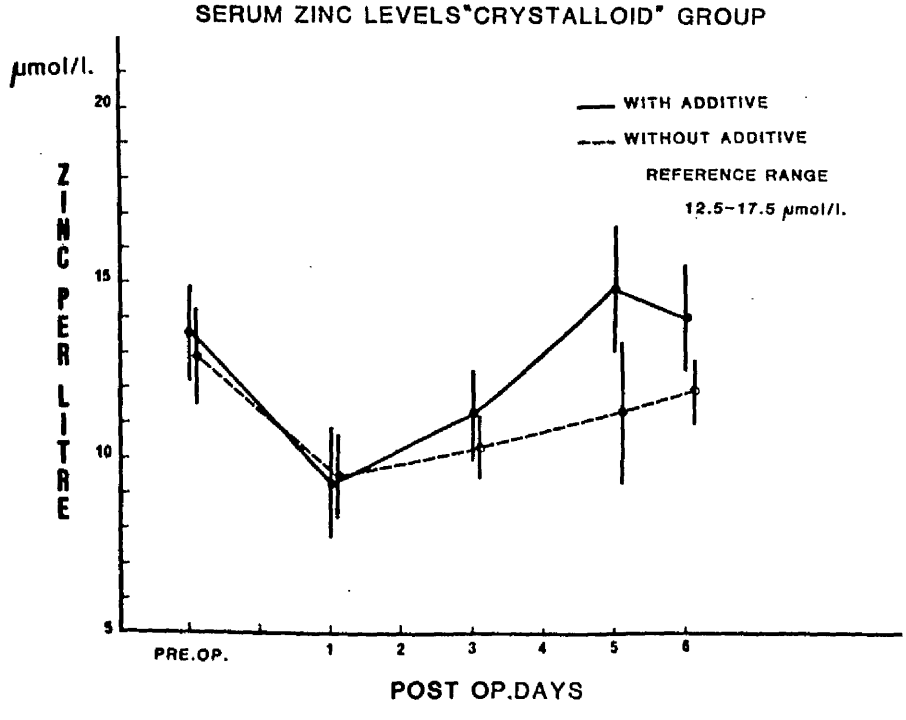


Figure 14

Comparison of serum zinc concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

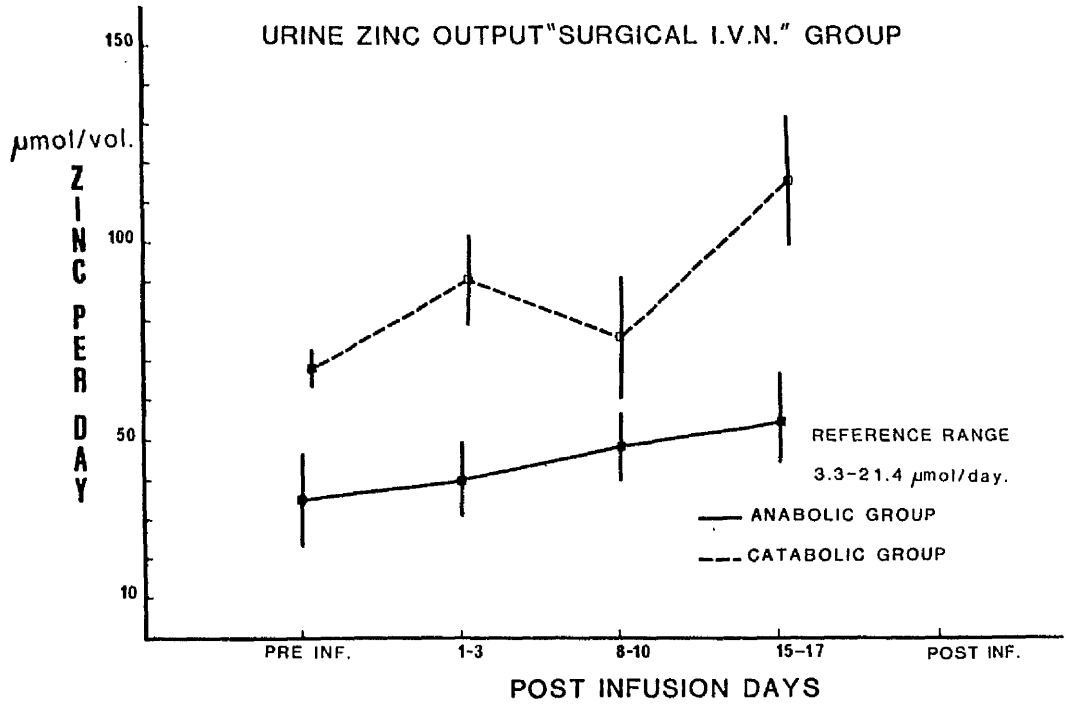


Figure 12

Excretion of zinc in ten anabolic patients and six catabolic patients receiving 4854 infusion.

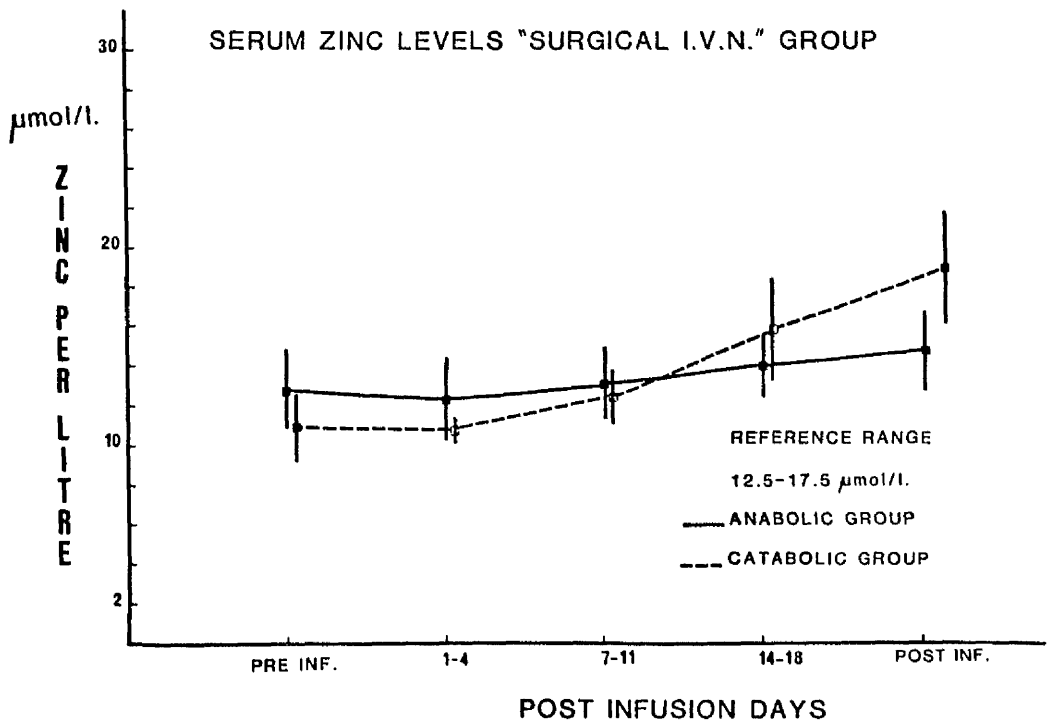


Figure 15

Serum zinc concetrations in ten anabolic patients and six catabolic patients receiving 4854 infusion.

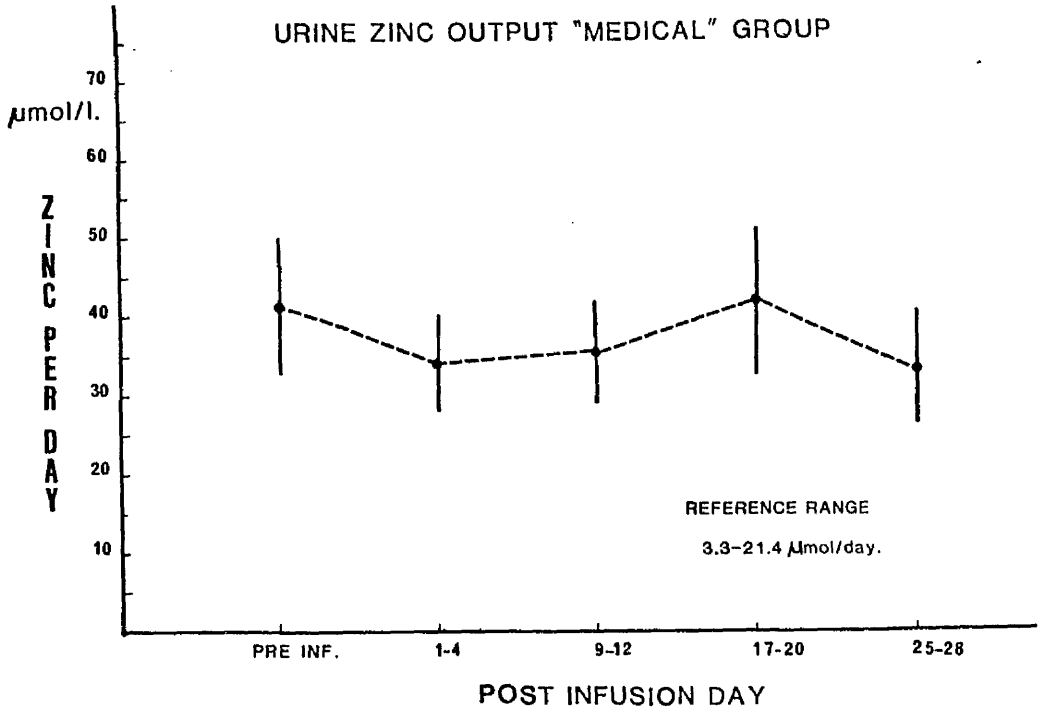


Figure 13

Excretion of zinc in four medical patients receiving 4854 infusion.

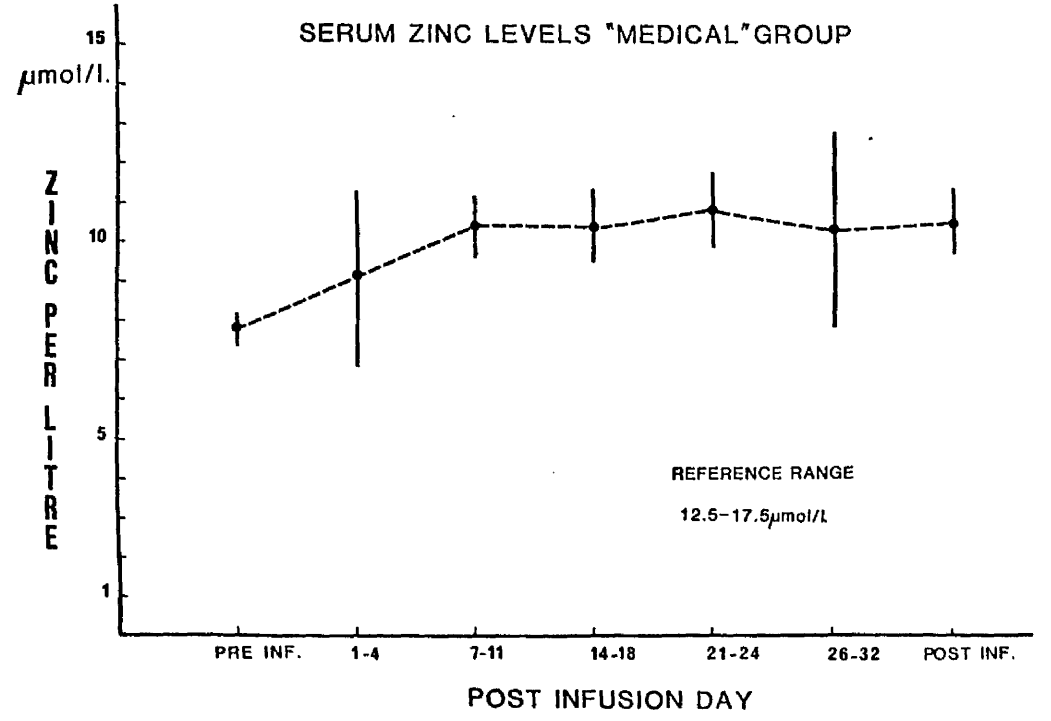


Figure 16

Serum zinc concentrations in four medical patients receiving 4854 infusion.

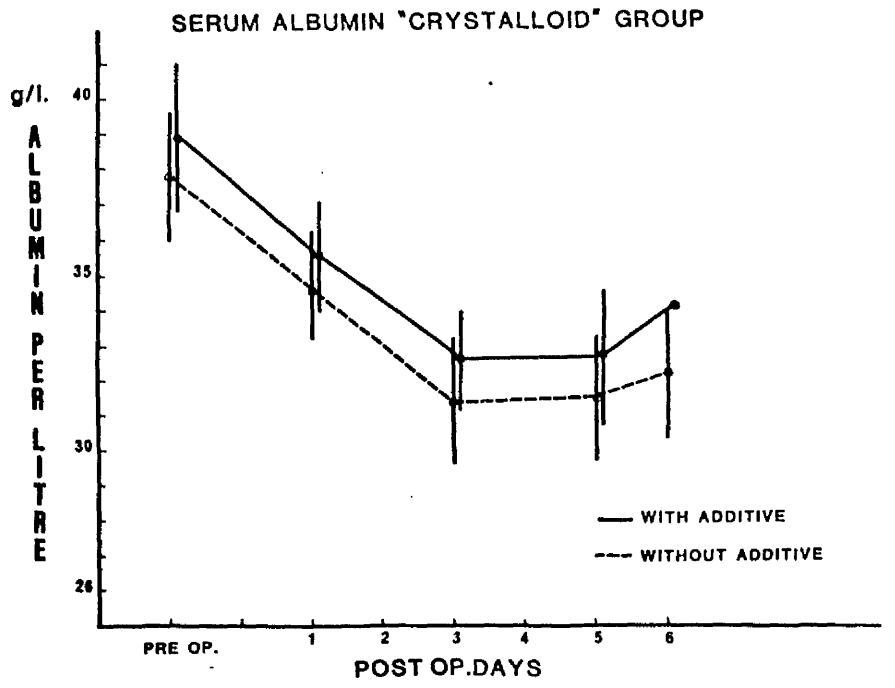


Figure 17

Comparison of serum albumin in two groups of ten patients with 4854 and without 4854 infusion.

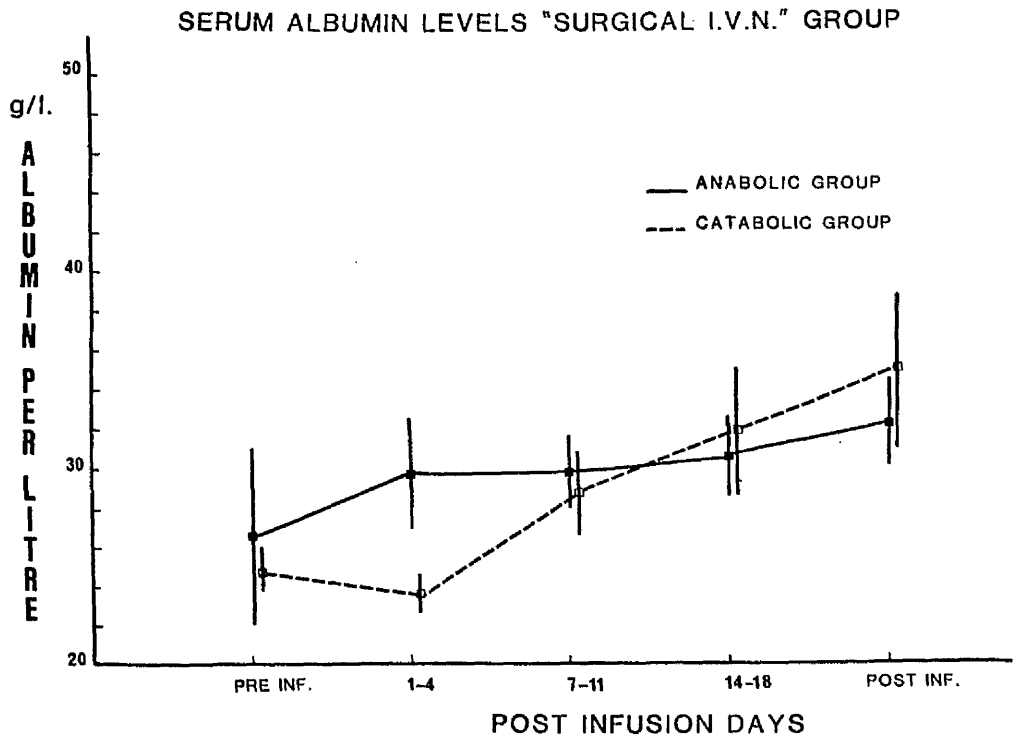


Figure 18

Serum albumin in ten anabolic patients and six catabolic patients receiving 4854 infusion.

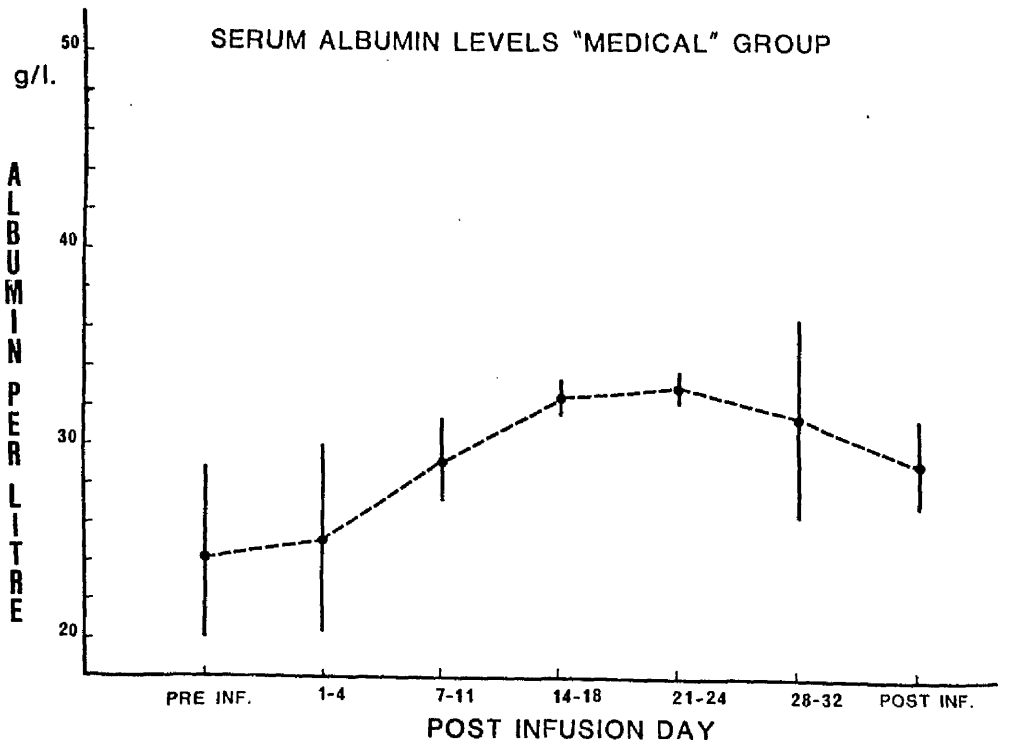


Figure 19

Serum albumin in four medical patients receiving 4854 infusion.

COPPER

4.2.1 Introduction

The existence of copper in both plant and animal tissues was recognised in the early 19th century. The concept of copper deficiency in man was not widely accepted. During the past few years, copper deficiency has been reported in premature infants, in malnourished infants fed exclusively by the intravenous route and also in adults. There are a number of enzymes which contain copper. These cuproenzymes play a vital role in many physiological functions in man and animals (Boyd O'Dell 1976). Copper is involved in the development and maintenance of cardiovascular and skeletal integrity, central nervous system structure and functions, and in iron metabolism.

Anaemia, defects in connective tissue formation, albunism n, brain disease (Menke's kinky hair syndrome) and muscle incoordination, are some of the many pathological manifestations caused by copper deficiency.

4.2.2. Body Composition

The whole body copper content of the adult is about 80 mg. Neonates have higher copper concentration than adults; 200 mg/kg body weight which then falls to about 30 mg/kg. The distribution of the total body copper among the tissue varies. Liver contains a significant amount of copper. In general terms, copper distribution can be described as:

Liver : 1.47 ± 3.9 µg/g wet weight

Brain : 5.6 ± 0.2 µg/g wet weight

Kidney : 2.1 ± 0.4 µg/g wet weight

Muscle : 0.7 ± 0.02 µg/g wet weight

In blood, copper occurs in erythrocytes (where it is believed to be mainly bound to super-oxide dismutase) and plasma. Plasma contains about 15-26 μmol/l of copper, but is affected by age and sex.

In plasma, 90% of copper is bound to caeruloplasmin.

4.2.3 Dietary Intake, Absorption and Excretion

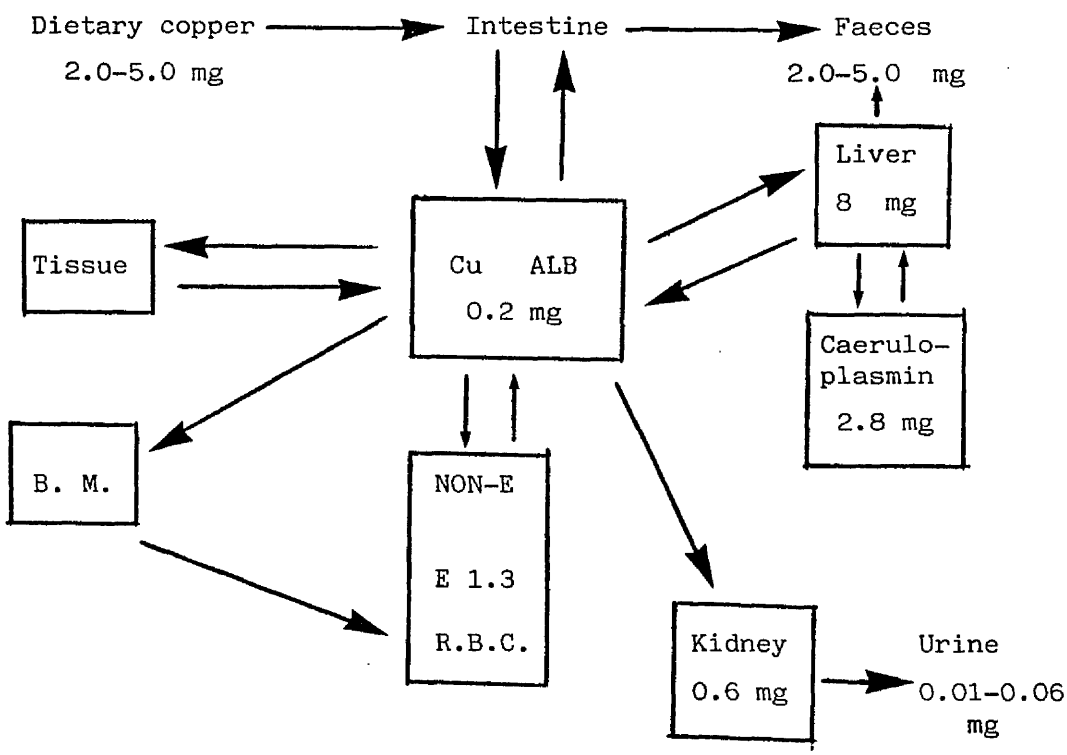
The recommended dietary intake for copper is 2 mg/day (31.5 μmol/l. The average diet contains 2-5 mg/day (31.5-78.7 μmol/day) copper. A diet which contains more vegetables and refined foods has less copper content. Certain foods such as shellfish and organ meat like liver and kidney, are rich in copper content.

Copper is mainly absorbed from the duodenum in man. Copper absorption and retention is affected by the chemical forms in which the metal is ingested, and by the dietary levels of several other minerals and organic substances. Inorganic compounds of zinc, cadmium and molybdenum in the presence of SO_4^{2-} excess iron and calcium, depress absorption of copper. Once copper is absorbed, it enters the blood plasma from the intestine and becomes loosely bound to serum albumin to form the small, direct reacting pool of plasma copper. In this form, it is distributed widely to the tissue and can pass readily into erythrocytes. Copper albumin serum pool also receives copper from tissue. The copper in caeruloplasmin does not appear to be readily available for exchange. Copper then reaches the liver and is incorporated into the mitochondria, microsomes, nuclei and soluble cell fractions. The copper is either stored in these sites or released for incorporation into caeruloplasmin and various copper-containing

enzymes. Caeruloplasmin is synthesised in liver and secreted into serum. The hepatic copper is also secreted into the bile and excreted via this route back to the intestinal contents. Much smaller amounts of copper also pass directly from the plasma into the urine or through the intestinal wall. A high proportion of ingested copper appears in faeces. Most of this normally consists of unabsorbed copper.

Intravenous injection of copper, resulting in elevated blood and tissue copper levels, is followed by a greater excretion of copper in bile, hence in faeces, but does not normally raise urinary output (Underwood 1977). Negligible amounts of copper are lost in sweat and comparatively small amounts in the normal menstrual flow.

Figure 10



Non-E = Non erythrocuproprotein

B.M. = Bone marrow

R.B.C. = Red blood cells

4.2.4 Biochemical Importance

There are a number of cuproproteins, in which there is a characteristic ratio between moles of protein and atoms of associated copper. The contained copper does not dissociate during isolation of the protein and they function as enzymes. These include caeruloplasmin, superoxide dismutase, cytochrome C oxidase, lysyl oxidase, tyrosinase and dopamine beta-hydroxylase. Copper metalloproteins exist in liver, red cells and other tissues. The incorporation of iron into haem requires the copper enzyme ferroxidase. The beta-globulin caeruloplasmin is able to oxidase aromatic amines in vitro. This protein may also serve as a transport protein for copper. The metal, although tightly bound to the protein, is taken up by specific cell receptors. From animal studies, the importance of copper to elastin and collagen synthesis, is clear as is the role of various copper enzymes in the central nervous system.

Inborn Genetic Errors

A) Menke's Syndrome

This is a fatal defect in young children, presenting as neurological damage and arterial and bone lesions, with [illegible], hair changes (steely or kinky hair). The disease may be due to failure of intestinal and other cell membrane transport. This condition cannot be reversed by copper therapy.

B) Hepatolenticular Disease (Wilson's Disease)

This is also a genetic error in which copper absorption in the gut is normal but intracellular copper metaboloism is abnormal. The failure of copper excretion in bile, leading to a gradual build-up of toxic levels in liver, then in kidney and brain.

4.2.5 Clinical Effects of Deficiency

A wide variety of disorders has been associated with dietary deficiency of copper. They include anaemia, depressed growth, bone disorders, depigmentation of hair and wool, heart failure, cardiovascular defects and gastrointestinal disturbances. The extent to which one of these dysfunctions is actually revealed depends upon the species, its age and sex.

Copper deficiency mainly occurs in premature babies and in infants suffering from malnutrition who have been fed exclusively on a cow milk diet. A marked anaemia which is resistant to iron therapy, neutropenia (less white cells) and signs of intestinal malabsorption with diarrhoea are reported. Children who fed intravenously with copper deficient/ regimens have similar signs and symptoms, but in addition, there are bone abnormalities. Adults receiving prolonged intravenous feeding may also develop copper deficiency. This deficiency can be corrected by supplying copper either orally or intravenously.

4.2.6. Biochemical Assessment of Copper Status

1) Serum copper measurement and /or assay of plasma caeruloplasmin gives a reasonable guide to copper status. Many clinical conditions tend to increase serum copper non-specifically. If serum copper is below 10 µmol/l, it suggests that copper deficiency may develop.
2) Abnormally high liver copper concentration is also a characteristic of a number of diseases.
3) Since urine copper is normally low (less than 1 µmol/day) is not a useful measure of copper deficiency. Also,, administration of chelating agents (such as amino-acid infusion) increases urinary copper (Tyrola et al 1982).

4) The direct measurement of red cell copper is possible and the activity of the red cell enzyme superoxide dismutase can be measured.

5) The copper protein, caeruloplasmin, appears to be involved in the conversion of iron from ferrous to ferric state. This is required for the transformation of iron into haemoglobin. A clinical effect of severe copper deficiency is therefore an iron deficiency anaemia.

4.2.7 Copper in Intravenous Nutrition

Long-term parenteral nutrition with infusion fluids which have not been supplemented with copper, may lead to deficiency. Low birth weight infants go into negative nitrogen balance from birth and there may also be substantial losses of trace minerals in excreta (Shaw 1973). Also, intestinal absorption of nutrients is poor. There have been some reports of copper deficiency in low birth weight infants (Al-Rashid et al 1971; Sann et al 1978). Severe hypocupraemia, neutropenia and extensive bone changes have been observed in infants and children receiving inadequate amounts of copper when fed intravenously (Karpel et al 1972; Heller et al 1978). Solomons et al and Fleming have shown that the administration of total parenteral nutrition without copper, resulted consistently in a decrease in serum levels. A dosage of 5 mg/day (20 μmol), was used by Dunlap et al (1974) to treat patients with haematological abnormalities and biochemical signs of copper deficiency. The AMA recommended intravenous supply is 50-1500 μg copper (8-24 μmol). In this study, 20 μmol (1.27 mg) copper per day was used intravenously.

4.2.8 Results of this Study

Urine Copper

A) Crystalloid Study

Urine excretion in both groups was at the same level at the start and on post-operative days 1, 2 and 3 (Figure 20). There was no significant difference between the groups on these days. However, on day 4, there was a significant increase. Patients given the additive (4854) excreted 0.84 $\pm$ 0.48 µmol/vol) which is above the normal range of (0.20-0.58 µmol/vol) (Table 24). Out of nine patients, three were within the normal range, while six were above. In the group without the additive, on day 4, excretion was (0.47 $\pm$ 0.19 µmol/vol) (Table 25). Out of ten patients, seven were within the normal range while three were above.

B) Surgical I.V.N

Both anabolic and catabolic surgical patients started with a high copper excretion (0.6 $\pm$ 0.9 µmol/vol) for anabolic patients and (1.6 $\pm$ 0.6 µmol/vol) for catabolic patients (Tables 26 and 27). There was no significant difference between the groups throughout the intravenous feeding period (Figure 21). The copper excretion throughout was above the normal range. In the anabolic group on infusion days 15-17, out of nine patients, one was within the normal range, while eight patients were above the normal range.

C) Medical I.V.N

Copper excretion prior to infusion in this group was normal, (Table 28), but was above the normal range through the infusion period (Figure 22). The copper excretion was constant during the study period. On days 1-4, out of four patients, two were within the normal range and two were above; days 9-12

TABLE 24

Copper in Urine, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (μmol/vol)			
			1	2	3	4
1	J.B	0.48	0.40	0.39	0.64	0.67
2	J.M	0.37	0.55	0.68	0.73	0.61
3	S.M	0.36	0.61	1.42	1.64	1.80
4	W.W	0.24	0.76	0.39	0.28	0.48
5	C.M	*	*	*	*	*
6	W.A	0.23	0.43	0.30	0.52	0.68
7	J.S	0.29	0.40	0.40	0.46	0.48
8	P.S	0.18	0.75	0.57	1.00	1.48
9	W.R	0.49	0.65	0.58	0.48	0.51
10	A.P	0.33	0.50	0.52	0.84	0.88
Mean		0.33	0.56	0.58	0.73	0.84
S.D		0.11	0.14	0.34	0.40	0.48

TABLE 25

Copper in Urine, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (μmol/vol)			
			1	2	3	4
1	M.M	0.06	0.28	0.12	0.26	0.24
2	A.C	0.31	0.76	0.47	0.38	0.47
3	W.L	0.39	0.31	0.40	0.27	0.60
4	J.M	0.30	0.22	0.69	0.30	0.20
5	M.M	0.20	0.21	0.71	0.53	0.49
6	H.S	0.52	0.53	0.33	0.49	0.56
7	J.M	0.81	0.28	0.68	0.68	0.34
8	T.C	0.46	0.52	*	0.54	0.33
9	R.M	0.44	1.01	0.64	*	0.60
10	H.S	0.48	1.25	1.20	0.81	0.84
Mean		0.40	0.54	0.66	0.47	0.47
S.D		0.20	0.36	0.37	0.19	0.19

* Urine contaminated

TABLE 26

Copper in Urine, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (μmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	W.M	-	2.19	2.54	1.54	-
2	J.W	-	1.49	1.08	0.93	0.43
3	J.T	-	0.81	0.91	-	-
4	I.P	0.38	0.33	0.29	0.42	-
5	D.M	0.35	1.24	1.04	0.68	0.66
6	J.N	-	1.10	1.05	1.30	-
7	M.K	2.41	2.58	1.61	1.40	-
8	M.G	0.36	0.43	1.03	0.73	-
9	W.W	-	0.60	0.80	1.28	-
10	A.G	1.39	-	-	1.12	-
Mean		0.6	0.8	0.8	0.6	0.54
S.D		0.9	0.8	0.7	0.5	-

TABLE 27

CATABOLIC GROUP

No.	Name	Pre-Infusion (μmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	A.G	1.3	1.0	0.7	-	-
2	A.M	2.21	2.46	5.51	-	-
3	D.M	-	2.30	2.90	-	-
4	H.W	-	1.20	1.30	1.04	-
5	I.C	2.14	3.25	3.20	1.90	0.74
6	G.R	-	0.94	1.04	1.10	-
Mean		1.60	1.50	2.00	1.30	0.74
S.D		0.60	1.00	1.80	0.40	-

TABLE 28

Copper in Urine, Medical I.V.N. Study

No.	Name	Pre-Infusion (µmol/vol)	Post-Infusion Days			
			Mean 1-4	9-12	17-20	25-28
1	R.M	-	1.55	1.89	-	-
2	P.P	-	0.58	0.46	0.9	1.0
3	E.I	-	1.41	1.54	1.37	1.07
4	J.C	0.44	0.57	0.48	0.75	-
Mean		0.44	1.03	1.09	1.01	1.04
S.D		-	0.46	0.63	0.26	0.04

were similar, while on days 17-20 all three patients were above the normal range and they remained the same until days 25-28.

Serum Copper

A) Crystalloid Study

Both groups, prior to operation, started within the normal range (with additive 18.3 $\pm$ 3.5 µmol/l; without additive 21.4 $\pm$ 3.7 µmol/l). Throughout the study both groups remained within the normal range (Figure 23). There was no significant difference between the groups on each day of the study (Tables 29 and 30). In both groups, on day 6, out of ten patients, nine were within the normal range while one was above.

Caeruloplasmin, in both groups, was of similar concentration (with additive 299.1 $\pm$ 119.9 mg/l; without additive 299.3 $\pm$ 103.8 mg/l, which was within the normal range (150-450 mg/l). The concentrations remained the same throughout the study (Figure 26). There was no significant difference between the groups on any day during the study.

B) Surgical I.V.N

Both anabolic and catabolic groups started with a normal copper concentration (Tables 31 and 32) and they stayed normal until days 14-18. However, when the infusion was stopped, the copper concentration had increased just above the normal range (Figure 24). There was no significant difference between the groups at any day during the study, but when the day prior to operation was compared with the last day of infusion, in both groups, there was a significant increased (P less than 0.01). In the anabolic group, out of five patients, four were above the normal range and one was within; while in the catabolic group, all three patients were above the

TABLE 29

Copper in Serum, Patients with 4854 Infusion

No.	Name	Pre-op	Days 1	Post-op 3	5	(μmol/l) 6
1	J.B	15.0	15.5	19.0	23.5	23.0
2	J.M	18.5	16.5	17.0	18.5	19.0
3	S.M	21.5	15.5	21.0	24.5	23.0
4	W.W	17.5	15.5	17.0	21.0	20.0
5	C.M	21.5	22.5	21.5	27.0	25.5
6	W.A	13.2	10.9	11.7	11.7	15.2
7	J.S	16.0	14.0	14.5	16.5	18.0
8	P.S	23.5	19.0	19.0	19.0	18.5
9	W.R	15.0	16.0	15.5	15.0	16.5
10	A.P	21.0	22.0	23.0	19.5	18.0
Mean		18.3	16.7	17.9	19.6	19.7
S.D		3.5	3.5	3.5	4.6	3.2

TABLE 30

Copper in Serum, Patients without 4854 Infusion

No.	Name	Pre-op	Days 1	Post-op 3	5	(μmol/l) 6
1	M.M	25.0	23.5	24.0	22.5	23.0
2	A.C	17.0	18.0	22.0	18.0	19.0
3	W.L	27.0	27.5	27.5	26.5	26.0
4	J.M	18.5	17.5	16.5	19.5	20.5
5	M.M	18.0	18.5	17.0	19.5	18.0
6	H.S	17.5	17.0	19.5	22.0	22.0
7	J.M	23.5	25.5	23.0	21.0	21.0
8	T.C	25.5	21.5	20.0	20.0	20.0
9	R.M	21.0	16.0	17.0	18.5	18.0
10	H.S	21.0	14.0	13.5	16.0	15.5
Mean		21.4	19.9	20.0	20.4	20.3
S.D		3.7	4.4	4.2	2.9	3.0

TABLE 29 IIA

Caeruloplasmin, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (mg/l)			
			1	3	5	6
1	J.B	419	355	411	482	525
2	J.M	447	218	300	334	316
3	S.M	172	140	186	272	246
4	W.W	220	198	200	190	236
5	C.M	-	380	-	-	394
6	W.A	-	70	46	110	230
7	J.S	250	148	230	148	250
8	P.S	226	226	198	244	198
9	W.R	270	200	236	160	250
10	A.P	390	310	382	306	254
Mean		299	225	243	250	290
S.D		104	98	110	115	99

TABLE 29 IIB

Caeruloplasmin, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (mg/l)			
			1	3	5	6
1	M.M	515	549	525	507	519
2	A.C	196	274	470	306	330
3	W.L	256	232	248	300	232
4	J.M	154	146	160	180	208
5	M.M	220	172	198	206	198
6	H.S	274	220	280	394	366
7	J.M	240	360	246	320	360
8	T.C	400	326	300	296	240
9	R.M	466	206	370	540	220
10	H.S	270	-	-	-	-
Mean		299	276	311	339	297
S.D		120	123	122	122	106

TABLE 31

Copper in Serum, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (μmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	18.5	21.8	-	29.2
2	J.W	22.7	21.6	21.2	20.9	23.2
3	J.T	-	21.9	20.0	-	-
4	I.P	19.5	18.0	-	19.5	-
5	D.M	18.5	17.5	19.0	-	20.0
6	J.N	11.5	19.0	20.0	25.4	-
7	M.K	14.0	-	16.0	19.8	-
8	M.G	19.0	21.0	25.0	22.0	-
9	W.W	17.0	18.3	19.1	21.5	28.8
10	A.G	24.5	-	25.0	24.5	28.5
Mean		18.0	19.1	20.7	21.4	25.6
S.D		4.10	1.60	2.90	2.40	3.90

TABLE 32

CATABOLIC GROUP

No.	Name	Pre-Infusion (μmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	15.0	14.0	19.5	-	-
2	A.M	-	23.5	23.9	26.5	-
3	D.M	13.5	21.0	24.5	-	-
4	H.W	19.0	15.5	14.5	23.0	26.5
5	I.C	19.5	19.5	16.5	25.0	26.0
6	G.R	-	16.0	23.5	23.8	22.0
Mean		16.5	18.0	19.8	24.3	24.7
S.D		3.00	3.60	4.20	1.50	2.30

normal range.

C) Medical I.V.N

In this group, the patients started at a normal serum copper concentration (11.8 ± 3.9 μmol/l) and then it rose steadily during the study period (Figure 25). Copper concentration at the end of the study was above the normal range (28.1 ± 3.8 μmol/l) (Table 33). Of three patients, two were above the normal range while one was at the upper limit of normal (23.0 μmol/l).

TABLE 33

Copper in Serum, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (μmol/l)					
			Mean 1-4	7-11	14-18	21-24	28-32	Stopping Value
1	R.M	13.5	23.5	-	17.0	-	-	23.0
2	P.P	-	12.5	16.5	20.0	22.0	17.0	29.0
3	E.I	15.5	16.7	19.3	21.7	23.0	27.1	32.3
4	J.C	6.5	-	13.5	14.0	-	-	-
Mean		11.8	17.6	16.4	18.2	22.5	22.1	28.1
S.D		3.9	4.5	2.4	2.9	0.5	5.1	3.8

4.2.9 Discussion

Copper Excretion

1) Crystalloid Study

Although copper losses by routes other than urine were not measured, patients apparently retained about 96% of the copper infused, and the total mean percentage of urine copper excretion was 3.4% during the study period. At the end of the study there was no significant difference in copper excretion between the groups given the additive and the group without additive infusion.

2) Surgical and Medical I.V.N

The mean percentage of urinary copper excretion in the surgical anabolic group was 5.5 $\pm$ 2.6%, while for the catabolic group it was 9.9 $\pm$ 6.0%, and for the medical patients it was 5.5 $\pm$ 2.6%. Copper excretion was higher in these patients compared to the crystalloid group, probably due to the presence of chelating agents such as amino-acids. Tyrala et al (1982) have shown in infants that copper excretion was increased when they were fed on amino-acids intravenously. Urinary copper excretion is increased during catabolic illness, and copper behaves in a similar way to zinc.

Carr et al (1975) reported increases in urine copper in children who were recovering from severe burning injury, the increase persisting for up to two months until healing occurred. This suggests that catabolism will increase the urine copper excretion.

In the present study, even although the urine copper excretion was increased, the total amount lost in urine never exceeded 2 μmol copper per day, therefore, this loss is unlikely

to induce copper deficiency. The main route of copper excretion normally is via bile into faeces and only a small amount is excreted in urine. The amount of faecal excretion varies with the dietary input and this is the main homeostatic mechanism.

Serum Copper

1) Crystalloid Study

Both groups started within the normal range. Serum copper concentration drops slightly after operation and then rises to within the normal range. This result was similar to that found by Hallbrook et al (1978). They studied forty-five patients subjected to surgical trauma. The patients were randomly distributed into two groups. One group received intravenously, a zinc and copper containing solution while the other group was without this additive. There was a fall of serum copper concentration in both groups after operation, and then it increased steadily. The fall was more pronounced in patients not receiving the additive. The duration of the present crystalloid study was not long enough to see a real change in copper concentration, since it is known that biochemical changes during copper deficiency develop slowly. Mills (1979) has shown that when animals are deficient in copper, the first change is in serum caeruloplasmin, then in tissue, tyrosinase, cytochrome oxidase, superoxide dismutase activity, and then clinical signs such as weight loss and anaemia.

In this study, all the patients remained within the normal range for serum copper. Serum caeruloplasmin showed similar changes.

B) Surgical and Medical I.V.N

Both groups did show a rise in serum copper concentration by the end of the study, when patients were either above the normal range or at the upper limit of the normal range. Also, there was a significant rise in caeruloplasmin level. The expected levels of serum copper and caeruloplasmin for surgical patients of this type are at the upper limit of normal, since an increase in caeruloplasmin is an acute phase response to the stress or surgical trauma. Aronsen et al (1972) studied twenty-nine patients undergoing surgical operation and found that the caeruloplasmin level starts increasing after the operation, and increases steadily for up to fourteen days.

The medical intravenous nutrition patients started with low normal serum copper levels, which is to be expected for patients not receiving any copper supplement (Hallbrook et al 1978). This group included patients with malabsorptive disease and possible history of increased faecal losses. After a month or more, an intravenous nutrition with 20 μmol (1.27 mg) copper per day additive, the average serum copper had risen to above the normal range, as expected in this sort of patient with a variety of stress and inflammatory illness.

Conclusions

Various authors have reported copper deficiency during intravenous nutrition (Askari et al 1979; Lowry et al 1979). Many patients were treated by oral copper supplement ranging from 2-5 mg/day (31.5-78.7 μmol/day) of copper. Opinion as to the intravenous requirement is varied. The American Medical Association

recommended 8-24 μmol of copper intravenously, but from the present study it seems that 20 μmol copper per day can maintain copper levels in serum and can correct the acquired deficiency as in the medical cases. No evidence of clinical copper deficiency or biochemical signs were observed in any patients. As with all other metals in individual cases where there is excessive faecal or fistula fluid loss, an increased supply of intravenous copper may be required, but for the majority of patients 20 μmol (1.27 mg) of copper is enough to maintain serum copper levels. Serum copper or serum caeruloplasmin seems a valid index of copper status.

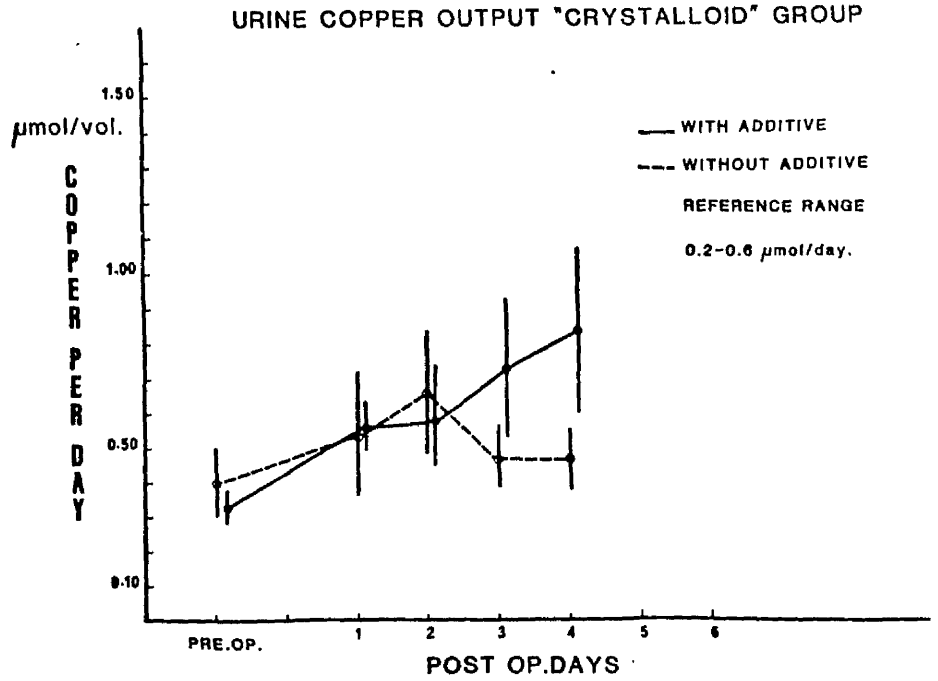


Figure 20

Comparison of copper excretion in two groups of ten patients, with 4854 and without 4854 infusion.

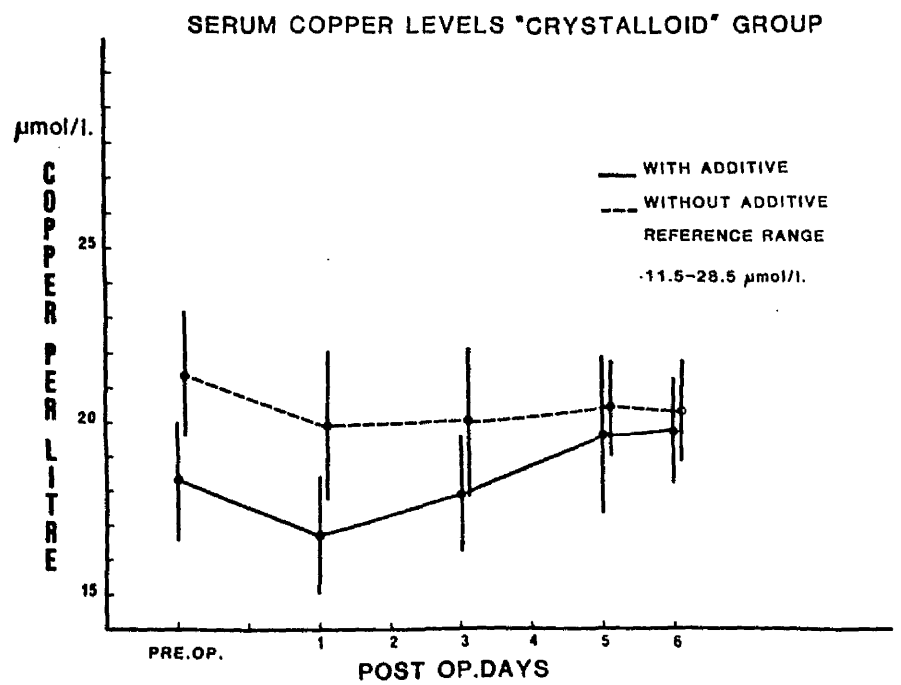


Figure 23

Comparison of serum copper concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

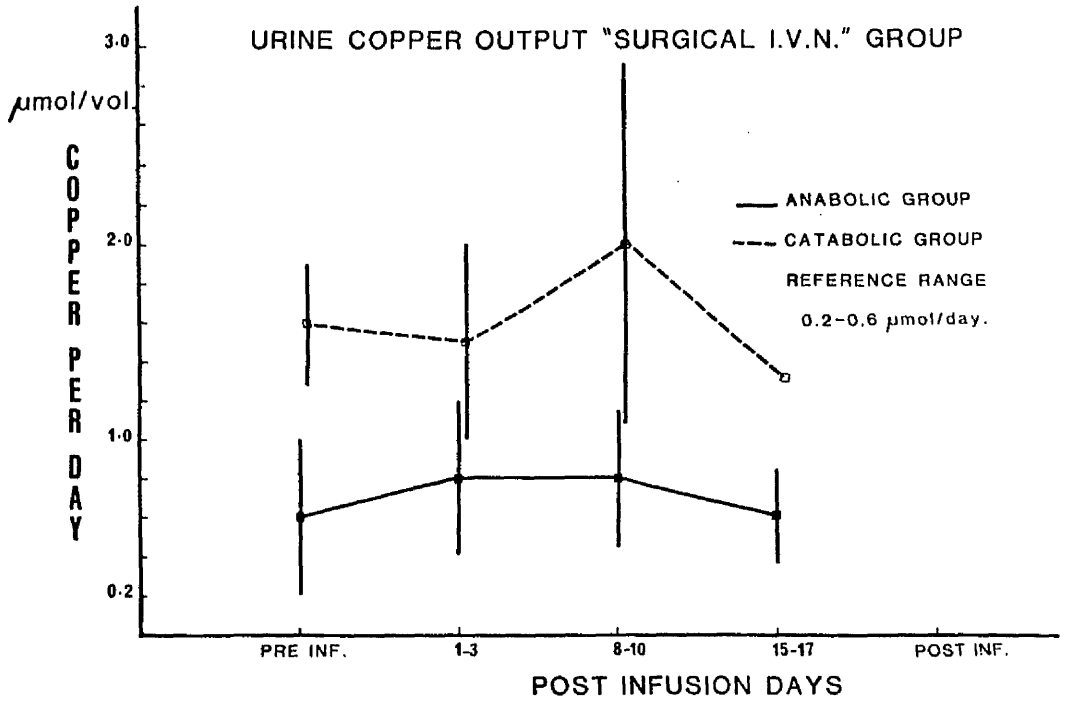


Figure 21

Excretion of copper in ten anabolic patients and six catabolic patients receiving 4854 infusion.

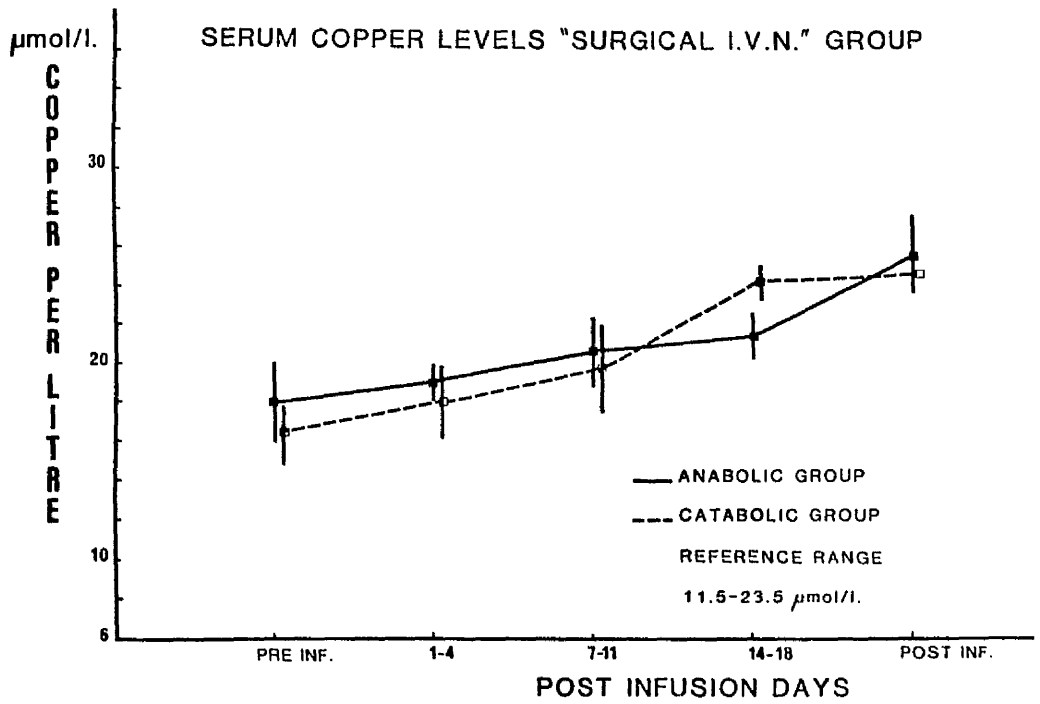


Figure 24

Serum copper concentrations in ten anabolic patients and six catabolic patients receiving 4854 infusion.

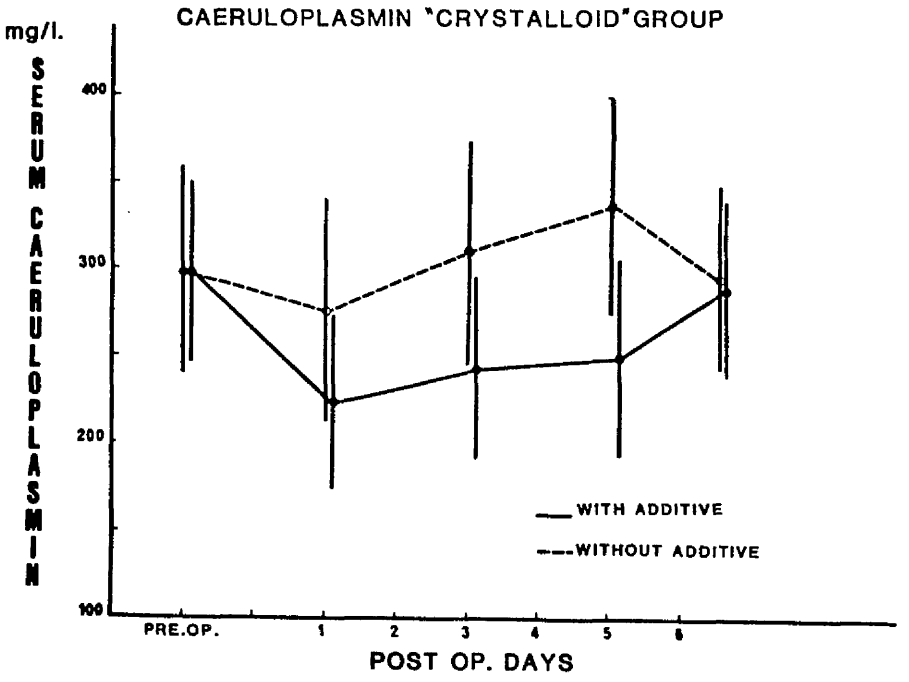


Figure 26

Comparison of serum caeruloplasmin concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

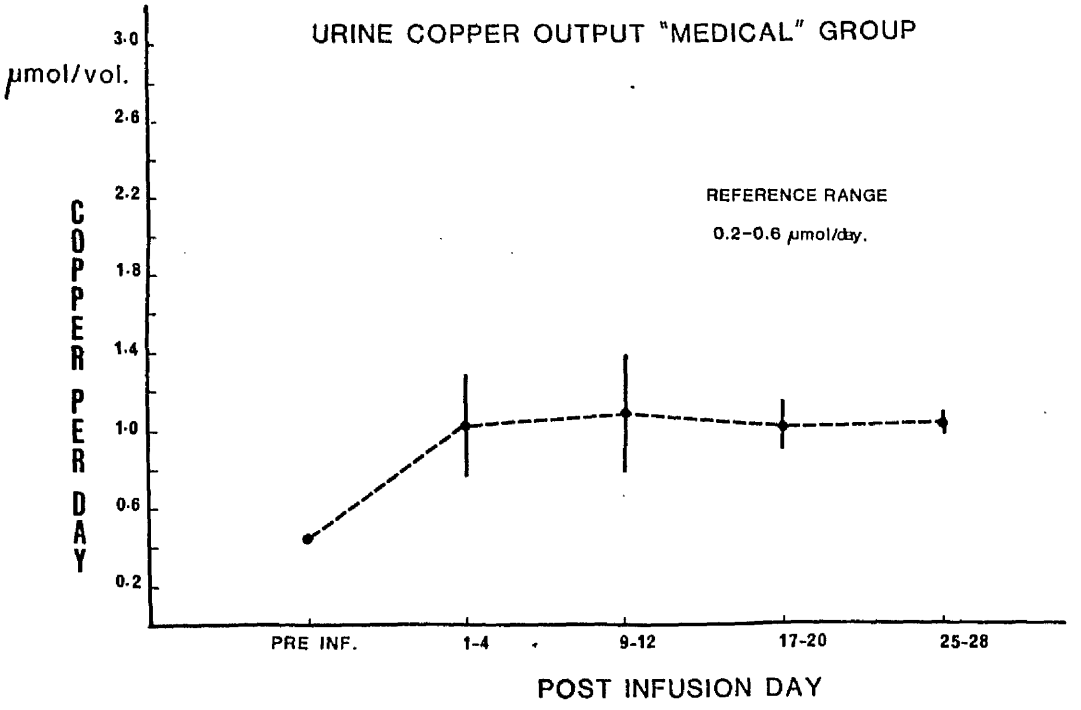


Figure 22

Excretion of copper in four medical patients receiving 4854 infusion.

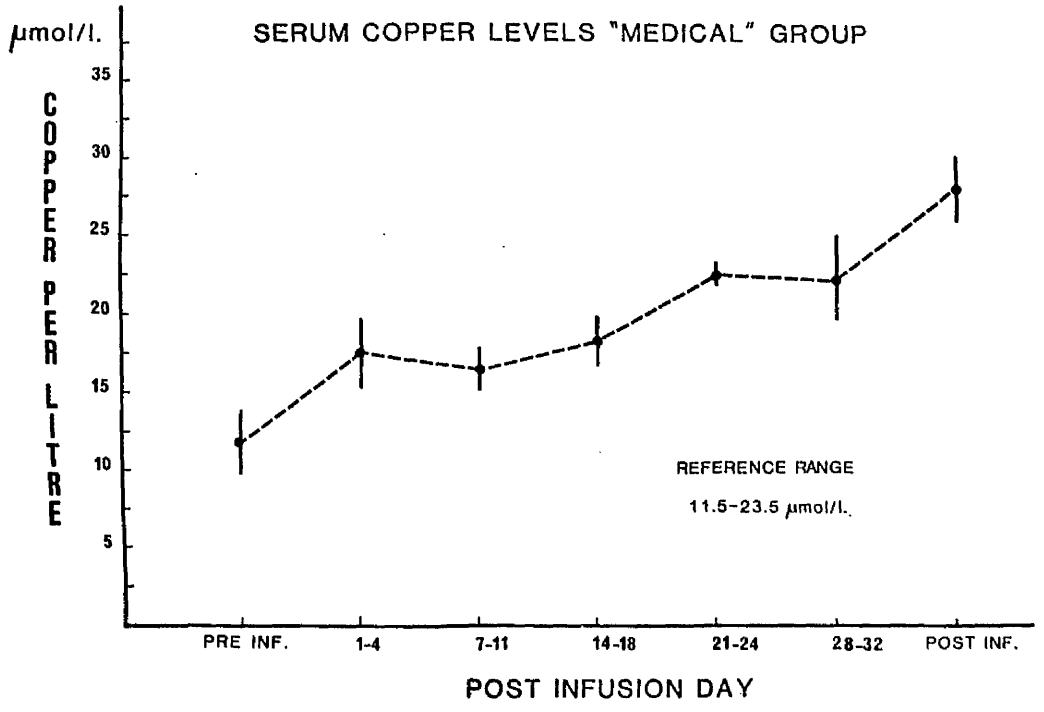


Figure 25

Serum copper concentrations in four medical patients receiving 4854 infusion.

MANGANESE

4.3.1 Introduction

Manganese is widely distributed in nature but occurs only in trace amounts in biological materials, particularly in animal tissue. Even when the concentrations are highest in liver and kidney, manganese is present only in amounts of two to three parts per million, that is, microgram per gram dry weight. Despite these very small amounts, it is clear that manganese plays several important roles in the maintenance of biological functions. The earliest studies were nutritional experiments which started about 1930 by McCollum, Elvehjem, Hart and others (Utter 1976). A few years later it was demonstrated that manganese prevented a skeletal abnormality in chickens. Since that time, manganese has been shown to be essential for many species of animals. Possible manganese deficiency in man has been reported (Doisy 1972).

Manganese is known to be a co-factor in a number of human enzymes, but no correlation between dietary intake and the activity of any of these enzymes has been demonstrated (Wenlock et al 1979).

4.3.2 Body Composition

The human body contains about 12-20 mg manganese. It is widely distributed in the body tissue and fluids. In the human, brain, kidney, pancreas and liver, in descending order, show higher manganese concentrations than other organs. Generally, higher manganese concentrations are seen in mitochondria compared to cytosol. Manganese concentration in blood and serum vary greatly. Whole blood contains about 9.0 µg/l (0.16 µmol/l), while serum contains about 0.5 µg/l (0.009

μmol/l). Serum manganese concentrations in normal humans have been variably reported, probably due to the contamination of blood during specimen collection (Versieck et al 1980).

4.3.3 Dietary Intake, Absorption and Excretion

The recommended dietary intake for manganese is 2.5-5 mg/day (41.5-91.0 μmol/day). The average daily intake in food is around 0.7-20 mg/day (10-300 μmol/day). Manganese content of food varies greatly. The highest concentration is in nut grain and cereals, while the lowest is in dairy products, meat, poultry, fish and sea-food. Relatively high concentrations of manganese were found in soluble ("Instant") coffee and tea. This accounts for 10% of the total daily intake (McLeod et al 1972). During the first week of life, an infant's manganese intake is low, 7 μg/day (0.13 μmol/day) producing a negative manganese balance, which is followed by a progressively increasing intake of manganese from infancy to two years. Despite this apparently diminished intake during early childhood, a relatively constant concentration of manganese is maintained in the liver throughout life.

The proportion absorbed is not certain but it has been estimated that a minimum of only 20 μg (0.36 μmol) must be retained to prevent deficiency. The precise mechanisms of absorption of manganese from the gastrointestinal tract, and the status of specific blood carrier for manganese is unclear at the present time. Although Cotzias et al (1960) proposed the existance of a specific manganese-carrying protein, called transmanganin, carrying one atom of manganese per molecule, there is not widespread agreement that manganese is associated with a specific protein in blood.

Absorbed manganese rapidly appears in the bile and is excreted almost exclusively in faeces. Smaller amounts are also excreted in other gastrointestinal secretions. Very little manganese is excreted in urine. The amount of faecal excretion varies with the dietary input, and this is the main homeostatic mechanism for maintaining balanced manganese tissue concentrations. This pathway is so effective that manganese toxicity is rare and has only been identified after chronic inhalation of large quantities of manganese dust during industrial exposure.

4.3.4 Biochemical Importance

Even although the manganous ion is known to be an activator of many enzymes, it is not possible at present to correlate the activity of manganese-dependent enzymes and the degree of deficiency. However, the skeletal abnormalities produced in animals appear to be caused by defective function of two manganese-dependent enzymes, a polysaccharide polymerase and galactotransferase, which are present in epiphyseal cartilege. These enzyme findings have not been made in man, but there have been some suggestions that an abnormality in manganese metabolism may be associated with the development of human mycopolysaccharidoses, which have many similar pathological features to the manganese deficiency state found in animals. Deficiency in animals has also been linked with sterility, possibly due to a reduced activity of mevalonate kinase, a manganese-dependent enzyme involved in cholesterol synthesis. A hypoglycaemic activity for manganese has also been suggested, but this is not proven.

Manganese seems to be intimately involved in the synthesis

of protein, DNA and RNA. The DNA manganese complex was first reported in 1957. They concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals. Since minute quantities of manganese were detected during the isolation of RNA and DNA, it was suggested that manganese may bear a functional relationship to protein synthesis and the transmission of genetic information. However, direct evidence of the in vivo role of manganese in mammalian protein biosynthesis is still limited, although in vitro evidence indicates that manganese is involved in protein synthesis (Leach 1976). It is however, possible that other divalent ions, such as magnesium, may interchange with manganese, hence major effects of manganese depletion may only become evident when this interchange does not occur, for example, due to simultaneous depletion of other divalent cations.

4.3.5 Clinical Effects of Deficiency

Manganese deficiency has been well documented in animals and is characterised by growth impairment, skeletal deformity, reproductive and neuromuscular abnormalities. Other defects in carbohydrate metabolism have been observed with manganese deficiency. Many of the guinea-pigs with a congenital deficiency have short survival times and exhibit aplasia or hypoplasia of pancreatic tissue. Furthermore, these animals which survive to adult age exhibit abnormal tolerance to intravenously administered glucose (Prasad 1976).

The only postulated case of human manganese deficiency was reported by Doisy (1972). While studying vitamin K deficiency in a volunteer under metabolic ward conditions, it was noted that the patient had weight loss, transient dermatitis, occasional nausea and vomiting, changes in hair and beard colour, and

slow growth of hair and beard. Protein synthesis seemed to be unaffected. The most striking finding was hypocholesterolaemia. These findings were due to the inadvertent failure to add manganese to the purified diet mixture. Supporting evidence that this clinical picture resulted from manganese deficiency was obtained by duplicating the results in chicks fed a purified diet deficient in both vitamin K and manganese (Burch et al 1975). These investigations suggest that manganese may be an essential trace element in man.

4.3.6 Biochemical Assessment of Manganese Status

Ideally, techniques such as metabolic balance studies should be performed, but that is not practicable for large-scale studies. Therefore, measurement of manganese in serum has to be used as a convenient guide to manganese status. The normal range for manganese in serum is 7-27 nmol/l (Table 6).

Studies performed in the early 1960s indicated that serum manganese may be increased following myocardial infarction or infectious hepatitis and it was proposed that manganese may be released from necrotic tissue. This observation has not been confirmed using careful sampling and analytical procedures.

4.3.7 Manganese in Intravenous Nutrition

Although the requirement for manganese has not been proven in man, the Food & Nutrition Board of the United States has recently provided guidelines for oral manganese 2.5-5 mg (46-91 μmol/day). An expert panel from the American Medical Association (1979) has recently suggested that for patients receiving intravenous nutrition, approximately 0.15-0.8 mg (3-13 μmol) manganese should be provided intravenously per day. For neonates, Shenkin et al (1978) suggested 0.3 μmol of manganese per kilogram per day intravenously.

In this study, 15 μmol (0.82 mg) per day of manganese was given intravenously to the patients.

4.2.8 Results of this Study

Urine Manganese

A) Crystalloid Study

Urine manganese excretion in both groups was similar at the start and on post-operative day 1 (Tables 34 and 35). There was no significant difference between the groups on these days, but there was a significant increase (P less than 0.05) between the groups on post-operative days 2, 3 and 4. Also in the group given additive, when the day prior to operation was compared with post-operative days 2, 3 and 4, there was a significant increase (P less than 0.05) (Figure 27).

At the end of the study, in the group given the additive, the mean urine manganese excretion was 76.7 $\pm$ 61.9 nmol/vol, and out of seven patients, four were within the normal range and three were above, while in the group without additive, excretion was 25.6 $\pm$ 13.1 nmol/l and all eight patients were within the normal range.

B) Surgical I.V.N

Both anabolic and catabolic surgical patients started within the normal range (51.0 $\pm$ 36.0 nmol/vol) for anabolic patients, and 19.0 $\pm$ 13.0 nmol/l for catabolic patients) (Tables 36 and 37). There was no significant difference between the groups throughout the intravenous feeding period (Figure 28). Manganese excretion was above the normal range in both groups on days 8-10 and 15-17. In the anabolic group on days 15-17, of nine patients, eight were above the normal range and one was within the range, while in the catabolic group, of four patients, two were within the normal range and two were above.

TABLE 34

Manganese in Urine, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (nmol/vol)			
			1	2	3	4
1	J.B	24.0	12.0	24.0	99.0	210.0
2	J.M	21.0	60.0	60.0	70.0	40.0
3	S.M	8.0	5.0	12.0	45.0	74.0
4	W.W	7.0	13.0	19.0	24.0	48.0
5	C.M	*	*	*	*	*
6	W.A	22.0	12.0	70.0	45.0	40.0
7	J.S	*	10.0	25.0	27.0	*
8	P.S	6.0	10.0	33.0	36.0	37.0
9	W.R	18.0	37.0	*	*	*
10	A.P	28.0	20.0	31.0	76.0	88.0
Mean		16.8	19.9	34.3	52.8	76.7
S.D		8.6	17,6	20.3	26.4	61.9

TABLE 35

Manganese in Urine, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (nmol/vol)			
			1	2	3	4
1	M.M	6.0	11.0	10.0	21.0	13.0
2	A.C	*	10.0	*	29.0	*
3	W.L	21.0	20.0	16.0	8.0	17.0
4	J.M	19.0	11.0	*	77.0	30.0
5	M.M	16.0	6.0	*	*	28.0
6	H.S	21.0	25.0	4.0	28.0	49.0
7	J.M	27.0	8.0	14.0	18.0	10.0
8	T.C	15.0	59.0	17.0	*	21.0
9	R.M	*	*	11.0	*	37.0
10	H.S	*	*	*	*	*
Mean		25.6	18.8	12.0	30.2	25.6
S.D		22.8	17.5	4.8	24.2	13.1

* Urine contaminated

TABLE 36

Manganese in Urine, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days Mean 1-3	8-10	15-17	Stopping Value
1	W.M	-	25.0	100.0	10.0	-
2	J.W	-	141.0	134.0	142.0	21.0
3	J.T	-	48.0	74.0	-	-
4	I.P	20.0	53.0	52.0	67.0	-
5	D.M	71.0	90.0	85.0	117.0	36.0
6	J.N	-	96.0	162.0	201.0	-
7	M.K	91.0	141.0	70.0	62.0	-
8	M.G	-	-	-	105.0	-
9	W.W	-	153.0	102.0	194.0	-
10	A.G	21.0	-	-	116.0	-
Mean		51.0	93.0	97.0	113.0	29.0
S.D		36.0	49.0	36.0	62.0	11.0

TABLE 37

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days Mean 1-3	8-10	15-17	Stopping Value
1	A.G	10.0	30.0	120.0	-	-
2	A.M	-	46.0	64.0	44.0	-
3	D.M	-	68.0	112.0	-	-
4	H.W	-	34.0	140.0	48.0	-
5	I.C	28.0	33.0	114.0	95.0	29.0
6	G.R	-	82.0	101.0	160.0	-
Mean		19.0	49.0	109.0	87.0	29.0
S.D		13.0	21.0	25.0	54.0	-

C) Medical I.V.N

This group started with high manganese excretion (109.0 $\pm$ 47.5 nmol/vol) (Table 38). It was steadily increased and then became stable after days 9-12 (Figure 29). Although manganese excretion was stable, it was above the normal range. All four patients were above the normal range through the intravenous feeding period.

Serum Manganese

A) Crystalloid Study

Both groups, prior to operation, started just above the normal range (with additive 14.2 $\pm$ 7.0 nmol/l; without additive 8.9 $\pm$ 4.4 nmol/l) (Tables 39 and 40). The patients without additive remained constant throughout the study; when each post-operative day was compared to the day prior to operation, there was no significant difference. The patients given additive show a steady increase in the serum manganese concentration (Figure 30). In this group, there was a significant difference between the day prior to operation and post-operative day 5 (P less than 0.01). Furthermore, when the groups were compared with one another on each particular day, there was a significant increase on day 3, and on day 5 (P less than 0.001). At the end of the study, after cessation of intravenous manganese by day 6, of ten patients, nine were within the normal range and only one was above. In the group not receiving intravenous manganese, of nine patients, six were within the normal range and three were sub-normal. In this group, two patients prior to operation started with sub-normal manganese serum concentrations and remained the same throughout the study.

TABLE 38

Manganese in Urine, Medical I.V.N. Study

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			
			Mean 1-4	9-12	17-20	25-28
1	R.M	-	175.0	183.0	-	-
2	P.P	-	480.0	60.0	75.0	105.0
3	E.I	157.0	179.0	143.0	160.0	207.0
4	J.C	62.0	68.0	79.0	115.0	-
Mean		109.5	225.5	116.2	116.7	156.0
S.D		47.5	153.5	49.3	34.7	51.0

TABLE 39

Manganese in Serum, Patients with 4854 Infusion

No.	Name	Pre-op	Days Post-op (nmol/l)			
			1	3	5	6
1	J.B	15.0	15.0	25.0	25.0	17.0
2	J.M	13.0	7.0	20.0	15.0	6.0
3	S.M	16.0	11.0	27.0	36.0	31.0
4	W.W	9.0	11.0	15.0	17.0	10.0
5	C.M	13.0	13.0	22.0	31.0	20.0
6	W.A	13.0	12.0	19.0	18.0	18.0
7	J.S	9.0	10.0	17.0	27.0	18,0
8	P.S	5.0	5.0	11.0	17.0	11.0
9	W.R	31.0	15.0	18.0	27.0	15.0
10	A.P	18.0	15.0	16.0	25.0	13.0
Mean		14.2	11.4	19.0	23.8	15.9
S.D		7.0	3.4	4.8	6.9	6.8

TABLE 40

Manganese in Serum, Patients without 4854 Infusion

No.	Name	Pre-op	Days Post-op (nmol/l)			
			1	3	5	6
1	M.M	20.0	18.0	18.0	16.0	16.0
2	A.C	10.0	11.0	6.0	6.0	6.0
3	W.L	7.0	2.0	7.0	9.0	4.0
4	J.M	5.0	5.0	5.0	5.0	5.0
5	M.M	12.0	13.0	7.0	11.0	23.0
6	H.S	7.0	5.0	4.0	7.0	9.0
7	J.M	7.0	7.0	5.0	2.0	-
8	T.C	7.0	8.0	4.0	12.0	9.0
9	R.M	6.0	4.0	4.0	7.0	7.0
10	H.S	8.0	10.0	9.0	9.0	10.0
Mean		8.9	8.3	6.9	8.4	9.9
S.D		4.4	4.8	4.2	3.9	6.1

B) Surgical I.V.N

Both anabolic and catabolic groups prior to the infusion, started within the normal range ((17.0 $\pm$ 10.0 nmol/l) for anabolic patients; 8.0 $\pm$ 6.0 nmol/l for catabolic patients) (Tables 41 and 42). In the anabolic group, serum manganese concentrations steadily increased throughout the feeding period, but there was a fall in serum manganese concentrations when feeding was stopped. The catabolic group was similar, but there was a sharp increase in manganese concentrations on days 1-4 (Figure 31). In both groups, when the day prior to operation was compared with the post-infusion day, there was a significant increase between the days on 7-11 and 14-18 (P less than 0.05), but there was no significant difference between the groups on any day during the study period. At the end of the study, on days 14-18, in the anabolic group all seven patients were above the normal range, and also in the catabolic group of four patients three were above the normal range while one was within the range.

C) Medical I.V.N

The medical patients prior to the infusion, started just above the normal range (33.7 $\pm$ 15.5 nmol/l) (Table 43). Serum manganese concentrations remained steady to days 14-18, then increased until the end of the study (Figure 32). All four patients were above the normal range during the intravenous feeding period.

4.3.9 Discussion

Urine Manganese

A) Crystalloid Study

The calculated percentage of urinary excretion was 0.3 $\pm$ 0.15% of the total infused dose of 60 umol (3.28 mg) of manganese.

TABLE 41

Manganese in Serum, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	25.0	44.0	-	17.0
2	J.W	5.0	31.0	34.0	34.0	10.0
3	J.T	-	10.0	27.0	-	-
4	I.P	17.0	24.0	-	32.0	-
5	D.M	31.0	24.0	25.0	-	20.0
6	J.N	13.0	29.0	42.0	44.0	-
7	M.K	27.0	-	69.0	102.0	-
8	M.G	7.0	40.0	16.0	33.0	-
9	W.W	25.0	35.0	38.0	41.0	24.0
10	A.G	13.0	-	27.0	47.0	25.0
Mean		17.0	27.0	36.0	48.0	19.0
S.D		10.0	9.0	15.0	25.0	6.0

TABLE 42

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	2.0	13.0	18.0	-	-
2	A.M	-	147.0	72.0	70.0	-
3	D.M	9.0	13.0	24.0	-	-
4	H.W	15.0	14.0	27.0	24.0	14.0
5	I.C	5.0	10.0	21.0	31.0	33.0
6	G.R	-	29.0	60.0	43.0	7.0
Mean		8.0	38.0	37.0	42.0	18.0
S.D		6.0	54.0	23.0	20.0	13.0

TABLE 43

Manganese in Serum, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (nmol/l)					
			Mean 1-4	7-11	14-18	21-24	28-32	Stopping Value
1	R.M	53.0	60.0	-	22.0	-	-	-
2	P.P	-	27.0	32.0	24.0	33.0	17.0	-
3	E.I	33.0	55.0	51.0	49.0	121.0	169.0	80.0
4	J.C	15.0	-	25.0	33.0	-	-	-
Mean		33.7	47.3	36.0	32.0	77.0	93.0	80.0
S.D		15.5	14.5	11.0	10.7	44.0	76.0	-

Thus, about 99% of infused manganese is retained by the patients. The main route of manganese output is via faeces. However, in these patients, faecal output was not measured, since faecal production was minimal. This suggests that the retention of manganese in these patients is very high.

B) Surgical and Medical I.V.N

The surgical anabolic patients had an average manganese output of 0.3 $\pm$ 0.13%, retaining therefore about 99% of infused dose over the study period. Catabolic patients excreted 0.1 $\pm$ 0.2% retaining the same amount as that of anabolic patients. Whereas the medical group excreted about 1.0 $\pm$0.28% of their infused manganese. This suggests that the urinary manganese excretion in all these patients is very low indeed.

Serum

A) Crystalloid Study

The patients from both groups prior to operation started within the normal range of serum manganese. The level drops after the operation as a response to stress or trauma. In the group without additive, the serum manganese concentration was constant, which was at the lower limit of the normal range throughout the study period, while in the group given additive serum manganese increases considerably until day 5, but the serum concentration fell substantially within twenty-four hours of stopping the infusion. Even although serum manganese levels were at the upper limit of the normal range, there were no clinical or biochemical signs of toxicity.

B) Surgical and Medical I.V.N

The anabolic and the catabolic patients started within the normal range, and values rose throughout the study to above

the normal range in virtually all surgical intravenous nutrition patients. After cessation of intravenous manganese, the serum manganese level drops considerably.

The medical patients started above the normal range and remained the same throughout the study. Even although the serum manganese levels were high, there were no clinical or biochemical signs of toxicity. In earlier studies, Addamel^{R} was used, providing 60 μmol (328 mg) of manganese per day. In these patients, manganese excretion was as high as about 649 nmol/volume; even then, in these patients there was no evidence of manganese toxicity.

Conclusions

From all these different studies it seems that manganese requirement for man is very low indeed. In this trial, manganese supply was at the upper limit of the recommended allowance, and 15 umol/day (0.82 mg) provided intravenously during this study, is too high for the majority of the patients. Probably only half of this amount, that is 7.5 μmol/day (0.41 mg), would be sufficient to maintain serum manganese concentrations within the normal range. The American Medical Association has suggested that patients receiving intravenous nutrition should receive 3-13 μmol/day.

One author has suggested that poor bone growth and healing is present in orthopaedic patients, who apparently have no detectable manganese or copper in serum (Saltman 1981).

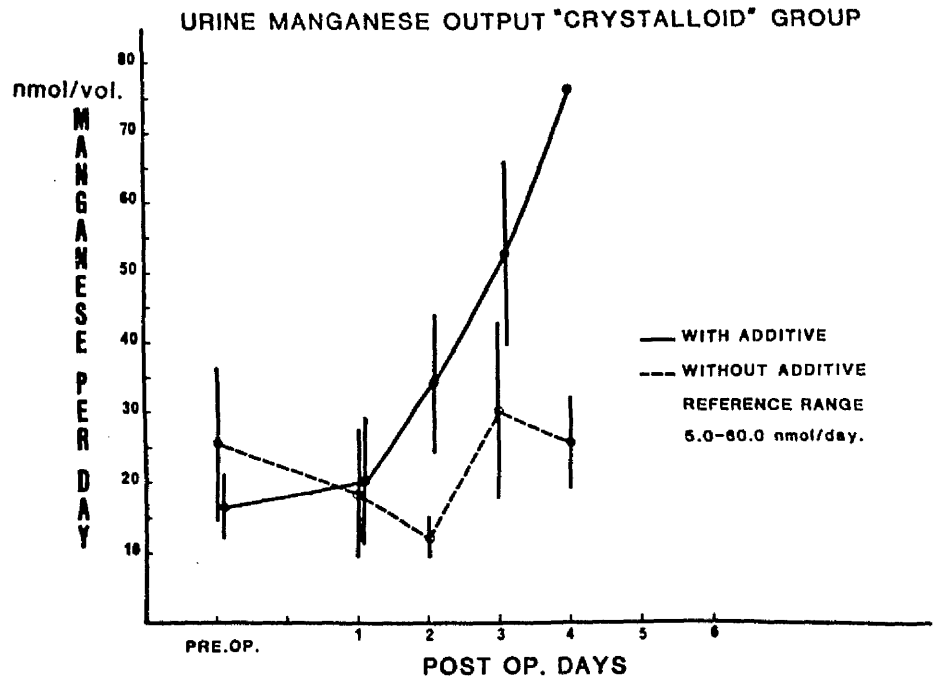


Figure 27

Comparison of manganese excretion in two groups of ten patients, with 4854 and without 4854 infusion.

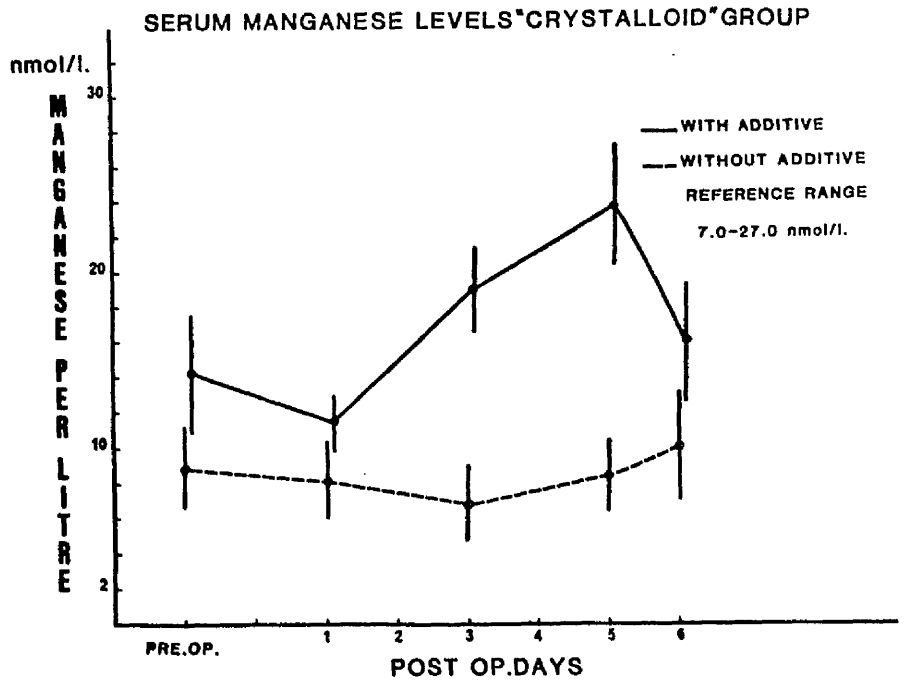


Figure 30

Comparison of serum manganese concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

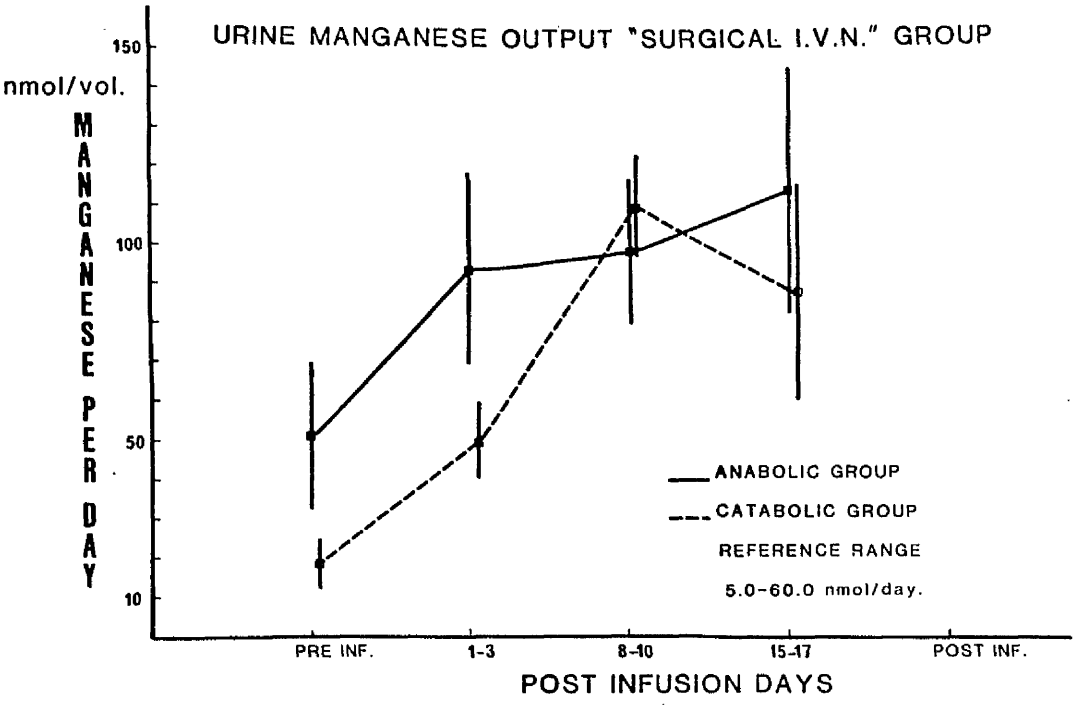


Figure 28

Excretion of manganese in ten anabolic patients and six catabolic patients receiving 4854 infusion.

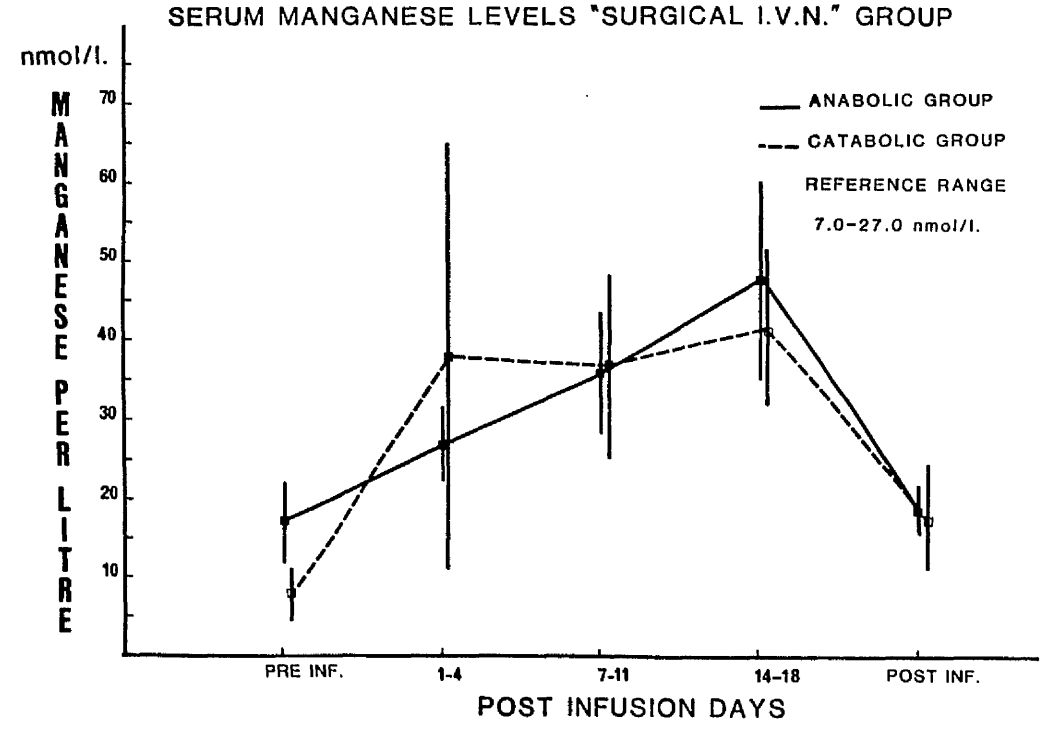


Figure 31

Serum manganese concentrations in ten anabolic patients and six catabolic patients receiving 4854 infusion.

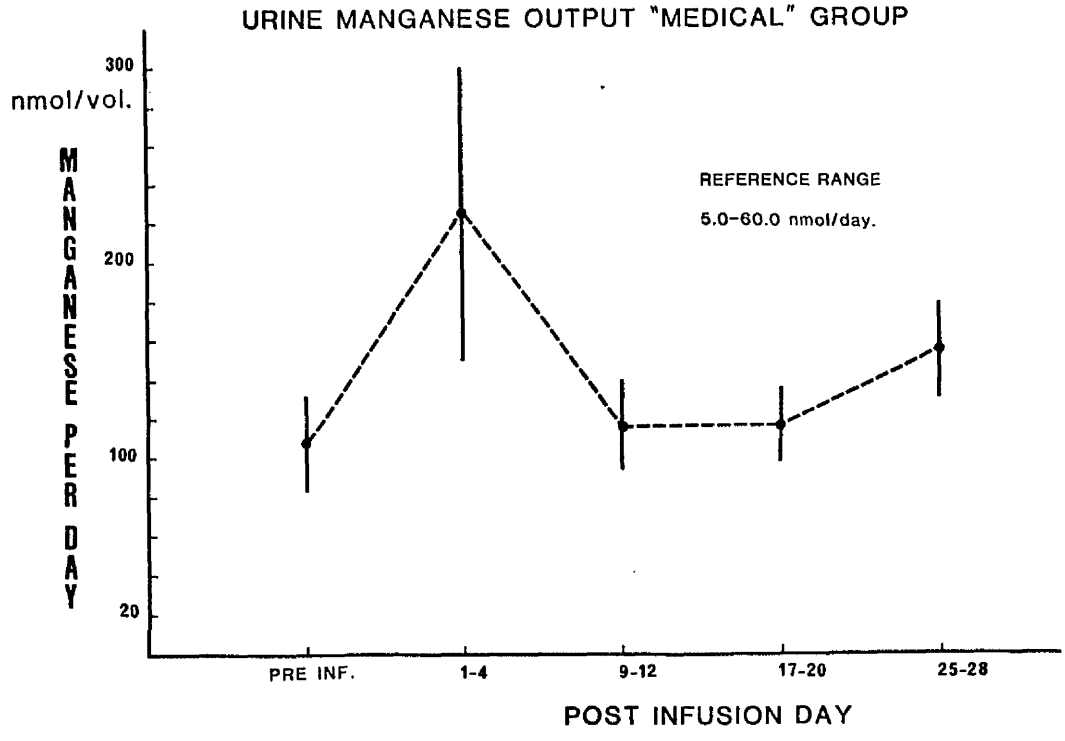


Figure 29

Excretion of manganese in four medical patients receiving 4854 infusion.

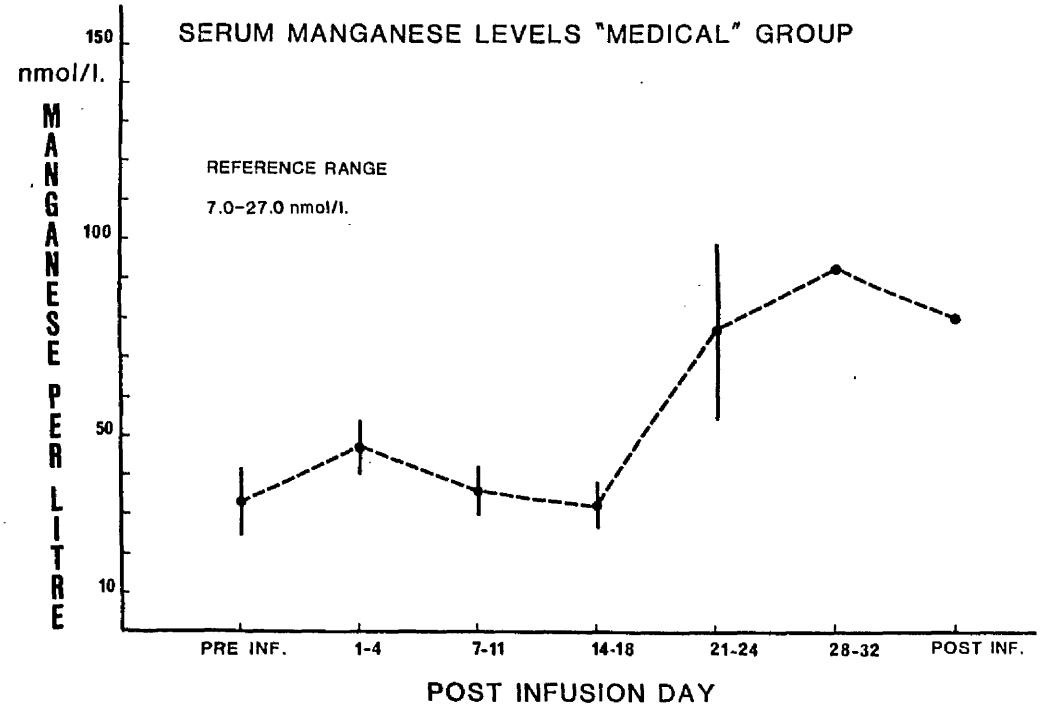


Figure 32

Serum manganese concentrations in four medical patients receiving 4854 infusion.

CHROMIUM

4.4.1 Introduction

Chromium is found in varying concentrations in air, water, soil and in most biological tissue. Its existence has been known for almost two centuries, and for most of the time it was thought only to be harmful. However, its role as an essential element for normal growth and development in animals has been well documented in the past two decades, and its essentiality for humans has been proposed recently.

Chromium was shown to be an essential trace element by Mertz and Schwarz (1979). They observed that rats fed with stock laboratory diets had impaired tolerance to a glucose load. This intolerance was reversed by an insulin potentiating factor which was present in brewer's yeast, meat and other foods. An organic chromium complex, of uncertain structure, known as the glucose tolerance factor (GTF) appears to act in a complex with insulin to facilitate the entry of glucose into cells. The essentiality of chromium for humans was subsequently demonstrated in 1975 (Jeejeebhoy et al 1975; Freund et al 1979), during prolonged intravenous feeding.

Chromium appears to act to potentiate the effects of insulin and therefore it has influence on carbohydrate lipid and protein metabolism.

4.4.2 Body Composition

The chromium concentration in different tissue have been reported to vary from less than one part per billion, that is microgram per litre, to several parts per million, that is milligrams per litre, wet weight (Mertz 1969), but this variation is partially attributable to discrepancies between

different analytical techniques. Approximately half of the naturally occuring chromium in liver has been reported to be concentrated in the nuclear fraction. With recent advances in atomic absorption techniques, it now appears that the previous published estimates of serum chromium concentrations were inaccurate. The total amount of chromium thought to be present in serum is around 2 nmol/l, that is 0.1 μg/l, which is near the detection limit of most commonly available methods. These values are lower by at least an order of magnitude than previous estimates (Versieck et al 1980). The concentration in normal urine is however, somewhat higher, around 10 nmol/l (6-43 nmol/24 hours) (Table 5).

4.4.3 Dietery Intake, Absorption and Excretion

The recommended dietary intake for chromium is around 50-200 μg/day (0.96-3.85 μmol). Minimum daily requirement for chromium is around 50 μg/day (0.96 μmol). There are few foodstuffs with appreciable amounts of chromium. It is possible that diets adequate in energy and protein are not necessarily optimal for chromium. Spices, brewer's yeast, liver and kidney are considered good sources but only small quantities are present in fish, vegetables and fruit. Western diet, where a high proportion of energy needs are met by refined carbohydrate, have lower total amounts of chromium than diets in other parts of the world.

Little work has been done on the intestinal absorption of chromium in man. Inorganic trivalent chromium salts are poorly absorbed by man and animals. Chromates are better absorbed but the preferred valency state for chromium in physiological conditions is Cr^{3+}, and it is likely that any chromates present in the diet are reduced in the gastro-

intestinal tract from Cr^{6+} to Cr^{3+}. Absorption of ^{51}Cr labelled chromic chloride is limited to approximately 0.5% (Hambidge 1981). In vitro investigations using the rat intestine suggest that Cr^{3+} may enter into the mucosal cell by a process of either simple or facilitated diffusion and subsequently be transported across the serosal surface by a more specific mechanism (Mertz et al 1971). The rate of absorption may be decreased by the presence of other metals and increased by various chelating agents. In contrast to the very restricted absorption of inorganic Cr^{3+}, 10-25% of chromium has been found to be absorbed from organic complexes in brewer's yeast.

It seems clear that at least two forms of chromium circulate in the plasma compartment. Some chromium is bound to transferrin in the beta-globulin fraction and is thought to be trivalent chromium. The other form is presumably GTF bound chromium, but definite studies on this point have yet to be done. There is ample evidence regarding dietary requirement for chromium, but little or no evidence regarding the necessity for certain species of chromium in diet. However, inorganic compounds must be converted to the biologically active form to function in vivo.

The major excretory route for chromium is by the kidney, and urine contains at least 80% of all excreted chromium. A small percentage is excreted via the intestine into faeces, which contains all unabsorbed chromium. Urinary chromium is derived from a relatively small fraction of the plasma chromium which is dialysable. Until the late 1970s, urine chromium excretion rates were thought to average from 5-10 ug daily in adults. However, as with plasma, recent advances in analytical techniques have led to a downward revision

of this figure. Hence, calculated requirements for intestinal absorption of chromium have decreased proportionately, and it now appears that man may need less than 1 μg/day to achieve chromium balance; and normal urinary excretion is around 10 nmol/l (0.5 μg/l) (Table 5).

4.4.4 Biochemical Importance

Various studies have shown some response to chromium in certain enzyme systems and pathways (Mertz 1969). Mathur and Doisy (1972) noted a marked shift in the hepatic intracellular distribution of chromium in rats under conditions of increased protein synthesis. The ^{51}Cr label shifted from nucleus to the cytoplasm in these experiments. The place of chromium in human nutrition remains uncertain. There is no known role for chromium other than in the form of glucose tolerance factor. Oral administration of chromium complex which is present in brewer's yeast and is known as the glucose tolerance factor has been shown to improve carbohydrate metabolism in diabetic subjects. When taken by normal persons, it can increase the proportion of cholesterol in high density lipoproteins (Mertz 1980; Riales et al 1981). The biochemical mechanisms underlying these effects are not understood. Variable reports have been made during clinical trials of inorganic chromium supplements given to diabetic patients and it seems that the complex which occurs naturally in yeast is required for the most efficient intestinal absorption of chromium. A major problem in all clinical studies is the difficulty of reliable determination of chromium in biological material and of the biologically active glucose tolerance factor. Most previous published estimates of chromium concentrations are inaccurate.

4.4.5 Clinical Effects of Deficiency

In experimental animals, diets low in chromium result in impaired growth, and reduced life-span with disturbances of glucose, lipid and protein metabolism. Rats deprived of adequate protein and of chromium, develop corneal lesions. The best evidence for the occurrence of human chromium deficiency states has been derived from the results of therapeutic trials of dietary supplementation with chromium. Subjects selected for these studies were chosen on the basis of impaired glucose tolerance, rather than biochemical evidence of chromium depletion. Most of the subjects improved. Therefore, the potential clinical importance of these findings is considerable (Hambidge 1981). A severe impairment of glucose tolerance, found in malnourished infants improves upon supplementation of the diet with 250 μg (4.8 μmol) of inorganic chromium per day (Saner 1980). Chromium deficiency has now been described in patients receiving prolonged parenteral nutrition. The first case described by Jeejeebhoy et al (1977) was a woman who developed unexpected weight loss and peripheral neuropathy after three and a half years of previously successful intravenous nutrition. She had marked glucose intolerance, resistant to insulin therapy. A negative chromium balance was found and low values of chromium demonstrated in whole blood and hair, relative to control samples. Infusion of inorganic chromium (150 μg/day or 4.8 μmol) for two weeks, resulted in clinical improvement, a reduction in plasma glucose levels with improvement in glucose tolerance and reduced requirement for insulin. Positive nitrogen balance was restored with body weight gain, and the peripheral neuropathy recovered after a few months of chromium therapy. In a later study,

Freund et al (1979) reported that a 45 year old woman who had been maintained for five months by intravenous nutrition after complete bowel resection, developed severe glucose intolerance, culminating in a hyperglycaemic hyperosmolar nonketotic coma. This patient lost body weight despite adequate energy input and exhibited a confusional state resembling hepatic encephalopathy. However, infusion of 150 µg chromium/day (2.88 µmol) was given and rapid improvement was observed. Within three to four days, glucose levels in blood returned to normal without the need of exogenous insulin. This patient also showed restoration of body weight on the same level of energy and protein intake as had been offered previously.

4.4.6 Biochemical Assessment of Chromium Status

The establishment of reliable and preferably simple, laboratory assays and criteria for the assessment of chromium nutrition, would obviously facilitate the detection and confirmation of chromium deficiency states. Unfortunately, no simple assay of proven validity is yet available and, even in the fully equipped research laboratory, major problems persist.

In the past, one outstanding problem has been the lack of analytical instrumentation and techniques of sufficient sensitivity and precision to allow accurate quantitative analysis of chromium concentrations of few parts per billion (ppb), that is micrograms per litre (µg/l) or less in biological materials. However, there has been very substantial progress recently in the development of more sensitive and sophisticated analytical systems, which have proved useful for the determination of chromium in biological samples. These include neutron activation analysis, stable isotope dilution techniques

and graphite furnace atomic absorption spectrophotometry with improved background correction systems.

The definition of chromium deficiency cannot rely solely upon determination of chromium in body tissue or fluids. The lowering of plasma insulin response to a glucose load, and /or improvement of glucose tolerance after a period of chromium supplementation is the most convincing evidence of prior chromium deficiency state. In normal individuals, there also should be a sharp increment in plasma and urine chromium levels within 30-120 minutes of a glucose load. Failure to observe this change suggests tissue deficiency of the glucose tolerance factor. Substantial GTF biological activity has been detected in hair follicles and there are some indications that measurement of the chromium content of hair can give a useful indication of body chromium status (Hambidge 1981). However, caution is necessary in interpreting these results, since in general, hair analysis probably provide only a rather crude index of trace element nutritional status.

Urine contains most of the chromium excreted by the body and the rate of urinary chromium excretion reflects in part the quantity of chromium in the diet. Furthermore, the majority of chromium present in the urine may be derived from biologically active components of plasma chromium. Therefore, it seems that measurement of increases in chromiun in serum and urine, after glucose loads, has to be used as the only practicable guide to chromium status.

4.4.7 Chromium in Intravenous Nutrition

Acute chromium deficiency has been described by two different groups, in patients receiving long-term parenteral nutrition. The cases are described in Section 4.4.5. This illustrates

the need to consider chromium requirement during intravenous nutrition. The use of 50% glucose as the sole energy source, may accentuate underlying deficiency by promoting urinary excretion of chromium. The American Medical Association recommended input is 15-20 μg Cr/day (0.28-0.38 μmol/day) during intravenous feeding. However, it is likely that the naturally occuring level of chromium, as a contaminant in amino-acid, carbohydrate and lipid solutions, is a considerable proportion of this requirement. Since urine chromium is increased during infusion of carbohydrates and any excess intravenous chromium will be rapidly excreted by the kidney, it is probably necessary to make some addition of chromium in intravenous feeding.

In this study, 0.4 μmol/day (20.8 μg/day) chromium was given intravenously to the patients.

4.4.8 Results of this Study

Urine Chromium

A) Crystalloid Study

Urine chromium excretion in both groups was within the normal range at the start of the study (22.1 $\pm$ 13.9 nmol/volume in the group with additive and 19.3 $\pm$ 9.9 mmol/volume for the group without additive). (Tables 44 and 45). There was a significant difference (P less than 0.05) between the groups on post-operative days 1, 2, 3 and 4. Also, in the group given additive, when the day prior to operation was compared with post-operative days 1, 2, 3 and 4, there was a significant increase (P less than 0.05) (Figure 33). The group without additive remained constant throughout the study. At the end of the study, in the group with additive, the mean urine chromium excretion was 127.4 $\pm$ 30.6 nmol/volume)

TABLE 44

Chromium in Urine, Patients with 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/vol)		
			1	2	3	4
1	J.B	50.0	70.0	100.0	140.0	120.0
2	J.M	20.0	70.0	120.0	120.0	100.0
3	S.M	11.0	130.0	120.0	110.0	140.0
4	W.W	11.0	45.0	83.0	100.0	100.0
5	C.M	*	*	*	*	*
6	W.A	19.0	45.0	81.0	106.0	122.0
7	J.S	11.0	66.0	131.0	133.0	140.0
8	P.S	17.0	100.0	101.0	134.0	185.0
9	W.R	19.0	58.0	86.0	83.0	87.0
10	A.P	41.0	87.0	128.0	239.0	153.0
Mean		22.1	74.6	105.6	129.4	127.4
S.D		13.9	27.4	19.7	44.9	30.6

TABLE 45

Chromium in Urine, Patients without 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/vol)		
			1	2	3	4
1	M.M	6.0	110.0	47.0	60.0	44.0
2	A.C	20.0	70.0	30.0	30.0	40.0
3	W.L	12.0	5.0	5.0	31.0	9.0
4	J.M	13.0	27.0	37.0	56.0	53.0
5	M.M	18.0	14.0	42.0	33.0	18.0
6	H.S	12.0	21.0	12.0	28.0	26.0
7	J.M	41.0	12.0	23.0	45.0	18.0
8	T.C	25.0	31.0	*	38.0	25.0
9	R.M	27.0	*	45.0	*	43.0
10	H.S	19.0	28.0	55.0	41.0	30.0
Mean		19.3	35.3	32.9	40.2	30.6
S.D		9.9	33.6	16.8	11.5	14.0

* Urine contaminated

and all nine patients were above the normal range, while in the group without additive, excretion was 30.6 ± 14.0 nmol/volume, and of ten patients, nine were within the normal range and one was above.

B) Surgical I.V.N

Both anabolic and catabolic surgical patients started above the normal range (185.0 ± 114.0 nmol/volume for anabolic patients and 141.0 ± 138.0 nmol/volume for catabolic patients). (Tables 46 and 47). There was no significant difference between the groups, and urine chromium excretion was above the normal range throughout the intravenous feeding period. At the end of the study, in both groups on days 15-17, all patients were above the normal range (Figure 34).

C) Medical I.V.N

This group started with a high chromium excretion (480 ± 369.0 nmol/volume) (Table 48). It was constant throughout the intravenous feeding period. Although chromium excretion was stable, it was above the normal range. All four patients were above the normal range during the study period.

Serum Chromium

A) Crystalloid Study

Both groups prior to operation started with barely detectable levels (with additive 5.2 ± 3.3 nmol/l; without additive 5.2 ± 2.2 nmol/l (Tables 49 and 50). The patients without additive remained constant throughout the study at very low levels. However, the patients with additive showed a sharp increase in serum chromium concentrations (Figure 36) after post-operative day 1. In this group there was a significant increase between the day prior to the operation and post-operative days 3, 5 and 6 (P less than 0.01). Furthermore,

TABLE 46

Chromium in Urine, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	W.M	300.0	280.0	450.0	-	-
2	J.W	-	169.0	312.0	271.0	61.0
3	J.T	-	222.0	330.0	-	-
4	I.P	157.0	298.0	268.0	418.0	-
5	D.M	314.0	282.0	428.0	294.0	-
6	J.N	-	255.0	247.0	309.0	-
7	M.K	224.0	403.0	405.0	407.0	-
8	M.G	40.0	146.0	193.0	355.0	-
9	W.W.	-	265.0	405.0	428.0	-
10	A.G	76.0	-	-	253.0	-
Mean		185.0	258.0	338.0	342.0	61.0
S.D		114.0	75.0	90.0	70.0	-

TABLE 47

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	A.G	300.0	280.0	450.0	-	-
2	A.M	54.0	62.0	336.0	331.0	-
3	D.M	-	182.0	368.0	-	-
4	H.W	-	288.0	287.0	365.0	-
5	I.C	68.0	176.0	345.0	338.0	-
6	G.R	-	528.0	565.0	478.0	-
Mean		141.0	253.0	392.0	378.0	-
S.D		138.0	158.0	100.0	68.0	-

TABLE 48

Chromium in Urine, Medical I.V.N. Study

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			
			Mean 1-4	9-12	17-20	25-28
1	R.M	-	305.0	493.0	-	-
2	P.P	-	300.0	313.0	355.0	355.0
3	E.I	111.0	378.0	436.0	422.0	356.0
4	J.C	849.0	720.0	659.0	778.0	-
Mean		480.0	426.0	475.0	518.0	356.0
S.D		369.0	173.0	124.0	186.0	0.5

TABLE 49

Chromium in Serum, Patients with 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/l)		
			1	3	5	6
1	J.B	8.0	11.0	74.0	81.0	100.0
2	J.M	4.0	4.0	75.0	107.0	70.0
3	S.M	7.0	14.0	97.0	156.0	120.0
4	W.W	7.0	6.0	65.0	96.0	67.0
5	C.M.	2.0	2.0	61.0	96.0	58.0
6	W.A	2.0	4.0	67.0	90.0	87.0
7	J.S	5.0	5.0	69.0	110.0	87.0
8	P.S	2.0	5.0	91.0	138.0	95.0
9	W.R	12.0	10.0	108.0	121.0	101.0
10	A.P	3.0	4.0	81.0	125.0	79.0
Mean		5.2	6.5	78.8	112.0	86.4
S.D		3.3	3.8	15.3	23.2	18.6

TABLE 50

Chromium in Serum, Patients without 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/l)		
			1	3	5	6
1	M.M	4.0	10.0	15.0	14.0	16.0
2	A.C	2.0	2.0	5.0	3.0	4.0
3	W.L	5.0	5.0	9.0	6.0	6.0
4	J.M	7.0	11.0	11.0	6.0	10.0
5	M.M	6.0	6.0	7.0	2.0	3.0
6	H.S	2.0	2.0	2.0	6.0	3.0
7	J.M	6.0	12.0	8.0	10.0	10.0
8	T.C	8.0	9.0	7.0	11.0	7.0
9	R.M	4.0	8.0	8.0	11.0	8.0
10	H.S	8.0	7.0	6.0	12.0	11.0
Mean		5.2	7.2	7.8	8.1	7.8
S.D		2.2	3.5	3.5	4.0	4.1

when the groups were compared with one another on post-operative days 3, 5 and 6, there was a significant difference (P less than 0.01). Although at the end of the study, after cessation of intravenous chromium on day 5, by day 6, serum chromium concentrations fell. All ten patients were still above the normal range, while in the group not given intravenous chromium, serum chromium remained unchanged.

B) Surgical I.V.N

Both anabolic and catabolic groups prior to infusion, started above the normal range (34.0 $\pm$ 21.0 nmol/1 for anabolic patients and 14.0 $\pm$ 11.0 nmol/1 for catabolic patients) (Tables 51 and 52). In both groups, serum chromium concentrations steadily increased during the study, but there was a fall in serum chromium concentration when provision of intravenous chromium was stopped. There was a sharp increase in serum chromium concentrations on days 14-18 in the anabolic group, while the catabolic group values on days 14-18 remained similar to those on days 7-11 (Figure 37). In both groups, when the day prior to infusion was compared with post-infusion days, there was a significant increase through the infusion period (P less than 0.05). Also, there was a significant difference between the groups on days 14-18 (P less than 0.05). The study ended on days 14-18. Measurements made at varying times thereafter, showed that all patients were above the normal range. (Seven patients in the anabolic group and four patients in the catabolic group).

C) Medical I.V.N

The medical patients prior to infusion, started with high serum chromium concentrations (29.0 $\pm$ 11.0 nmol/1) (Table 53). Serum chromium concentrations increased steadily through-

TABLE 51

Chromium in Serum, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	76.0	129.0	-	89.0
2	J.W	12.0	58.0	84.0	106.0	64.0
3	J.T	-	6.0	62.0	-	-
4	I.P	11.0	82.0	-	140.0	-
5	D.M	40.0	49.0	74.0	-	133.0
6	J.N	31.0	-	204.0	239.0	-
7	M.K	34.0	-	132.0	219.0	-
8	M.G	27.0	75.0	44.0	162.0	-
9	W.W.	78.0	84.0	97.0	110.0	76.0
10	A.G	42.0	-	146.0	350.0	127.0
Mean		34.0	61.0	108.0	189.0	98.0
S.D		21.0	28.0	50.0	87.0	31.0

TABLE 52

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	10.0	30.0	50.0	-	-
2	A.M	-	22.0	72.0	70.0	-
3	D.M	31.0	37.0	113.0	-	-
4	H.W	11.0	33.0	60.0	105.0	43.0
5	I.C	5.0	24.0	39.0	73.0	62.0
6	G.R	-	13.0	113.0	89.0	-
Mean		14.0	27.0	75.0	84.0	53.0
S.D		11.0	9.0	32.0	16.0	13.0

TABLE 53

Chromium in Serum, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (nmol/l)					Stopping Value
			Mean 1-4	7-11	14-18	21-24	28-32	
1	R.M	14.0	35.0	-	61.0	-	-	-
2	P.P	-	78.0	86.0	116.0	126.0	87.0	-
3	E.I	39.0	66.0	105.0	132.0	137.0	159.0	76.0
4	J.C	35.0	-	94.0	122.0	-	-	-
Mean		29.0	60.0	95.0	108.0	132.0	123.0	76.0
S.D		11.0	18.0	7.8	28.0	6.0	36.0	-

out the intravenous feeding period (Figure 38). After cessation of intravenous chromium infusion, serum chromium concentrations fell rapidly. All four patients were above the normal range throughout the study period.

4.4.9 Discussion

Chromium Excretion

A) Crystalloid Study

Although chromium losses by routes other than urine were not measured, the calculated percentage of urinary excretion was 27.3 $\pm$ 6.1%, of the total infused dose of 1.6 μmol chromium over the study period, patients apparently retained about 72% of the infused chromium. In the group with additive, the urinary excretion was much higher over four days. Also in the unsupplemented group, urine excretion increased slightly on post-operative days 2 and 3, indicating a small metabolic response to the operation, or possibly to the provision of intravenous glucose solution enhancing the chromium output.

B) Surgical and Medical I.V.N

In the surgical group, the anabolic patients had an average chromium output of 77.0 $\pm$ 14.8%, retaining therefore about 23% of the infused dose, whereas the more catabolic surgical patients excreted some 83.6 $\pm$ 25.3% of the infused chromium, retaining about 16% only of the chromium infusion. Urine chromium excretion was high indeed in the medical patients, about 115.5%. It should be considered that chromium present in (4854) trace element additive, is hexavalent whereas the biologically essential species or form of chromium is generally considered to be trivalent. The extent to which the hexavalent chromium is converted to trivalent chromium, either in a three-litre bag or in vivo, is not known.

Serum Chromium

A) Crystalloid Study

The patients from both groups prior to operation, started at low levels of serum chromium. In the group without additive, the serum chromium concentrations remained barely detectable, while there was a substantial increase in serum chromium concentrations in the group with additive, after post-operative day 1, with levels as high as 150 nmol/l (after four days) being achieved. Serum chromium concentrations fell rapidly within twenty-four hours after cessation of the chromium infusion.

B) Surgical and Medical I.V.N

The anabolic and the catabolic surgical patients had detectable levels of serum chromium, which increased steadily throughout the study. After cessation of the intravenous chromium, the serum chromium level dropped rapidly. The high serum chromium concentrations suggest that the increased urinary chromium losses resulted from the rise in serum chromium concentrations.

The medical patients had clearly measurable serum chromium concentrations, which increased throughout the intravenous feeding period. Even although chromium concentrations were high in all these different patients, there were no clinical signs of toxicity.

Conclusions

From the three studies, it seems that the chromium requirement for man is very low indeed. It seems likely that chromium provided in (4854) trace metal additive at 0.4 μmol/day (400 nmol/day) is excessive for the majority of the patients, especially since it adds to the chromium present in the amino-

acid solutions, as a contaminant. Therefore, probably only half of the amount, that is 0.2 μmol/day (200 nmol/day) would be sufficient to maintain serum chromium concentrations during intravenous nutrition. The American Medical Association recommendation for adult patients receiving intravenous nutrition is 280-380 nmol/day (15-20 μg/day) of chromium.

In this study, for trials, initially 400 nmol/day was used, which was at the upper end of the AMA range. It is now suggested that 200 nmol/day is adequate.

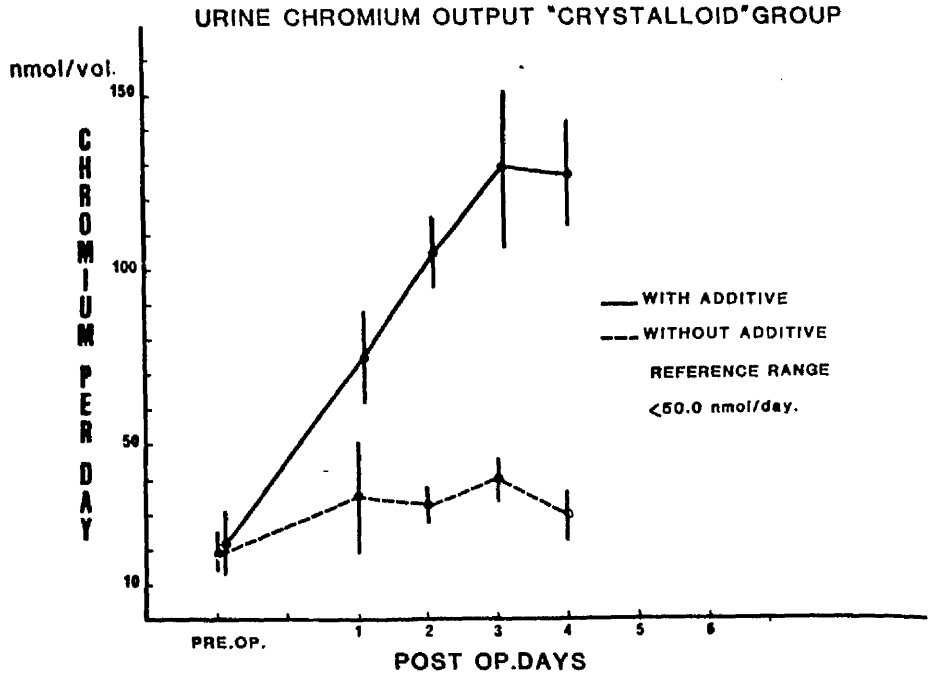


Figure 33

Comparison of chromium excretion in two groups of ten patients, with 4854 and without 4854 infusion.

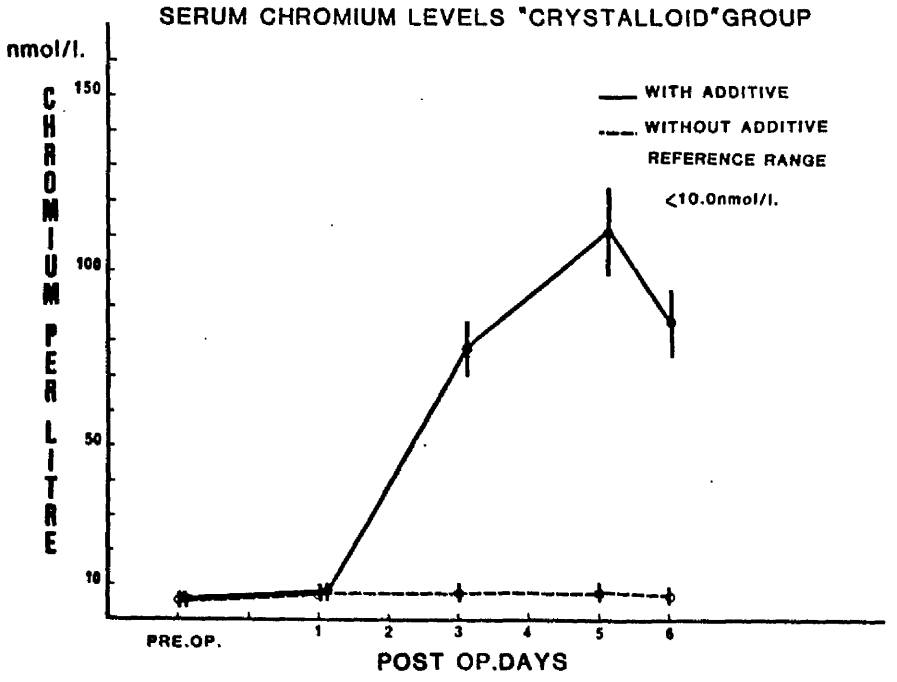


Figure 36

Comparison of serum chromium concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

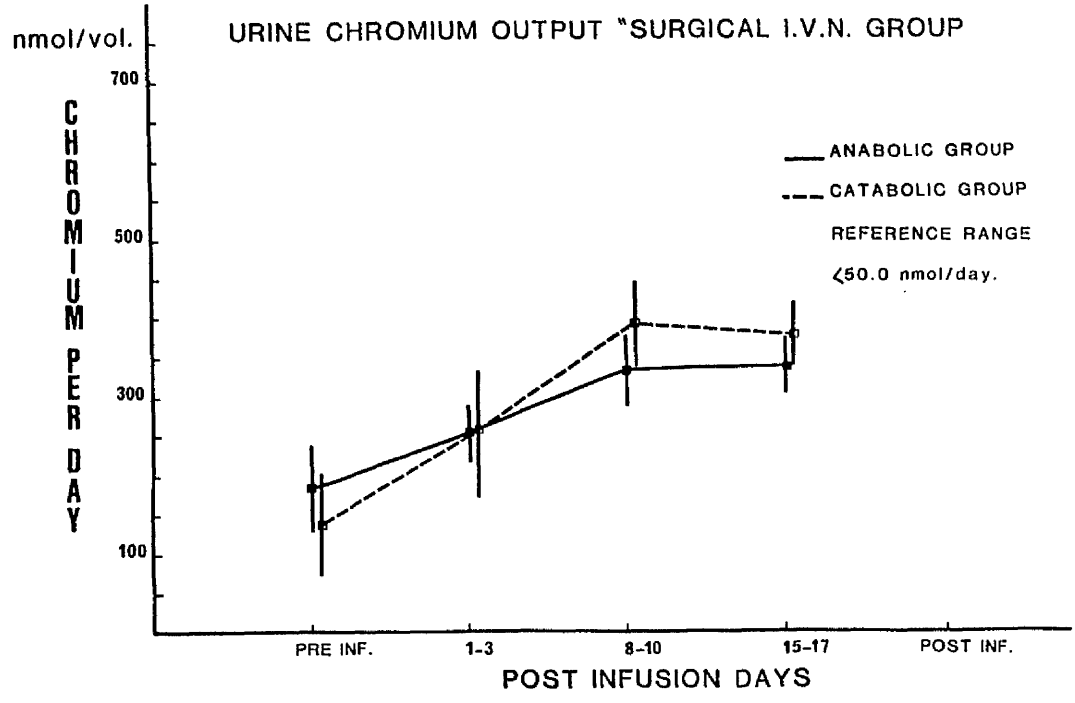


Figure 34

Excretion of chromium in ten anabolic patients and six catabolic patients receiving 4854 infusion.

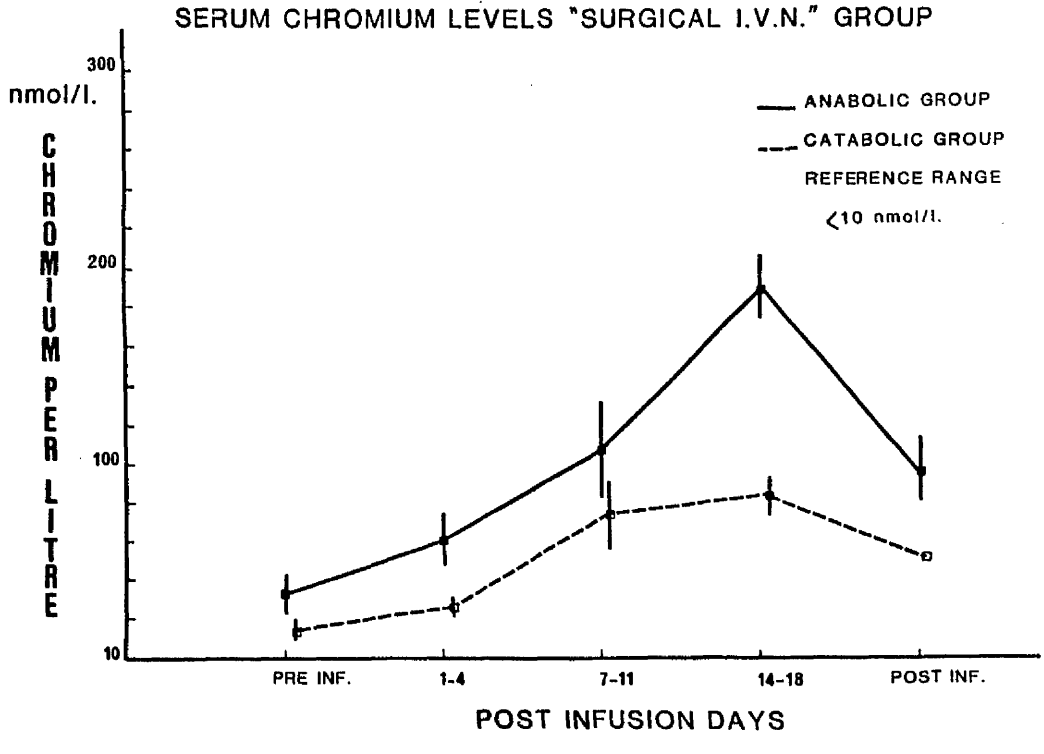


Figure 37

Serum chromium concentrations in ten anabolic patients and six catabolic patients, receiving 4854 infusion.

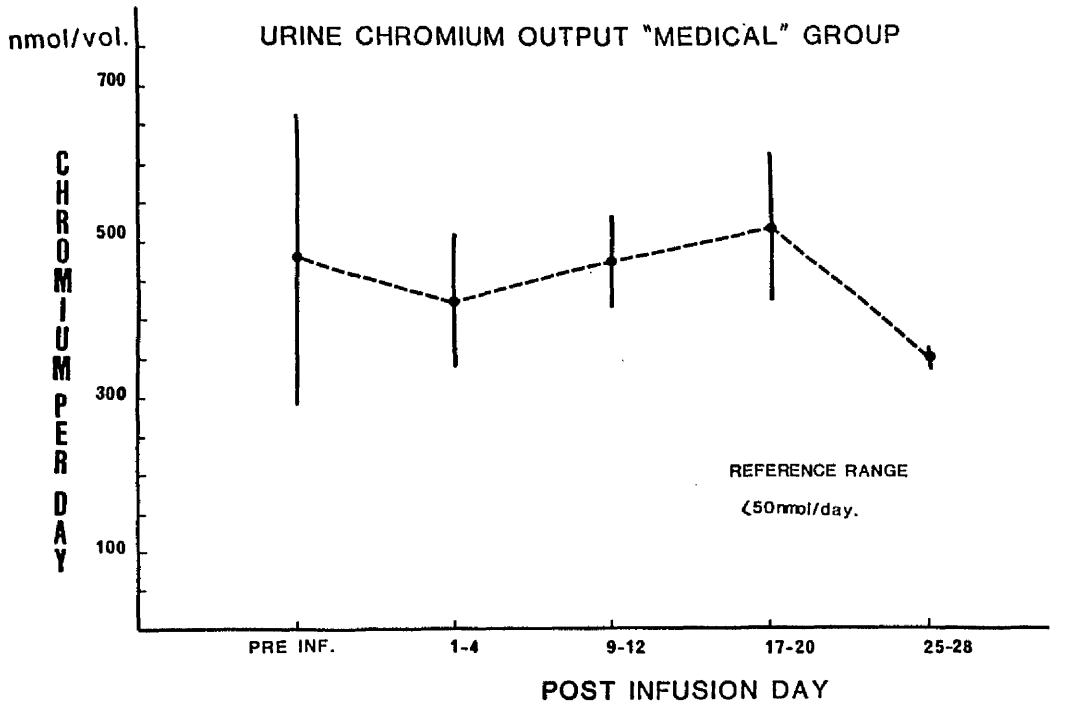


Figure 35

Excretion of chromium in four medical patients receiving 4854 infusion.

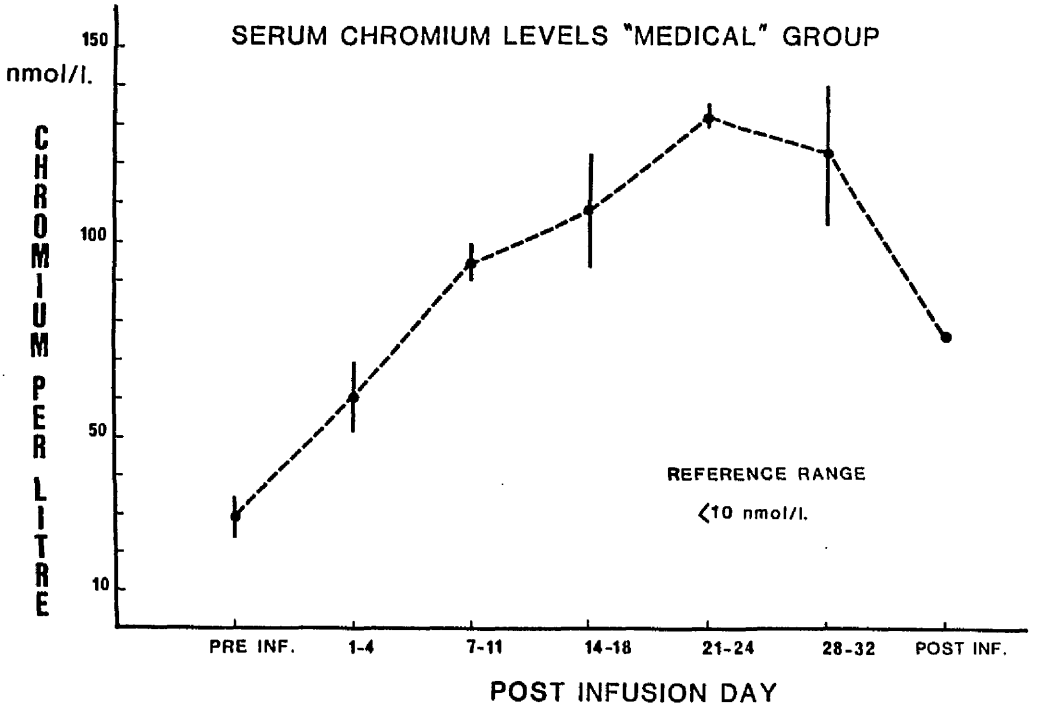


Figure 38

Serum chromium concentrations in four medical patients receiving 4854 infusion.

SELENIUM

4.5.1 Introduction

Selenium has been shown to be an essential element for several animal species. Selenium occurs in all cells and tissues of the animal body in concentrations which vary with the level and chemical form of selenium in the diet. The economic importance of selenium in the nutrition of farm livestock is well appreciated and has prompted basic studies of selenium biochemistry (Underwood 1977). The essential role of selenium in human nutrition is only recently recognised (Young 1981). Previously, more interest was expressed in the potential toxic effect of this metalloid.

Selenium has been shown to be an essential constituent of erythrocyte glutathione preoxidase in several species, and such an enzyme is known to be present in human red cells and plasma. Dietary deficiency of selenium (together with vitamin E deficiency) induces muscular dystrophy (ruminants), pancreatic degeneration and increased capillary permeability (poultry) and liver necrosis (rats).

Chinese investigators have shown that selenium deficiency is one of the principal factors responsible for Keshan disease, a cardiomyopathy which affects persons (mainly children and young women) living in rural areas of a selenium-deficient zone in China.

4.5.2 Body Composition

The whole body content of selenium is in the region of 2-10 mg (25-125 μmol/l). By analogy with animal studies, human liver and kidney will contain most selenium with lesser amounts in skeletal muscle, skin and bone. The concentration of selenium in blood is highly responsive to the changes

in selenium levels in the diet over a wide range. The selenium levels in whole blood from 210 male donors in the United States were reported to range from 0.10-0.34 μg/ml (0.001-0.004 μmol). Some evidence was obtained of a geographic pattern reflecting established regional differences in the selenium levels in crops. Dickson et al (1967) reported the values of selenium in blood, in Canada, which were similar to the above values. From data obtained in New Zealand, a country with extensive areas of low selenium soil, levels of normal New Zealand people were reported as 0.68 μg Se/ml (0.009 μmol). Whole blood selenium concentrations are higher than plasma or serum concentrations because of the higher concentration in the red cell, of the selenoenzyme glutathione peroxidase.

4.5.3 Dietary Intake, Absorption and Excretion

The dietary content of selenium is dependent upon the soil of the region where plant and animal foodstuffs were originally produced. In areas such as North America, dietary intake is from 50-200 μg/day (0.63-2.5 μmol/day), whereas in New Zealand, where soil selenium is particularly low, diets only supply 20-50 μg/day (0.25-0.6 μmol/day). Fish and other seafoods are particularly good food sources, as are animal liver and kidney. Cereal products and vegetables contain variable amounts due to geographical variation in the soil content of selenium. The American recommended intake is 50-200 μg (0.6-2.5 μmol/day) selenium. Mixed diets normally provide this amount. U.K. surveys do not suggest any generalised lack in the average diet.

The role of selenium in human nutrition is complex and inter-related with vitamin E, and possibly other "anti-oxidant"

factors. The chemical form of selenium varies greatly and can affect its availability from diet, hence biological action. Salts like sodium selenite or selenomethionine, are well tolerated.

Comparatively little attention has been paid to the intestinal absorption of selenium. About 50% of selenium from the diet is absorbed by the gut. It is believed from animal studies that absorbed selenium is at first carried mainly in the plasma from which it enters all tissues including bone, hair and leukocytes. Selenite, selenium has to undergo a chemical transformation by the erythrocytes in order to bond by the plasma proteins. The process of expulsion of selenium from the erythrocytes depends upon adequate glutathione levels in these cells. Selenium is transported in blood plasma bound to a variety of proteins, including lipoproteins. The average plasma concentrations lie in the range 42-190 μg Se/l (530-2406 nmol/l). Values depend upon the age of the population studied as well as recent dietery selenium intake.

The main route of selenium excretion is in urine, probably as organo-selenium complexes, the total amount excreted being related to current dietary selenium intake. Available human data shows that 11.2% of the ^{75}Se, in an intravenous dose of (^{75}Se)-L-Selenomethionine appeared in the urine five days after administration, while 2.5% appeared in faeces (Burk 1976). Measurement of urinary selenium content has long been used in screening for selenium toxicity by estimating the urinary output. Values above 100 μg Se/l (1266 nmol/l) are considered as evidence of excessive exposure to selenium.

4.5.4 Biochemical Importance

The biochemical role of selenium compounds is complex, but one important action is as "antioxidants". A selenoprotein, glutathione peroxidase is found in most tissues and body fluids. This enzyme is a part of the cellular antioxidant system, and acting together with the tocophorols (vitamin E), protects all membranes, and other intracellular structures, from damage by peroxides and other active species generated during oxidative metabolism. This is the best known biochemical role of selenium.

A further hypothesis has been put forward by Diplock (1974); selenium and particularly selenide, is presented as having a role in the electron transfer functions associated with mitochondria containing non-haem iron proteins.

Also, selenium can be used for modification of heavy metal toxicity. The biochemical mechanisms by which selenium reduces cadmium and mercury toxicity are unknown, but the diminution of metal toxicity by physiological quantities of selenium has important implications. These metals are becoming more widespread in the environment, so adequate selenium nutrition is important to avoid toxicity, particularly in animals.

4.5.5 Clinical Effects of Deficiency

Selenium is necessary for growth and fertility in animals and for the prevention of various disease conditions which show a variable response to vitamin E. These are liver necrosis in rats and other species, exudative diathesis and pancreatic fibrosis in poultry, muscular dystrophy (white muscle disease) in lambs, calves and other species, and hepatosis dietetica in pigs.

Human evidence of selenium deficiency was previously restricted to studies of children suffering from protein calorie malnutrition. Addition of extra selenium to an otherwise adequate diet led to improved weight gain. Recently, there has been considerable speculation that suboptimal selenium intake may contribute to the incidence of certain forms of cancer (Schrauzer 1976) and cardiovascular disease. These speculations are based on epidemiological data and variables other than selenium levels in the soil could be responsible for the correlations.

More recently however, symptomatic selenium deficiency has been observed in patients fed intravenously using nutrients low in selenium (Rijj 1979). One case reported from New Zealand was of a woman who required long-term intravenous feeding following surgery and developed severe muscle pain relieved by intravenous supply of selenium as selenium-methionine. A patient in America receiving intravenous feeding (Johnson 1981) presented with a cardiomyopathy which was similar to that reported in Keshan disease, an endemic condition found in areas of China, which has been linked to dietary selenium deficiency. Fell et al (1981) reported a patient with biochemical signs of selenium depletion, which was corrected by oral intake of 2 mg sodium selenite/day (900 μg or 11.3 μmol/day). No other biochemical or haematological changes resulted from this therapy, nor were there any apparent clinical benefits associated with improvement in selenium status.

4.5.6 Biochemical Assessment of Selenium Status

Adequate means of assessing selenium in individuals are needed for population surveys and to diagnose selenium deficiency (or toxicity). Blood levels of selenium have been widely

used as an index of selenium status, since the development of sensitive fluorometric assays, and atomic absorption spectrophotometry using a hydride generation system. Plasma or serum levels of selenium may be a more realistic indicator of selenium status, than whole blood selenium. Also, twenty-four hour urine determination of selenium is a guide as to the dietary selenium intake. Furthermore, the activity of selenium containing enzyme erythrocyte glutathione peroxidase falls in response to a selenium deficient diet and rises with adequate selenium intake.

4.5.7 Selenium in Intravenous Nutrition

In hospital practice, the groups of patients at risk of selenium deficiency, are those with pre-existing dietary lack, who present with extensive malabsorption and gastrointestinal disease, severe enough to require supplementary feeding. Nutrients commonly used in intravenous feeding are low in selenium content. Biochemical evidence of low concentrations of selenium in plasma and urine, together with a reduced glutathione peroxidase activity, has been found in various categories of inflammatory bowel disease (Fell et al 1980).

Since the intestinal absorption of selenium is efficient, it is possible to administer sodium selenite or selenomethionine orally and 1 mg sodium selenite (450 μg:5.7 μmol) per day, were suggested as a reasonable therapeutic dosage. However, in cases of severe intestinal dysfunction, when oral administration is not possible, intravenous supply of selenomethionine or sodium selenite can be used (Rijj 1981). The amount required intravenously has not been determined. In this study, 0.4 μmol, that is 32 μg/day of selenium was provided intravenously.

4.5.8 Results of this Study

Urine Selenium

A) Crystalloid Study

Urine selenium excretion in both groups prior to operation was within the normal range (Tables 54 and 55) (632.0 $\pm$ 560 µmol/volume) for the group with additive and (411 $\pm$ 390.0 nmol/volume for the group without additive). When the day prior to operation was compared with the post-operative days, there was no significant rise. Also, when both groups were compared, on each particular day, there was no significant difference between the groups, except on post-operative day 4, when there was a significant difference (P less than 0.05). The group without additive remained within the lower limit of the normal range through the study (Figure 39). At the end of the study, in the group with additive, the mean urine selenium excretion was (682.0 $\pm$ 385.0 nmol/volume) and of nine patients, seven were within the normal range and two were above. In the group without additive, urinary excretion was (368.0 $\pm$ 207.0 nmol/volume) and of ten patients, eight were within the normal range, one was above and one was subnormal. In this group, one patient started with subnormal selenium concentrations and remained the same through the study.

B) Surgical I.V.N

Prior to infusion, at the beginning of the study, the catabolic group started within the normal range (642.0 $\pm$ 59.0 nmol/volume), while the anabolic group was below the normal range (102.0 $\pm$ 19.8 nmol/volume) (Tables 56 and 57). After infusion on days 1-3, the anabolic group came within the lower limit of the normal range, and remained the same throughout the study (Figure 40). The catabolic group remained constant

TABLE 54

Selenium in urine, Patients with 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/vol)		
			1	2	3	4
1	J.B	660	680	640	780	670
2	J.M	440	350	330	350	190
3	S.M	2060	540	660	700	970
4	W.W	463	1798	773	693	1496
5	C.M	*	*	*	*	*
6	W.A	517	493	399	452	549
7	J.S	275	289	296	328	338
8	P.S	288	627	517	381	459
9	W.R	727	2059	1312	744	788
10	A.P	262	528	449	699	675
Mean		632	818	597	570	682
S.D		560	644	312	187	385

TABLE 55

Selenium in Urine, Patients without 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/vol)		
			1	2	3	4
1	M.M	340	610	220	380	340
2	A.C	140	330	250	220	350
3	W.L	420	1000	1060	770	450
4	J.M	355	518	660	513	246
5	M.M	233	339	516	194	338
6	H.S	466	577	487	490	888
7	J.M	1474	501	750	507	293
8	T.C	337	372	410	262	342
9	R.M	173	272	220	*	361
10	H.S	167	305	149	141	76
Mean		411	482	472	386	368
S.D		390	218	288	203	207

* Urine contaminated

TABLE 56

Selenium in Urine, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	W.M	-	420	480	440	-
2	J.W	-	545	-	457	170
3	J.T	-	217	-	-	-
4	I.P	88	211	219	378	-
5	D.M	116	316	264	288	-
6	J.N	-	-	-	-	-
7	M.K	-	-	-	-	-
8	M.G	-	-	-	-	-
9	W.W	-	195	207	208	-
10	A.G	-	-	-	-	-
Mean		102	317	293	354	170
S.D		20	141	127	105	-

TABLE 57

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			Stopping Value
			Mean 1-3	8-10	15-17	
1	A.G	600	505	480	-	-
2	A.M	-	-	-	-	-
3	D.M	-	-	-	-	-
4	H.W	-	374	235	297	-
5	I.C	683	838	623	694	290
6	G.R	-	946	1110	1312	-
Mean		642	666	632	768	290
S.D		59	270	405	511	-

during the intravenous feeding period. In both groups, when the day prior to infusion was compared with post-infusion days, there was no significant increase. At the end of the study, in the anabolic group the mean urinary selenium excretion was 354.0 $\pm$ 105.0 nmol/volume, and all five patients were within the normal range, while in the catabolic group, the excretion was (768.0 $\pm$ 511.0 nmol/volume), and of three patients, two were within the normal range and one was above.

C) Medical I.V.N

This group started within the normal range of urine selenium excretion (262.0 $\pm$ 131.0 nmol/volume) (Table 58). On post-infusion days 1-4 there was a decrease in urine selenium excretion which then increased later in the study (Figure 41). At the end of the study, selenium excretion was 213.0 $\pm$ 28.0 nmol/volume and both patients were within the normal range.

Serum Selenium

A) Crystalloid Study

Both groups prior to operation started within the normal range (with additive 1526.0 $\pm$ 282.0 nmol/l, and without additive 1070.0 $\pm$ 398.0 nmol/l) (Tables 59 and 60). Throughout the study, both groups remained within the normal range (Figure 42). There was a significant difference between the groups through the study (P less than 0.05), but in each group, when the day prior to operation was compared with the post-operative days, there was no significant increase. At the end of the study, on post-operative day 6, in the group given additive the serum selenium concentration was 1539.0 $\pm$ 283.0 nmol/l, and of ten patients, seven were within the normal range and three were above, while in the group without additive,

TABLE 58

Selenium in Urine, Medical I.V.N. Study

No.	Name	Pre-Infusion (nmol/vol)	Post-Infusion Days			
			Mean 1-4	9-12	17-20	25-28
1	R.M	-	150.0	280.0	-	-
2	P.P	-	145	127	195	185
3	E.I	393	312	279	349	240
4	J.C	131	170	185	302	-
Mean		262	194	218	282	213
S.D		131	69	65	64	28

TABLE 59

Selenium in Serum, Patients with 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/l)		
			1	3	5	6
1	J.B	1560	1320	1330	1360	1460
2	J.M	1320	1040	1220	1300	1360
3	S.M	1320	1390	1650	1710	1870
4	W.W	2040	1750	1800	2030	1990
5	C.M	1610	1250	1320	1420	1620
6	W.A	1393	1089	1304	1418	1456
7	J.S	1279	1013	1114	1241	1203
8	P.S	1170	1190	1340	1440	1470
9	W.R	1683	1595	1633	1392	1823
10	A.P	1887	1963	823	823	1140
Mean		1526	1360	1353	1413	1539
S.D		282	318	284	310	283

TABLE 60

Selenium in Serum, Patients without 4854 Infusion

No.	Name	Pre-op	Days	Post-op (nmol/l)		
			1	3	5	6
1	M.M	1130	1140	1080	1010	1110
2	A.C	1190	1010	1240	1060	1110
3	W.L	1620	1390	1420	1550	1490
4	J.M	1600	1270	1330	1340	1370
5	M.M	1050	850	820	850	840
6	H.S	1420	1040	1060	1190	1250
7	J.M	887	1064	1010	760	856
8	T.C	595	562	608	747	761
9	R.M	570	469	304	557	545
10	H.S	633	508	494	697	722
Mean		1070	930	937	976	1005
S.D		398	323	371	313	308

selenium concentration was 1005.0 ± 308.0 nmol/l and of ten patients, nine were within the normal range and one was subnormal.

B) Surgical I.V.N

Both anabolic and catabolic groups started with a normal serum selenium concentration. Anabolic group 976.0 ± 291.0 nmol/l, and catabolic group 1041.0 ± 254.0 nmol/l (Tables 61 and 62). Both groups remained constant, within the normal range, throughout the intravenous feeding period. After cessation of intravenous selenium, in both groups selenium concentration increased (Figure 43). When the groups were compared with each other, on each particular day, there was no significant difference. Also, when the day prior to infusion was compared with post-infusion days, there was no significant increase. At the end of the study, on days 14-18, the selenium concentration in the anabolic group was 903.0 ± 286.0 nmol/l, and all three patients were within the normal range, while the serum selenium concentration of the catabolic group was 879.0 ± 421.0 nmol/l. Out of four patients, three were within the normal range and one was subnormal.

C) Medical I.V.N

The patients in this group started below the serum selenium normal range (467.0 ± 50.0 nmol/l) (Table 63). After the intravenous feeding on days 1-4, serum selenium concentration increased steadily to days 14-18, and then there was a decrease (Figure 44). The patients were within the lower limit of the normal range on days 7-11 and 14-18. At the end of the study, two patients were subnormal.

TABLE 61

Selenium in Serum, Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	W.M	-	1150	-	-	1240
2	J.W	1240	1430	1275	1220	1329
3	J.T	-	534	622	-	-
4	I.P	714	615	-	823	-
5	D.M	1216	937	982	-	836
6	J.N	-	-	-	-	-
7	M.K	-	-	-	-	-
8	M.G	-	-	-	-	-
9	W.W	735	735	729	665	940
10	A.G	-	-	-	-	-
Mean		976	900	902	903	1086
S.D		291	343	291	286	236

TABLE 62

CATABOLIC GROUP

No.	Name	Pre-Infusion (nmol/l)	Post-Infusion Days			Stopping Value
			Mean 1-4	7-11	14-18	
1	A.G	1020	970	1040	-	-
2	A.M	-	925	849	798	-
3	D.M	-	-	-	-	-
4	H.W	798	659	583	317	-
5	I.C	1304	1152	1228	1160	1215
6	G.R	-	570	950	1241	1469
Mean		1041	855	930	879	1342
S.D		254	238	239	421	180

TABLE 63

Selenium in Serum, Medical I.V.N. Study

No.	Name	Pre-Inf	Post-Infusion Days (nmol/l)						
			Mean	1-4	7-11	14-18	21-24	28-32	Stopping Value
1	R.M	410		410	-	460	-	-	-
2	P.P	-		420	480	700	580	430	-
3	E.I	460		510	490	436	384	403	456
4	J.C	532		-	608	583	-	-	-
Mean		467		447	526	545	482	417	456
S.D		50		45	58	106	98	14	-

4.5.9 Discussion

Selenium Excretion

A) Crystalloid Study

The calculated percentage of urinary selenium excretion was 166.7 ± 84.7% of the total infused dose of 1.6 μmol of selenium over the study period, with no retention of selenium. Urine selenium excretion was normal in both groups; about 200-800 nmol/day pre-operation. This was not affected either by the operation of by extra selenium supplementation.

B) Surgical and Medical I.V.N

In the surgical group, the anabolic patients had an average selenium output of 80.3 ± 31.0%, retaining therefore about 21% of the infused dose, whereas the catabolic surgical patients excreted about 180.8 ± 105.9%, with no retention at all. Urine selenium excretion was less in medical patients, about 56.4 ± 16.3%, with higher retention than surgical patients, some 43% of the total infused amount. This is in contrast to other patients who show a deterioration in selenium status on total parenteral nutrition without supplement. Lane et al (1981) studied seven intravenous nutrition patients for about one month. These patients had inflammatory bowel disease or short gut syndrome. In all these patients, they found lower serum selenium levels than in normals.

Analyses were performed on specimens from only six patients from the anabolic group and four patients from the catabolic group for technical reasons.

Serum Selenium

A) Crystalloid Study

Serum selenium was significantly higher throughout the study in the group receiving additive. The pre-operative day

and post-operative day 1 were however, significantly different in the two groups, which indicates some pre-existing difference in selenium status. Provision of extra supplementation had no effect on the serum selenium level, which remained fairly constant in both groups.

B) Surgical and Medical I.V.N

Serum selenium concentrations were well maintained in the surgical anabolic and the catabolic groups throughout the intravenous feeding period. Even although the retention was greater in the medical patients, selenium levels were at the lower limit of the normal range.

Conclusions

Selenium behaves differently than other elements such as zinc, copper and manganese. These elements show a sharp decrease after operation as a response to stress, but there was no such decrease in selenium concentrations. Also, no consistent trend was found during the study, to suggest the retention of selenium. There were analytical difficulties while determining the selenium values. Problems of ventilation made the analysis a health risk, since the headaches and nausea were experienced, possibly due to the release of volatile selenium compounds. Furthermore, no external quality control reference material was available for confirmation of the urine and serum selenium results. Recently, a technique using electrothermal atomisation for plasma selenium has been adopted, and will be used for future studies.

As far as it can be judged from the different studies, provision of 0.4 umol (31.58 μg) selenium per day does not affect pre-existing selenium status. However, none of the patients had evidence of clinical deficiency. Therefore,

no recommendation can be made as to the intravenous supply of selenium necessary to prevent or to correct the selenium depletion.

Investigations using red cell or plasma glutathione peroxidase were also unsatisfactory for technical reasons, but should be used for future investigations.

At present, patients with measurable biochemical signs of selenium depletion, could be treated with an oral preparation.

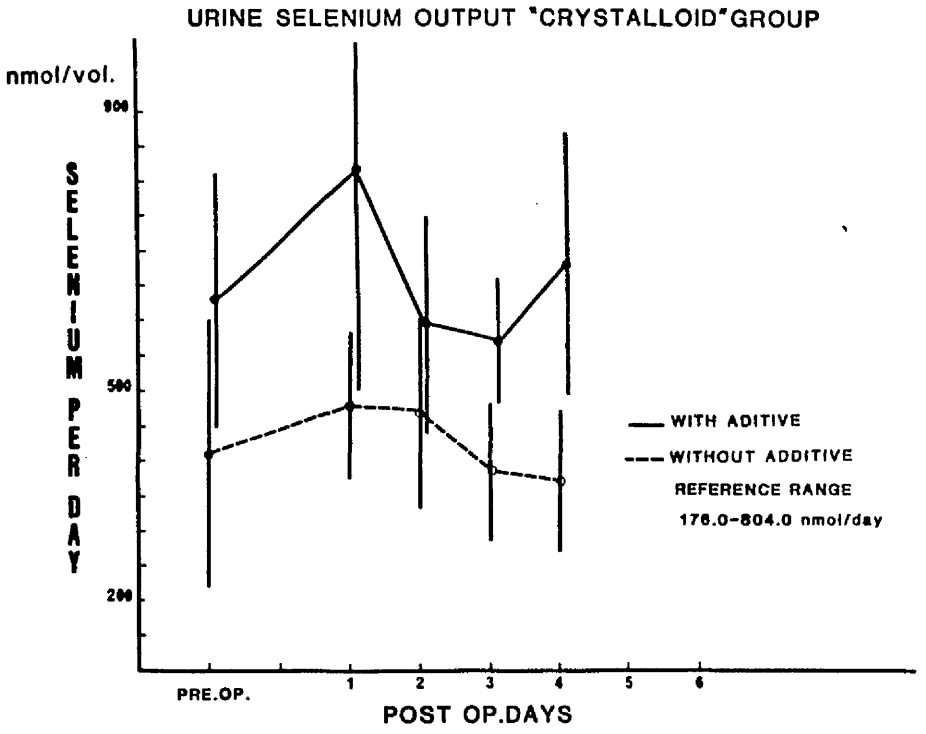


Figure 39

Comparison of selenium excretion in two groups of ten patients, with 4854 and without 4854 infusion.

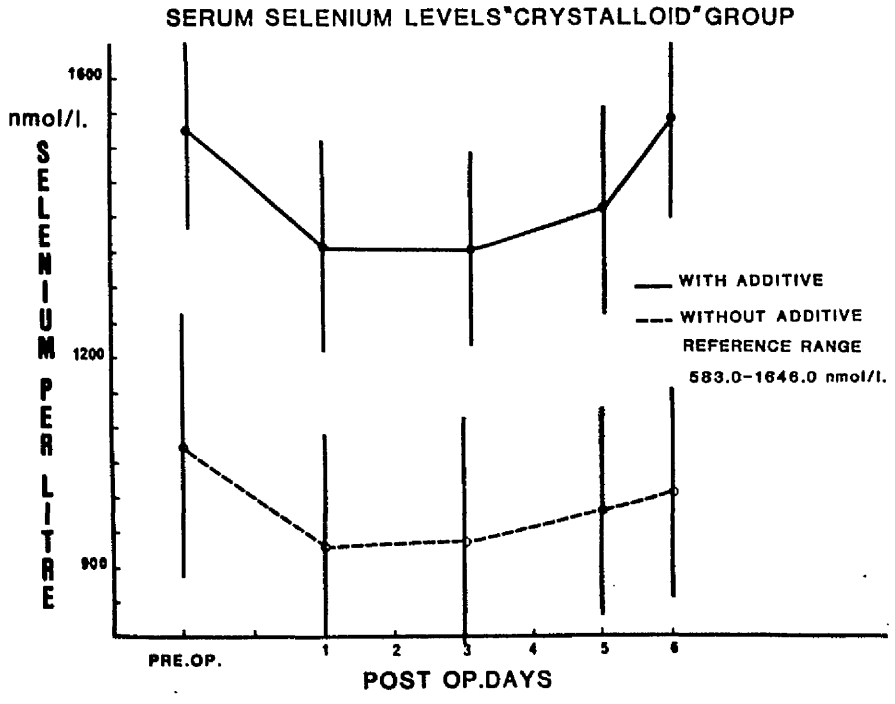


Figure 42

Comparison of serum selenium concentrations in two groups of ten patients, with 4854 and without 4854 infusion.

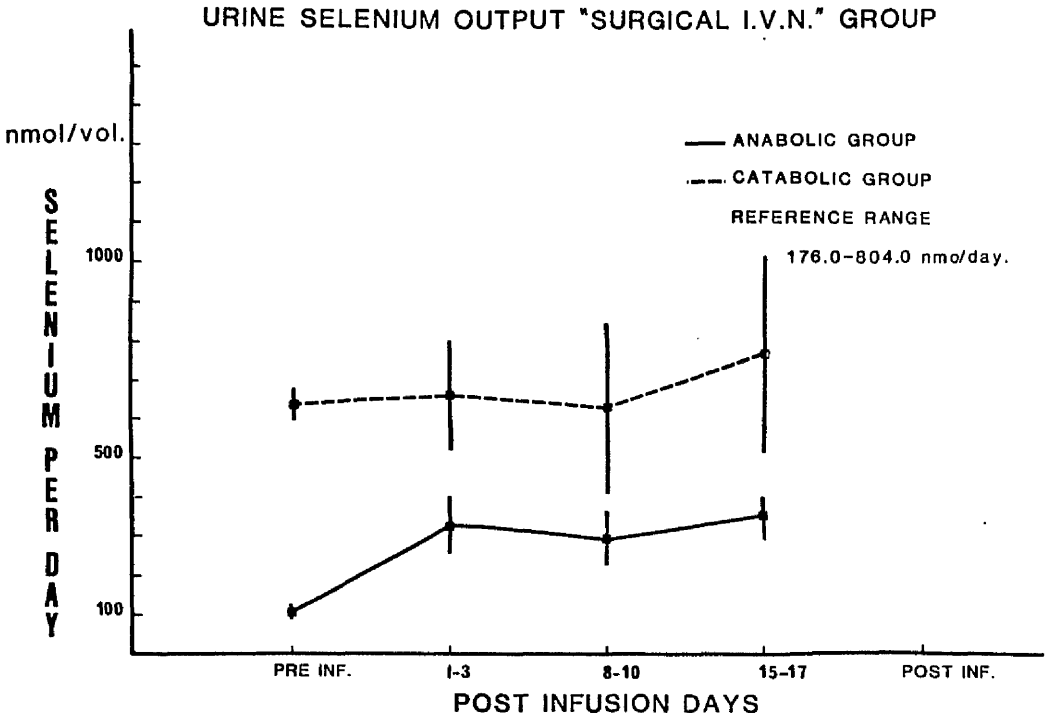


Figure 40

Excretion of selenium in ten anabolic patients and six catabolic patients receiving 4854 infusion.

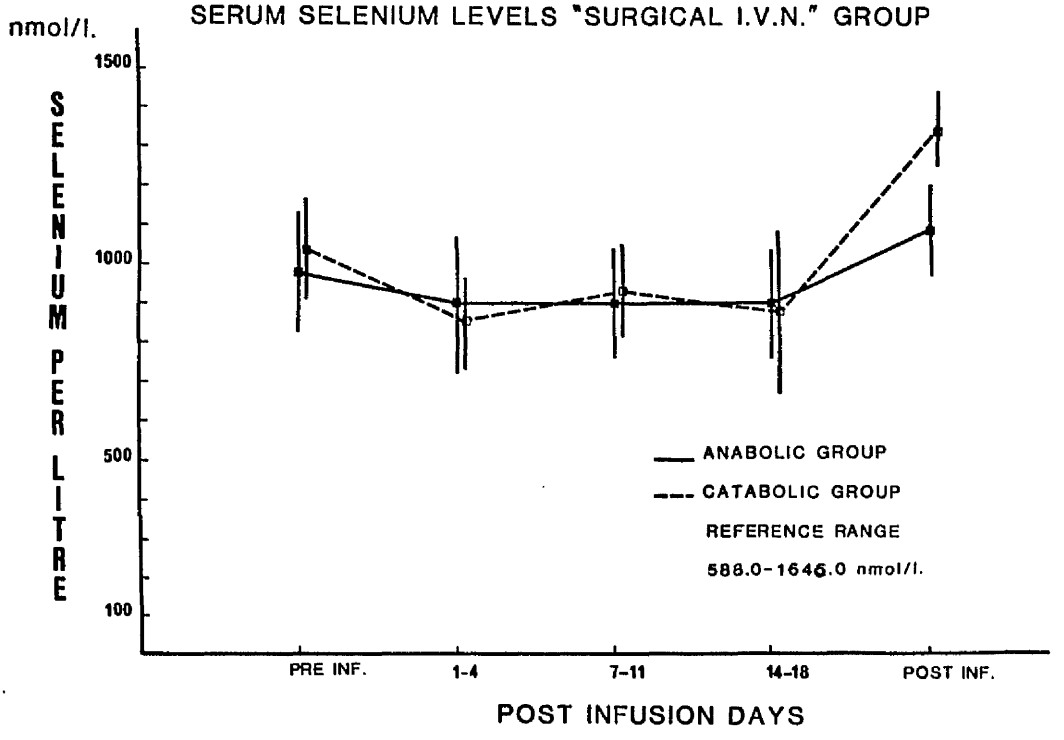


Figure 43

Serum selenium concentrations in ten anabolic patients and six catabolic patients receiving 4854 infusion.

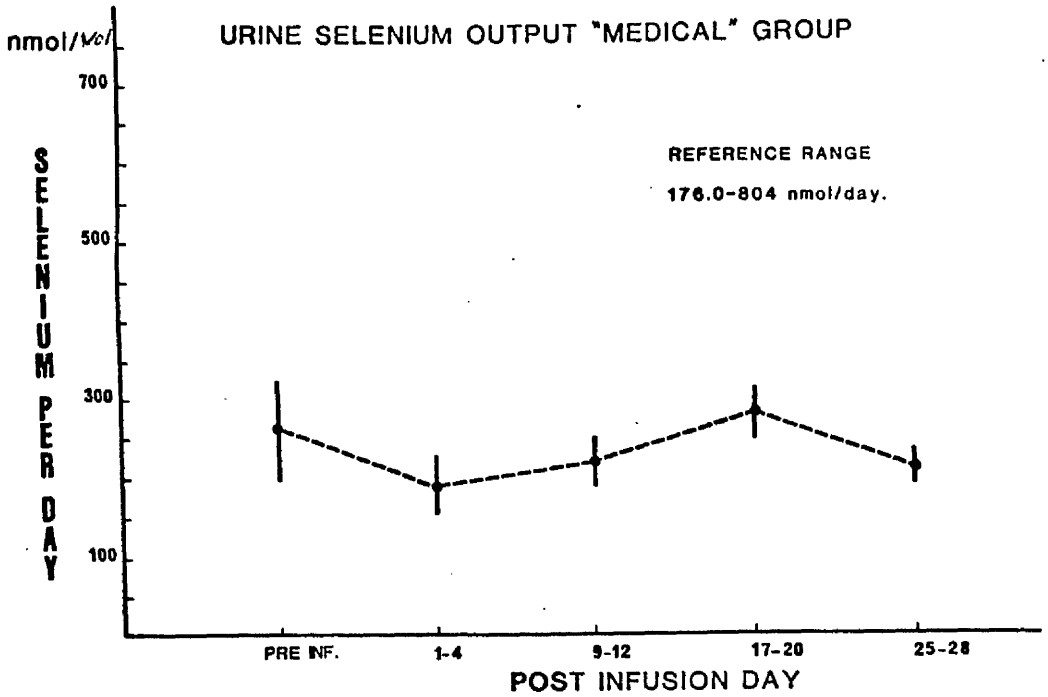


Figure 41

Excretion of selenium in four medical patients receiving 4854 infusion.

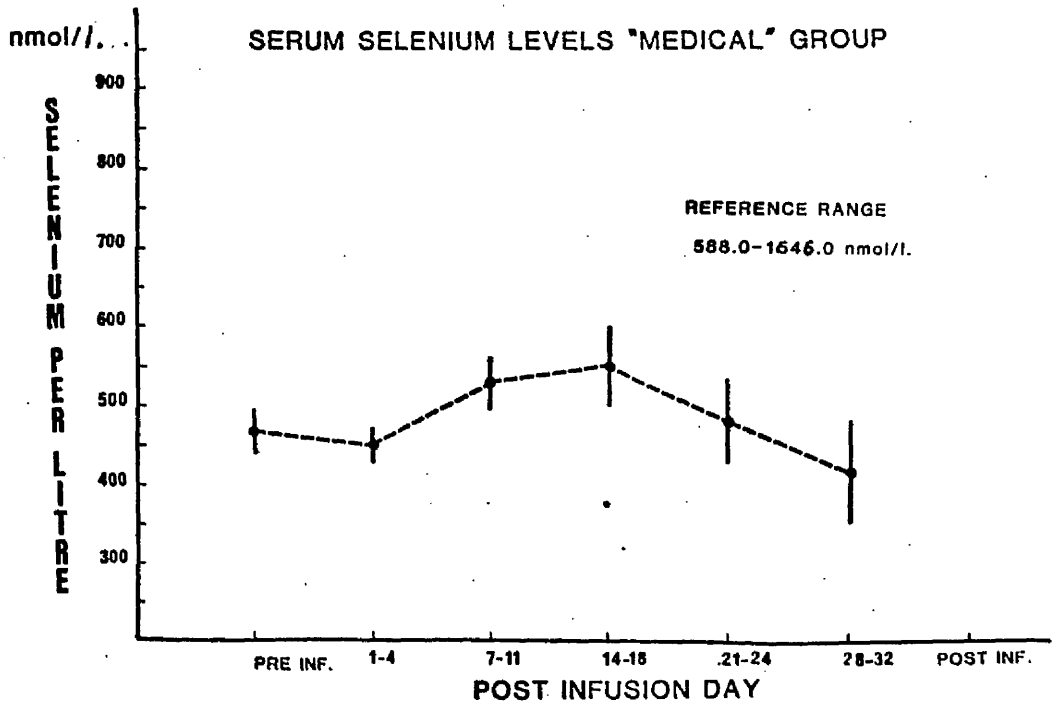


Figure 44

Serum selenium concentrations in four medical patients receiving 4854 infusion.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 5

5.1 CONCLUSIONS AND RECOMMENDATIONS

It is established that clinically obvious signs of essential trace element deficiency can occur during prolonged intravenous feeding. This has been recognised worldwide as greater numbers of patients are being managed for longer periods by intravenous nutrition using purified amino-acid mixtures and energy substrates lacking the former "contamination levels" of some trace metals. Thus, clinical signs, reversible upon the supply of zinc, copper, manganese, chromium, selenium and molybdenum, have been reported. Furthermore, as the biological importance of the fifteen or so essential trace elements in diet are further understood, it is certain that optimal utilisation of the amino-acids, carbohydrate and lipids given intravenously, will require the appropriate amount and appropriate molecular species of most of these elements. Complete parenteral nutrition should aim at providing intravenously the same quantity and form of all nutrients present in, and absorbed from, the normal diet. Present knowledge does not permit such an ideal regimen and estimates of requirements have to be based on empirical evidence, and any proposed intravenous therapy must above all be shown to be safe, and free from obvious toxic side-effects. Since the "therapeutic index" of some metals, especially when given intravenously, is low, most recommendations are probably cautious, aiming to supply the minimal maintenance level to avoid development of gross deficiency disease.

The trace metal formation 4854 investigated in this thesis, was based upon previous clinical and biochemical

studies of Addamel^{R} (Table 2) and the metabolic balance studies by Jacobson and Wester (1977). This led to proposals by Shenkin & Wretlind (1978) for various levels of supply of nutrients, including essential trace elements, for different catagories of patients. Additionally, the recommendations of the American Medical Association (1979) were considered and incorporated. From the studies reported in this thesis, it can be stated that:

1) The 4854 mixture is stable and compatible with other intravenous fluids, such as saline, glucose, amino-acids and lipids.
2) A total of some 559 patient days, in 40 patients, were experienced and no instance of toxicity from infusion of 4854 was recorded.
3) The retention of the infused metals was clearly better when given in saline or glucose rather than with amino-acids.

For individual elements, the following can be concluded:

a) Zinc

100 umol (6.54 mg) per day is sufficient for most patients. This level is in line with the AMA recommendations (2.5-4.0 mg). The amount supplied in 4854 is significantly more than in Addamel^{R}, which supplied only 20 μmol (1.31 mg) per day, and was clearly unable to maintain zinc status in some patients. 100 μmol zinc per day is a safe dosage and will correct mild degrees of zinc deficiency. However, for patients with extensive fistula and/or faecal losses, who are receiving substantial intravenous energy and amino-acid inputs, increased amounts of zinc may be required. Checks upon serum and urine zinc will give a guide as to the progress of each patient.

b) Copper

20 umol (1.27 mg) copper per day can maintain serum copper levels and will slowly correct a pre-existing copper depletion. This level is in line with the AMA recommendations (8-24 μmol) and is four times the amount in Addamel^{R} (5 μmol) which was unable to maintain serum copper levels in all patients.

The clinical and haematological consequences of copper deficiency in man are much slower to develop than those for zinc. However, since an enhanced production of caeruloplasmin appears to be a normal response to stress, injury and infection, it is important to ensure sufficient copper supply to permit increased synthesis of this acute phase reactant. Recently, there has been a suggestion that copper deficiency, as with zinc deficiency, will impair the immune response, and therefore increase the likelihood of infection during recovery.

c) Manganese

Provision of 15 umol (0.82 mg) of manganese per day still seems excessive, even although this is much lower than the amounts given in Addamel^{R} (60 μmol; 3.3 mg). The AMA guidelines propose 3-13 μmol/day. Since in the present study, serum and urine levels of manganese were substantially above the reference values, it is proposed that only 7.5 μmol manganese (0.41 mg) per day is required. No reports of manganese toxicity have appeared following the widespread use of Addamel^{R}, confirming the view that manganese is not a particularly toxic metal. It is clear that the human requirement for manganese is very small indeed, and that little convincing biochemical or clinical evidence of deficiency has yet been published. Nevertheless, provision of some manganese during intravenous nutrition would seem justified in view of its

established biochemical importance.

d) Chromium

Study of this metal continues to be hindered by the lack of adequately sensitive and specific methods of analysis. However, atomic absorption procedures described in Section 2.1, now allow measurement of normal urine chromium excretion and will detect increases in serum chromium reliably. As with manganese, human chromium requirements are extremely low. Provision of 0.4 μmol (20.8 mg) chromium per day, quite definitely increases both serum and urine chromium levels well above normal. Also, unavoidable contamination of amino-acid mixtures during manufacture means that variable additional amounts of chromium will be infused. The AMA recommendation was 0.28-0.38 μmol (15-20 μg) chromium per day. It is therefore proposed that only some 0.2 μmol (10.4 mg) of chromium should be added to intravenous regimens.

The biochemical importance of chromium is established and the clinical effects of severe deficiency are serious. Therefore, modest provision of chromium should be made and individual patients should be investigated when unexpected signs of insulin resistance develop.

e) Selenium

Evidence of the intravenous requirement of selenium has not been obtained during this study. Provision of 0.4 μmol (31.58 μg) of selenium per day did not alter selenium levels in serum or urine in a consistent manner. No patients were studied who had a pre-existing depletion so that it is not clear if this level of provision would have corrected biochemical abnormalities.

It is proposed to leave the amount supplied at the present

value, since this is non-toxic and should supply basal needs. For individual patients with severe malabsorptive states and biochemical evidence of low serum and urine selenium and low glutathione peroxidase activity, a possible option is oral provision of sodium selenite which is efficiently absorbed from the gut.

Further improvements in selenium assay and the use of glutathione peroxidase measurements are required for future studies.

Future Investigations

Future study of the amended "4854" mixture is required:

1) Wherever possible, a number of more complete metabolic balance studies should be attempted. This will involve the assay of metal output in fistula fluid and faeces for different categories of patient.
2) Direct measurement of metals in tissue, body fluids and excreta should be considered along with other appropriate "markers" of intracellular biochemical function.

Zinc

Leucocyte zinc levels; liver, muscle and skin biopsy and activity of zinc metalloenzymes like thymidine kinase.

Copper

Liver biopsy, copper assay, activity of copper enzymes such as red cell superoxide dismutase.

Chromium

Effect of glucose load upon serum and urine chromium levels should give an indication of tissue chromium reserves.

Selenium

Systematic study of red cell and plasma GSHPx activity will complement direct selenium measurements.

3) Extension of studies to include the other components of 4854, namely molybdenum, iodide and fluoride, will depend upon the availability of suitable analytical techniques. Possible work could include:

a) Iodine

Use of standard thyroid function tests during prolonged intravenous nutrition. Interpretation may be difficult due to changes induced by illness or stress.

b) Fluoride

Direct measurement of serum and urine fluoride using an ion selective electrode should be possible. Some study of bone biopsy and histopathology might be advisable in patients receiving long-term intravenous nutrition, including infusions of fluoride.

c) Molybdenum

Direct measurement of serum molybdenum is difficult and requires specialist neutron activation techniques. Molybdenum excretion is mainly via the urine, probably as the molydate anion. Sufficiently sensitive and specific electrothermal atomisation and atomic absorption spectrophotometric methods may become available. However, indirect estimation of molybdenum status could be more readily obtained by assay of molybdenum dependent enzymes such as xanthine oxidase and sulphite oxidase. Additionally, the urinary excretion of uric acid and the urinary ratios of sulphate to sulphite can be measured.

APPENDIX

APPENDIX

Patients with 4854 Infusion

No.	Name	Pre-Op	Post-Op Days			
			1	2	3	4 (24 hour urine volume/ml)
1	J.B	883	569	1151	2360	2389
2	J.M	1933	815	2047	1927	1204
3	S.M	1093	771	1656	2819	4627
4	W.W	1053	1798	2760	3463	3182
5	C.M	*	*	*	*	*
6	W.A	1435	1540	2132	1373	2348
7	J.S	736	1383	3111	3317	3677
8	P.S	1021	1781	2722	2787	3078
9	W.R	998	2321	2158	2195	1557
10	A.P	889	2277	2363	3801	4019

Patients without 4854 Infusion

No.	Name	Pre-Op	Post-Op Days			
			1	2	3	4 (24 hour urine volume/ml)
1	M.M	1567	2751	2038	2349	3374
2	A.C	1565	1378	476	384	677
3	W.L	3012	807	1053	3831	4130
4	J.M	1076	720	2868	4273	4092
5	M.M	1793	434	3227	2766	3077
6	H.S	1035	1603	1948	2579	3290
7	J.M	1501	500	1537	1668	1379
8	T.C	1686	2353	3475	3195	1317
9	R.M	642	1024	1179	*	2842
10	H.S	1589	839	2915	2312	2723

*Urine contaminated

Patients with 4854 Infusion

Urine Creatinine

No.	Name	Pre-Op	Post-Op Days (mmol/volume)			
			1	2	3	4
1	J.B	18.2	18.8	20.3	19.1	15.8
2	J.M	9.7	8.7	8.6	8.9	7.9
3	S.M	10.7	10.3	12.1	10.1	11.6
4	W.W	8.0	23.0	15.2	12.1	12.1
5	C.M	*	*	*	*	*
6	W.A	11.0	12.5	12.4	11.1	10.1
7	J.S	15.9	10.2	10.6	9.3	9.9
8	P.S	3.8	6.9	6.3	4.2	6.5
9	W.R	10.9	24.4	14.0	10.5	10.9
10	A.P	10.6	14.6	11.1	11.4	10.0

Patients without 4854 Infusion

Urine Creatinine

No.	Name	Pre-Op	Post6-Op Days (mmol/volume)			
			1	2	3	4
1	M.M	8.0	13.8	7.1	10.3	10.8
2	A.C	7.0	15.7	12.1	11.4	15.9
3	W.L	11.1	16.5	20.5	14.9	11.7
4	J.M	8.1	11.9	15.8	12.4	11.5
5	M.M	12.9	10.3	22.6	10.0	12.3
6	H.S	13.4	16.5	15.2	16.0	18.8
7	J.S	22.5	6.2	15.7	10.0	6.1
8	T.C	15.0	13.9	13.2	12.5	11.7
9	R.M	9.6	14.3	10.5	*	9.7
10	H.S	11.1	16.4	17.5	10.6	8.7

Zinc in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (µmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	30.7	29.6	30.5	38.1	-	-	-	38.1	-	-	-	32.4	-	-	-	-
2	J.W	-	40.3	-	-	-	51.2	45.0	64.4	-	-	-	-	-	46.7	49.1	79.4	53.6
3	J.T	-	29.7	26.8	27.6	31.8	33.2	45.8	-	36.2	53.0	61.6	49.8	-	-	-	-	-
4	I.P	34.1	18.3	21.0	-	-	-	-	-	-	24.4	18.1	-	-	-	-	-	32.3
5	D.M	64.1	-	68.6	43.1	-	-	-	54.1	70.4	105.1	58.9	34.7	44.8	85.4	73.9	55.1	92.5
6	J.N	-	-	-	38.4	30.1	9.3	-	-	-	-	39.1	44.4	26.2	52.1	19.3	50.2	59.2
7	M.K	-	-	-	-	157.0	132.5	72.9	-	-	-	54.2	-	150.0	92.6	103.5	92.7	112.4
8	M.G	10.5	-	-	-	-	-	-	-	-	-	-	-	-	48.0	46.3	-	-
9	W.W	-	-	-	71.3	-	-	-	-	-	52.3	48.0	73.9	-	76.2	-	-	43.9
10	A.G	32.4	-	-	-	-	-	-	-	-	-	-	-	-	26.1	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/volume)																
		17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	W.M	-	stop	18.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	52.5	43.4	26.3	35.1	27.8	12.7	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	42.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	31.0	37.0
5	D.M	23.1	63.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	J.N	-	30.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	114.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	66.7	-	-	66.0	-	62.6	54.5	59.9	34.7	28.0	46.4	45.8	60.7	69.0	-	45.9
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Zinc in Urine : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (µmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A.G	75.1	77.4	55.8	-	-	-	76.0	91.0	61.4	-	-	-	-	-	-	-	-
2	A.M	73.2	72.6	-	-	-	-	-	72.8	61.0	-	-	-	-	77.2	-	-	-
3	D.M	58.1	-	-	-	-	-	-	41.8	66.4	-	-	-	-	-	-	-	-
4	H.W	-	93.5	90.0	-	-	93.6	-	-	-	41.9	-	-	-	-	-	68.0	88.3
5	I.C	67.4	109.0	-	-	131.8	120.0	98.3	117.8	124.0	102.2	120.0	-	135.1	-	-	136.5	132.1
6	G.R	-	-	-	-	112.7	129.7	135.1	144.3	-	-	-	-	-	105.0	-	-	-

CONT...

cont

No.	Name	Post-Infusion Days (μmol/volume) 17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	I.C	127.4	140.0	-	stop	61.1	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	137.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Zinc in Serum : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (μmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	W.M	-	-	-	-	9.0	-	9.0	11.5	-	15.0	11.8	stop	11.8	-	-	-	-
2	J.W	21.5	-	-	-	21.3	-	19.5	-	16.7	-	-	-	17.3	-	16.4	stop	-
3	J.T	11.0	-	-	-	-	-	10.5	-	-	10.5	-	-	-	-	-	-	-
4	I.P	12.0	9.5	9.0	-	-	-	-	-	-	-	-	-	-	-	-	15.5	-
5	D.M	16.5	10.0	-	-	-	11.5	-	-	-	-	14.0	-	-	-	-	-	-
6	J.N	8.5	-	-	-	-	12.5	-	-	13.5	-	-	14.5	-	-	-	-	13.0
7	M.K	13.0	-	-	-	-	20.0	-	-	-	-	-	18.0	-	-	-	-	17.5
8	M.G	7.5	9.5	-	-	-	-	8.5	-	-	-	-	-	7.0	-	-	-	-
9	W.W	15.5	-	-	-	14.7	-	16.0	-	-	15.0	-	-	14.0	-	-	12.8	-
10	A.G	12.0	-	-	-	-	-	11.0	-	-	-	-	-	14.0	stop	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	W.M	16.5	-	-	-	12.8	-	-	-	-	-	-	-	-	-	-
2	J.W	-	19.7	-	-	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	-	-	-	-	-	-	13.0	-	-	-	-	-	13.5	15.5	15.5
5	D.M	-	-	-	stop	-	13.5	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	16.0	-	-	-	-	15.0	-	-	-	-	-
8	M.G	8.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	14.3	-	-	13.0	-	-	14.7	12.3	-	stop	13.7	-	-	-
10	A.G	10.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Zinc in Serum : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (μmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	A.G	13.0	-	-	11.5	-	-	13.5	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	11.2	-	10.0	-	-	8.5	-	-	-	10.5	-	-	-
3	D.M	7.5	11.0	-	-	-	-	-	11.5	-	-	-	-	-	-	-	-	-
4	H.W	14.0	-	-	10.0	-	11.0	-	-	-	12.0	-	-	-	22.5	stop	-	-
5	I.C	10.5	-	-	12.5	-	-	14.5	-	-	-	-	-	15.0	-	-	-	-
6	G.R	-	-	-	10.0	-	13.5	-	-	-	16.5	-	-	15.5	-	-	17.0	stop

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	13.5	-	-	-	-	-	-	-	-	-	-	-
5	I.C	16.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	22.5	-	-	-	-	-	-	-	20.5	-	-	-	-

Copper in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (μmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	1.84	2.07	2.65	2.91	-	-	-	2.54	-	-	-	2.13	-	-	-	-
2	J.W	-	1.49	-	-	-	1.17	1.01	1.08	-	-	-	-	-	0.75	0.90	0.96	0.76
3	J.T	-	1.04	0.72	0.68	0.81	1.16	1.25	-	0.64	0.96	1.14	0.82	-	-	-	-	-
4	I.P	0.38	0.24	0.42	-	-	-	-	-	-	0.37	0.20	-	-	-	-	-	0.40
5	B.M	0.35	-	1.33	1.16	-	-	-	0.55	0.93	1.30	0.89	0.48	0.38	0.51	0.58	0.43	0.92
6	J.N	-	-	-	1.10	1.51	0.80	-	-	-	-	1.05	-	0.61	0.91	0.58	2.03	-
7	M.K	2.41	-	2.58	-	3.54	2.69	1.58	-	-	-	1.61	2.09	1.58	1.48	1.53	1.35	1.57
8	M.G	0.36	0.63	-	0.22	-	-	-	0.94	1.03	-	-	-	-	0.79	0.73	-	-
9	W.W	-	-	-	0.60	-	-	-	-	-	0.91	0.69	0.57	-	1.46	-	-	0.62
10	A.G	1.39	-	-	-	-	-	-	-	-	-	-	-	-	1.12	-	-	-

CONT ...

cont ...

No.	Name	Post-Infusion Days (μmol/volume) 17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	W.M	-	-	0.95	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	1.07	1.39	0.84	0.82	0.72	stop	0.43	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	0.43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.42	0.64
5	D.M	stop	0.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	J.N	-	0.58	0.33	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	1.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	0.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	1.94	-	-	1.22	-	0.71	0.96	1.11	0.79	0.38	0.76	0.25	0.36	0.94	-	0.77
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Copper in Urine : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (μmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A.G	1.30	1.00	1.00	-	-	-	1.00	1.30	0.70	-	-	-	-	-	-	-	-
2	A.M	2.21	2.46	-	-	-	-	-	5.99	5.51	-	-	-	-	3.48	-	-	-
3	D.M	-	3.08	1.57	-	-	-	-	2.05	2.86	-	-	-	-	-	-	-	-
4	H.W	-	1.07	1.34	-	-	3.20	-	-	-	0.61	2.02	-	-	-	-	1.15	0.93
5	I.C	2.14	3.25	-	-	3.09	4.63	3.04	3.38	3.06	2.26	4.40	-	2.10	-	-	2.00	2.10
6	G.R	-	-	-	-	0.94	1.09	2.57	-	-	-	1.04	-	-	1.19	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/volume)																
		17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	I.C	1.70	2.10	1.71	stop	0.74	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	1.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Copper in Serum : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (μmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	W.M	-	-	-	-	18.5	-	19.5	22.5	-	23.5	25.3	stop	27.2	-	-	-	-
2	J.W	22.7	-	-	-	21.6	-	20.7	-	21.7	-	-	-	20.7	-	21.0	stop	-
3	J.T	-	21.9	-	-	-	-	19.5	-	-	20.5	-	-	-	-	-	-	-
4	I.P	19.5	18.0	18.0	-	-	-	-	-	-	-	-	-	-	-	-	19.5	-
5	D.M	18.5	17.5	-	-	-	19.5	-	-	-	-	18.5	-	-	-	-	-	-
6	J.N	11.5	-	-	-	-	19.0	-	-	20.0	-	-	21.3	-	-	-	-	29.5
7	M.K	14.0	-	-	-	-	16.0	-	-	-	-	-	18.0	-	-	-	-	21.5
8	M.G	19.0	21.0	-	-	-	-	25.0	-	-	-	-	-	22.0	-	-	-	-
9	W.W	17.0	-	-	-	18.3	-	18.7	-	-	19.5	-	-	-	-	-	21.5	-
10	A.G	24.5	-	-	-	-	-	25.0	-	-	-	-	-	24.5	stop	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/l) 21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	W.M	30.5	-	-	-	30.0	-	-	-	-	-	-	-	-	-	-
2	J.W	-	23.2	-	-	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	-	-	-	-	-	-	16.0	-	-	-	-	-	24.0	20.7	18.5
5	D.M	-	-	-	stop	-	20.0	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	22.5	-	-	-	-	22.5	-	-	-	-	-
8	M.G	25.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	21.4	-	-	22.5	-	-	22.0	23.3	-	stop	28.8	-	-	-
10	A.G	28.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Copper in Serum : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	post-Infusion Days (μmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	A.G	15.0	-	-	14.0	-	-	19.5	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	23.5	-	23.3	-	-	24.5	-	-	-	26.5	-	-	-
3	D.M	13.5	21.0	-	-	-	-	-	24.5	-	-	-	-	-	-	-	-	-
4	H.W	19.0	-	-	15.5	-	11.0	-	-	-	14.5	-	-	-	23.0	stop	-	-
5	I.C	19.5	-	-	19.5	-	-	16.5	-	-	-	-	-	25.0	-	-	stop	-
6	G.R	-	-	-	16.0	-	16.5	-	-	-	23.5	-	-	22.5	-	-	25.0	-

CONT ...

cont

No.	Name	Post-Infusion Days (μmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	26.5	-	-	-	-	-	-	-	-	-	-	-
5	I.C	26.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	25.0	-	-	-	stop	-	-	-	22.0	-	-	-	-

Manganese in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	9	15	50	70	-	-	-	100	-	-	-	910	-	-	-	-
2	J.W	-	141	-	-	-	113	89	134	-	-	-	-	-	102	94	104	196
3	J.T	-	69	122	26	54	78	81	-	34	109	78	80	-	-	-	-	-
4	I.P	20	32	74	-	-	-	-	-	-	71	33	-	-	-	-	-	44
5	D.M	71	-	-	90	-	-	-	239	104	206	65	79	82	139	142	110	124
6	J.N	-	-	-	96	-	104	-	-	-	-	162	-	57	329	67	201	-
7	M.K	91	-	141	-	314	236	100	-	-	-	70	121	139	65	91	42	76
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	137	105	-	-
9	W.W	-	-	-	153	-	-	-	-	-	82	122	85	-	82	-	-	194
10	A.G	21	-	-	-	-	-	-	-	-	-	-	-	-	116	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (nmol/volume)																
		17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	W.M	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	125	99	58	74	42	stop	21	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	98	91
5	D.M	stop	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	J.N	-	95	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	67	-	251	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	304	-	-	213	-	226	167	184	109	116	173	-	142	112	168	192
10	A.G	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Manganese in Urine : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A.G	10	530	30	-	400	-	-	120	-	-	-	-	-	-	-	-	-
2	A.M	393	46	-	-	-	-	-	136	64	-	-	-	-	44	-	-	-
3	D.M	-	84	51	-	-	-	-	186	112	-	-	-	-	-	-	-	-
4	H.W	-	34	124	-	-	60	-	-	-	38	267	-	-	-	-	48	-
5	I.C	28	33	-	-	103	79	82	156	122	109	112	-	112	-	-	83	99
6	G.R	-	-	-	-	82	153	156	-	-	-	101	-	-	108	-	-	-

Chromium in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	16	230	280	250	-	-	-	250	-	-	-	200	-	-	-	-
2	J.W	-	169	-	-	-	334	315	312	-	-	-	-	-	265	210	288	259
3	J.T	-	154	217	294	311	284	396	-	247	342	400	316	-	-	-	-	-
4	I.P	157	239	356	-	-	-	-	-	-	300	235	-	-	-	-	-	353
5	D.M	314	-	-	282	-	-	-	261	532	473	278	358	230	438	341	294	-
6	J.N	-	-	-	255	212	79	-	-	-	-	247	-	146	316	148	309	-
7	M.K	224	-	403	-	446	383	-	-	-	-	254	555	453	387	378	344	456
8	M.G	40	167	-	124	-	-	-	96	193	-	-	-	-	340	345	-	-
9	W.W	-	-	-	265	-	-	-	-	-	389	421	442	-	486	-	-	428
10	A.G	76	-	-	-	-	-	-	-	-	-	-	-	-	253	-	-	-

Manganese in Serum : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	W.M	-	-	-	-	25.0	-	-	44.0	-	-	47.0	stop	25.0	-	-	-	-
2	J.W	5.0	-	-	-	31.0	-	33.0	-	35.0	-	-	-	36.0	-	31.0	stop	-
3	J.T	-	10.0	-	-	-	-	25.0	-	-	28.0	-	-	-	-	-	-	-
4	I.P	17.0	17.0	31.0	-	-	-	-	-	-	-	-	-	-	-	-	32.0	-
5	D.M	31.0	24.0	-	-	-	31.0	-	-	-	-	18.0	-	-	-	-	-	-
6	J.N	13.0	-	-	-	-	29.0	-	-	42.0	-	-	36.0	-	-	-	-	44.0
7	M.K	27.0	-	-	-	-	66.0	-	-	-	-	-	71.0	-	-	-	-	102.0
8	M.G	7.0	40.0	-	-	-	-	16.0	-	-	-	-	-	33.0	-	-	-	-
9	W.W	25.0	-	-	-	35.0	-	36.0	-	-	39.0	-	-	36.0	-	-	46.0	-
10	A.G	13.0	-	-	-	-	-	27.0	-	-	-	-	-	47.0	stop	-	-	-

CONT...

cont

No.	Name	Post-Infusion Days (nmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	W.M	-	-	-	-	9.0	-	-	-	-	-	-	-	-	-	-
2	J.W	-	10.0	-	-	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	-	-	-	-	-	-	53.0	-	-	-	-	-	25.0	69.0	33.0
5	D.M	-	-	-	stop	-	20.0	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	91.0	-	-	-	-	82.0	-	-	-	-	-
8	M.G	35.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	39.0	-	-	40.0	-	-	38.0	37.0	-	stop	24.0	-	-	-
10	A.G	25.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Manganese in Serum : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	A.G	2.0	-	-	13.0	-	-	18.0	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	147.0	-	70.0	-	-	73.0	-	-	-	70.0	-	-	-
3	D.M	9.0	13.0	-	-	-	-	-	24.0	-	-	-	-	-	-	-	-	-
4	H.W	15.0	-	-	14.0	-	27.0	-	-	-	28.0	-	-	-	24.0	stop	-	-
5	I.C	5.0	-	-	10.0	-	-	21.0	-	-	-	-	-	31.0	-	-	stop	-
6	G.R	-	-	-	29.0	-	46.0	-	-	-	60.0	-	-	44.0	-	-	43.0	stop

CONT ...

cont

No.	Name	Post-Infusion Days (nmol/l) 21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	M.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	14.0	-	-	-	-	-	-	-	-	-	-	-
5	I.C	33.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	7.0	-	-	-	-	-	-	-	9.0	-	-	-	-

Chromium in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	16.0	230.0	280.0	250.0	-	-	-	250.0	-	-	-	200.0	-	-	-	-
2	J.W	-	169.0	-	-	-	334.0	315.0	312.0	-	-	-	-	-	265.0	210.0	288.0	259.0
3	J.T	-	154.0	217.0	294.0	311.0	284.0	396.0	-	247.0	342.0	400.0	316.0	-	-	-	-	-
4	I.P	157.0	239.0	356.0	-	-	-	-	-	-	300.0	235.0	-	-	-	-	-	353.0
5	D.M	314.0	-	425.0	282.0	-	-	-	261.0	532.0	473.0	278.0	358.0	230.0	-	438.0	341.0	294.0
6	J.N	-	-	-	255.0	212.0	79.0	-	-	-	-	247.0	-	146.0	-	316.0	148.0	309.0
7	M.K	224.0	-	403.0	-	446.0	383.0	-	-	-	-	254.0	555.0	453.0	-	387.0	378.0	344.0
8	M.G	40.0	167.0	-	124.0	-	-	-	96.0	193.0	-	-	-	-	340.0	345.0	-	-
9	W.W	-	-	-	265.0	-	-	-	-	-	389.0	421.0	442.0	-	486.0	-	-	128.0
10	A.G	76.0	-	-	-	-	-	-	-	-	-	-	-	-	253.0	-	-	-

CONT ...

cont

No.	Name	Post-Infusion Days (nmol/volume)																
		17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	W.M	-	-	88.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	266.0	157.0	80.0	196.0	170.0	stop	61.0	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	483.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	393.0	403.0
5	D.M	stop	91.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	J.N	-	166.0	70.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	421.0	-	428.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	364.0	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	521.0	-	-	487.0	-	438.0	449.0	409.0	241.0	419.0	357.0	369.0		354.0	404.0	304.0
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Chromium in Urine : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A.G	300	250	310	-	-	-	400	500	450	-	-	-	-	-	-	-	-
2	A.M	54	62	-	-	-	-	-	337	335	-	-	-	-	331	-	-	-
3	D.M	-	173	191	-	-	-	-	213	368	-	-	-	-	-	-	-	-
4	H.W	-	206	369	-	-	-	-	-	-	205	369	-	-	-	-	372	357
5	I.C	68	176	-	-	347	392	356	369	384	300	352	-	334	-	-	294	350
6	G.R	-	-	-	-	528	912	765	-	-	-	565	-	-	458	-	-	-

Cont

No.	Name	Post-Infusion Days (nmol/volume) 17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	I.C	371.0	471.0	381.0	stop	60.0	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	495.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Chromium in Serum : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	W.M	-	-	-	-	76.0	-	-	120.0	-	-	137.0	stop	135.0	-	-	-	-
2	J.W	12.0	-	-	-	58.0	-	74.0	-	93.0	-	-	-	98.0	-	113.0	stop	-
3	J.T	-	6.0	-	-	-	-	53.0	-	-	71.0	-	-	-	-	-	-	-
4	I,P	11.0	76.0	88.0	-	-	-	-	-	-	-	-	-	-	-	-	140.0	-
5	D.M	40.0	49.0	-	-	-	101.0	-	-	-	-	47.0	-	-	-	-	-	-
6	J.N	31.0	-	-	-	-	142.0	-	-	204.0	-	-	210.0	-	-	-	-	267.0
7	M.K	34.0	-	-	-	-	117.0	-	-	-	-	-	146.0	-	-	-	-	219.0
8	M.G	27.0	75.0	-	-	-	-	44.0	-	-	-	-	-	162.0	-	-	-	-
9	W.W	78.0	-	-	-	84.0	-	94.0	-	-	99.0	-	-	108.0	-	-	112.0	-
10	A.G	42.0	-	-	-	-	-	146.0	-	-	-	-	-	350.0	stop	-	-	-

CONT...

cont

No.	Name	Post-Infusion Days (nmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	W.M	-	-	-	-	43.0	-	-	-	-	-	-	-	-	-	-
2	J.W	-	64.0	-	-	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	-	-	-	-	-	-	132.0	-	-	-	-	-	105.0	219.0	315.0
5	D.M	-	-	-	stop	-	133.0	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	208.0	-	-	-	-	169.0	-	-	-	-	-
8	M.G	242.0		-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	65.0	-	-	104.0	-	-	102.0	98.0	-	stop	76.0	-	-	-
10	A.G	127.0		-	-	-	-	-	-	-	-	-	-	-	-	-

Chromium in Serum ; Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	A.G	10.0	-	-	30.0	-	-	50.0	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	22.0	-	34.0	-	-	38.0	-	-	-	48.0	-	-	-
3	D.M	31.0	37.0	-	-	-	-	-	113.0	-	-	-	-	-	-	-	-	-
4	H.W	11.0	-	-	33.0	-	74.0	-	-	-	60.0	-	-	-	105.0	stop	-	-
5	I.C	5.0	-	-	24.0	-	-	39.0	-	-	-	-	-	73.0	-	-	stop	-
6	G.R	-	-	-	13.0	-	76.0	-	-	-	113.0	-	-	89.0	-	-	90.0	-

CONT ...

cont

No.	Name	Post-Infusion Days (nmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	43.0	-	-	-	-	-	-	-	-	-	-	-
5	I.C	62.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	58.0	-	-	-	-	-	-	-	58.0	-	-	-	-

Selenium in Urine : Surgical I.V.N. Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	W.M	-	430	430	400	400	-	-	-	480	-	-	-	510	-	-	-	-
2	J.W	-	545	-	-	-	458	450	408	-	-	-	-	-	387	393	403	486
3	J.T	-	236	204	211	218	191	-	-	-	-	-	-	-	-	-	-	-
4	I.P	88	156	265	-	-	-	-	-	-	242	196	-	-	-	-	-	464
5	D.M	116	-	420	212	-	-	-	305	212	359	221	207	181	336	216	272	303
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	-	-	195	-	-	-	-	-	237	177	190	-	190	-	-	208
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

cont

No.	Name	Post-Infusion Days (nmol/volume)																
		17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	W.M	-	-	440	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	481	473	259	349	337	stop	170	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	291	-	-	-	-	-	-	-	-	-	-	-	-	-	-	253	424
5	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	208	-	-	216	-	203	203	192	167	274	225	250	231	283	-	258
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Selenium in Urine : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/volume) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	A.G	600	520	490	-	-	-	490	560	480	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	362	385	-	-	310	-	-	-	154	315	-	-	-	-	318	276
5	I.C	683	838	-	-	731	858	753	761	724	496	648	-	726	-	-	698	727
6	G.R	-	-	-	-	946	1048	1172	-	-	-	1190	-	-	1463	-	-	-

cont

		Post-Infusion Days (nmol/volume)																
No.	Name	17	18	19	20	21	22	23	24	25	26	27	28	30	31	32	35	36
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	M.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	I.C	658	707	766	stop	290	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	1161	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Selenium in Serum : Surgical I.V.N.Study

ANABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	W.M	-	-	-	-	1150	-	-	-	-	-	-	stop	1240	-	-	-	-
2	J.W	1240	-	-	-	1430	-	1260	-	1290	-	-	-	1290	-	1150	stop	-
3	J.T	-	534	-	-	-	-	534	-	-	709	-	-	-	-	-	-	-
4	I.P	714	583	646	-	-	-	-	-	-	-	-	-	-	-	-	823	-
5	D.M	1216	937	-	-	-	735	-	-	-	-	1228	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	735	-	-	-	735	-	735	-	-	722	-	-	684	-	-	646	-
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CONT ...

cont

		Post-Infusion Days (nmol/l)														
No.	Name	21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	W.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	J.W	-	1329	-	-	-	-	-	-	-	-	-	-	-	-	-
3	J.T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	I.P	-	-	-	-	-	-	709	-	-	-	-	-	418	380	-
5	D.M	-	-	-	stop	-	836	-	-	-	-	-	-	-	-	-
6	J.N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	M.K	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	M.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	W.W	-	659	-	-	684	-	-	588	840	-	stop	940	-	-	-
10	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Selenium in Serum : Surgical I.V.N. Study

CATABOLIC GROUP

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	6	7	8	10	11	12	13	14	16	17	18	20
1	A.G	1020	-	-	970	-	-	1040	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	925	-	874	-	-	823	-	-	-	798	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	798	-	-	659	-	532	-	-	-	583	-	-	-	317	stop	-	-
5	I.C	1304	-	-	1152	-	-	1228	-	-	-	-	-	1160	-	-	stop	-
6	G.R	-	-	-	570	-	1013	-	-	-	950	-	-	1000	-	-	1482	-

CONT ...

cont

No.	Name	Post-Infusion Days (nmol/l)														
		21	23	24	26	27	28	29	30	33	34	35	41	63	101	148
1	A.G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	D.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	H.W	-	-	-	735	-	-	-	-	-	-	-	-	-	-	-
5	I.C	1215	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	G.R	-	-	1304	-	-	-	stop	-	-	-	1469	-	-	-	-

Zinc in Urine : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (µmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	R.M	-	-	-	38.3	38.8	41.0	-	-	-	-	40.6	52.1	54.8	-	-	-	-	-
2	P.P	-	-	-	31.2	18.4	20.9	-	-	-	-	21.6	14.6	18.9	-	-	-	-	17.6
3	E.I	58.8	48.3	-	54.6	49.4	54.1	58.7	49.0	-	48.5	40.7	50.4	43.4	44.7	48.7	48.2	35.0	-
4	J.C	24.2	32.0	-	15.2	17.8	-	-	-	25.5	-	-	-	28.6	27.2	25.9	-	-	49.6

No.	Name	Post-Infusion Days (µmol/l) 18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	15.5	-	-	-	-	-	9.2	17.9	20.5	-	-	-	-	24.5	18.6	-	-	-
3	E.I	59.3	64.4	55.9	41.6	52.5	48.0	-	51.6	-	44.6	-	54.0	50.4	33.8	38.6	33.9	36.0	30.9
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Zinc in Serum : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (µmol/l) 1	3	4	7	10	11	13	14	17	18	21
1	R.M	6.8	-	-	6.0	-	-	-	8.5	-	-	-	-
2	P.P	-	-	-	6.0	7.5	-	9.0	-	-	9.0	-	-
3	E.I	9.0	-	15.3	-	12.3	11.5	-	-	11.8	13.3	-	12.5
4	J.C	7.5	-	-	-	-	11.0	-	-	-	-	11.5	-

No.	Name	Post-Infusion Days (µmol/l) 24	25	28	31	34	35	38	39	43	45	52	59
1	R.M	-	*7.5	-	-	-	-	-	12.5	-	-	-	-
2	P.P	9.0	-	-	6.5	4.0	-	*8.5	-	-	10.5	11.5	-
3	E.I	12.7	-	15.3	-	-	10.7	-	-	*9.5	-	8.5	7.0
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-

*After cessation of infusion

Copper in Urine : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (μmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	R.M	-	-	-	1.66	1.43	1.27	-	-	-	-	1.77	1.90	2.00	-	-	-	-	-	-
2	P.P	-	-	-	0.83	0.32	1.40	-	-	-	-	0.55	0.32	0.50	-	-	-	-	0.61	1.19
3	E.I	4.58	1.83	-	1.21	1.18	1.24	2.47	1.29	-	1.60	1.05	1.92	1.60	0.76	0.98	1.25	0.95	-	1.51
4	J.C	0.44	0.28	-	0.74	0.40	-	-	-	0.65	-	-	-	0.48	0.64	1.30	-	-	0.75	-

No.	Name	Post-Infusion Days (μmol/l) 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	-	-	-	-	-	0.50	0.84	1.15	-	-	-	-	0.63	0.73	-	-	-	-
3	E.I	1.60	1.01	0.62	1.04	1.21	-	0.91	-	1.23	-	1.04	1.31	0.98	1.42	1.03	1.14	1.26	-
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Copper in Serum : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (µmol/l) 1	3	4	7	10	11	13	14	17	18	21
1	R.M	13.5	-	-	23.5	-	-	-	17.0	-	-	-	-
2	P.P	-	-	-	12.5	15.7	-	17.5	-	-	20.0	-	-
3	E.I	15.5	-	16.7	-	18.5	20.0	-	-	21.3	22.1	-	22.6
4	J.C	6.5	-	-	-	-	13.5	-	-	-	-	14.0	-

No.	Name	Post-Infusion Days (µmol/l) 24	25	28	31	34	35	38	39	43	45	52	59
1	R.M	-	*15.0	-	-	-	-	-	23.0	-	-	-	-
2	P.P	22.0	-	-	17.5	16.5	-	*15.0	-	-	26.0	32.0	-
3	E.I	23.3	-	27.1	-	-	31.7	-	-	*31.3	-	32.6	32.0
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-

*After cessation of infusion

Manganese in Urine : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	R.M	-	-	-	190	160	150	-	-	-	-	150	140	260	-	-	-	-	-	-
2	P.P	-	-	-	670	480	850	-	-	-	-	560	60	60	-	-	-	-	60	90
3	E.I	157	189	-	173	174	169	158	173	-	200	117	141	115	115	96	159	74	-	148
4	J.C	62	79	-	61	63	-	-	-	93	-	-	-	79	100	93	-	-	115	-

No.	Name	Post-Infusion Days (nmol/l) 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	-	-	-	-	-	60	150	60	-	-	-	-	40	50	-	-	-	-
3	E.I	184	148	185	144	303	-	198	-	215	-	309	285	170	222	208	244	167	-
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Manganese in Serum : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	3	4	7	10	11	13	14	17	18	21
1	R.M	53.0	-	-	60.0	-	-	-	22.0	-	-	-	-
2	P.P	-	-	-	27.0	33.0	-	31.0	-	-	24.0	-	-
3	E.I	33.0	-	55.0	-	51.0	50.0	-	-	44.0	54.0	-	106.0
4	J.C	15.0	-	-	-	-	25.0	-	-	-	-	33.0	-

No.	Name	Post-Infusion Days (nmol/l) 24	25	28	31	34	35	38	39	43	45	52	59
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	-	33.0	-	-	18.0	25.0	-	-	-	-	-	-
3	E.I	135.0	-	169.0	-	-	121.0	-	-	*134.0	-	86.0	73.0
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-

*After cessation of infusion

Chromium in Urine : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	R.M	-	-	-	310	300	300	-	-	-	-	380	440	660	-	-	-	-	-	-
2	P.P	-	-	-	360	240	440	-	-	-	-	310	300	330	-	-	-	-	380	330
3	E.E	111	292	-	449	393	420	426	366	-	551	340	444	408	381	359	323	255	-	436
4	J.C	849	905	-	585	670	-	-	-	732	-	-	-	659	627	611	-	-	778	-

No.	Name	Post-Infusion Days (nmol/l) 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	-	-	-	-	-	340	340	370	-	-	-	-	430	430	-	-	-	-
3	E.E	434	396	261	372	331	-	351	-	361	-	438	432	327	386	360	399	352	-
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Chromium in Serum : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	3	4	7	10	11	13	14	17	18	21
1	R.M	14.0	-	-	35.0	-	-	-	61.0	-	-	-	-
2	P.P	-	-	-	78.0	75.0	-	97.0	-	-	116.0	-	-
3	E.I	39.0	-	66.0	-	95.0	115.0	-	-	131.0	133.0	-	127.0
4	J.C	35.0	-	-	-	-	94.0	-	-	-	-	122.0	-

No.	Name	Post-Infusion Days (nmol/l) 24	25	28	31	34	35	38	39	43	45	52	59
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	126.0	-	-	87.0	45.0	-	-	-	-	-	-	-
3	E.I	147.0	-	159.0	-	-	132.0	-	-	*127.0	-	94.0	57.0
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-

*After cessation of infusion

Selenium in Urine : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	R.M.	-	-	-	150	150	170	-	-	-	-	200	1400	360	-	-	-	-	-	-
2	P.P	-	-	-	180	110	200	-	-	-	-	100	120	160	-	-	-	-	220	170
3	E.E	393	279	-	299	357	343	337	286	-	348	249	312	205	286	304	268	199	-	358
4	J.C	131	148	-	196	167	-	-	-	204	-	-	-	185	200	211	-	-	302	-

No.	Name	Post-Infusion Days (nmol/l) 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	-	-	-	-	-	170	190	180	-	-	-	-	200	-	-	-	-	-
3	E.E	365	325	220	337	270	-	275	-	204	-	342	270	172	226	248	266	220	-
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Selenium in Serum : Medical I.V.N. Study

No.	Name	Pre Inf	Post-Infusion Days (nmol/l) 1	3	4	7	10	11	13	14	17	18	21
1	R.M	410	-	-	410	-	-	-	460	-	-	-	-
2	P.P	-	-	-	420	480	-	480	-	-	700	-	-
3	E.I	460	-	510	-	520	460	-	-	480	391	-	403
4	J.C	532	-	-	-	-	608	-	-	-	-	583	-

No.	Name	Post-Infusion Days (nmol/l) 24	25	28	31	34	35	38	39	43	45	52	59
1	R.M	-	-	-	-	-	-	-	-	-	-	-	-
2	P.P	580	-	-	430	300	-	-	-	-	-	-	-
3	E.I	365	-	403	-	-	353	-	-	*329	-	456	-
4	J.C	-	-	-	-	-	-	-	-	-	-	-	-

*After cessation of infusion

REFERENCES

REFERENCES

Al-Rashid, R.A. & Spangler, J. (1971). Neonatal copper deficiency. New England Journal of Medicine, 285, 841-843.

Annual Report, Kabi-Vitrum. Contamination due to steel needle.

Arakawa, T., Tamura, T., Igarashi, H., Suzuki, & Sandstead, H. (1976). Zinc deficiency in two infants during total parenteral alimentation for diarrhoea. Ann. J. Clinical Nutrition, 29, 197-204.

Aronsen, K.F., Ekelund, G., Kindmark, C.O. & Laurell, C.B. (1972). Sequential changes of plasma proteins after surgical trauma. Scand. J. Clin. Lab. Invest, 29, No. 124, 127-136.

Askari, A., Calvin, L., Long, W.S. & Blackmore. (1979). Urinary zinc, copper, nitrogen and potassium losses in response to trauma. Journal of Parenteral and Enteral Nutrition, No. 3, 151-155.

Becker, W.M. & Hoekstra, W.G. (1971). The intestinal absorption of zinc; intestinal absorption of metal ions. Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides, 229-256. Edited by S.C. Skoryina, D. Waldron. Pergamon Press., McGill University, Montreal.

Behne, D (1980). Problems of sampling and sample preparation for trace element analysis in the health sciences. Trace Element and Analytical Chemistry in Medicine and Biology, 769-782

Boyd, L.O'Dell. (1976). Biochemistry of copper. Medical Clinics of North America, Vol. 60, No.4, 687-703.

Burch, R.E., Hahn, H.K.J. & Sullivan, J.F. (1975). Newer aspects of the roles of zinc, manganese and copper in human nutrition. Clin. Chem, 21, No. 4, 501-520.

Burk, R.F. (1976). Selenium in man. In Trace Elements in Human Health and Disease, Vol. II, 105-133. Essential & Toxic Elements. Ed. A.S.Prasad. Academic Press, New York.

Carr, G. & Wilkinson, A.W. (1975). Zinc and copper urinary excretions in children with burns and scalds. Clinica Chimica Acta, 61, 199-204.

Cotzias, G.C. & Bertinchamps, J. (1960). Transmanganin, the specific manganese carrying protein of human plasma. Journal of Clinical Investigation, 39, 979.

Dickson, R.C. & Tomlinson, R.N. (1967). Selenium in blood and human tissue. Clin. Chim. Acta, 16, 311-321.

Diplock, A.T. (1974). The nutritional and metabolic roles of selenium and vitamin E. Proc. Nutr. Soc, 33, 315-321.

Doisy, E.A. (1972). Micronutrient controls on biosynthesis of clotting proteins and cholesterol. Trace Subs in Environmental Health, VI, Proceedings of University of Missouri 6th Annual Conference on Trace Substances in Environmental Health, University of Missouri, Columbia, 193.

Dunlap, W.M., James, G.W. & Hume, D.M. (1974). Anaemia and neutropenia caused by copper deficiency. Annals of Internal Medicine, 80, 470-476.

Fell, G.S. & Burns, R.R. (1978). Zinc and other trace elements. Advances in Parenteral Nutrition, 241-263. Ed. I.D.A. Johnston, Lancaster, MRP Press.

Fell, G.S., Shenkin, A. & Halls, D.J. (1980). Trace element analysis as a diagnostic tool in clinical medicine. Trace Element Analytical Chemistry in Medicine and Biology, 217-232. Ed. Peter Bratter, New York.

Fell, G.S., Stromberg, P., Main, A., Spooner, R.J., Campbell, R. & Russell, R. (1981). Biochemical signs of human selenium depletion. Proc. Nutr. Soc, 40, 76A.

Fodor, L., Eschner, J., Dick, W. & Ahnefeld. (1972). The clinical significance of the zinc deficiency syndrome. Relationship between zinc deficiency and hypo-protein anaemia. Anaesthetist, 21, 456-459.

Freund, H., Atamian, S. & Fischer, J.E. (1979). Chromium deficiency during total parenteral nutrition. J. Med Associaton, 241, No. 5, 496-498.

Golden, M.H.N., Golden, B.E., Harland, P.S. & Jackson, A.A. (1978). Zinc and immunocompetence in protein energy malnutrition. Lancet, 1226-1227.

Gordon, W. & White, P.J. (1975). Zinc deficiency in total parenteral nutrition. S. Afr. Med. J, 54, 823-824.

Guidelines for essential trace element preparations for parenteral use. (1979). A statement by an expert panel, AMA Department of Food & Nutrition. Journal of American Medical Association, 241, No.19, 2051-2054.

Hallbook, T. & Hedelin, H. (1978). Changes in serum zinc and copper, induced by operative trauma and effects of per- and post-operative zinc infusion. Acta Chir. Scand, 144, 423-426.

Halls, D.J. & Fell, G.S. (1980). The determination of manganese in urine and serum. Trace Element Analytical Chemistry in Medicine and Biology, 265-272.

Halls, D.J. et al. (1981). Determination of copper in urine by graphite furnace atomic absorption spectrometry. Clinical Chimica Acta, 114, 21-27

Hambidge, K.M. (1981). Chromium. Disorders of Mineral Metabolism, Vol.I: Trace Minerals, 271-294. Ed. A. Bronner & J.W.Coburn, Academic Press. A subsidiary of Harcourt brace Jovanovich Publishers, New York.

Heller, R.M., Kirchner, S.G., O'Neill, J.A., Hough, A.J., Howard, L., Kramer, S.S. & Green, H.L. (1978). Skeletal changes of copper deficiency in infants receiving prolonged total parenteral nutrition. Journal of Paediatrics, 92, 947-949.

Jacobson, S. & Wester, P.O. (1977). Balance study of twenty trace elements during total parenteral nutrition in man. British Journal of Nutrition, 37, 107-126.

Jeejeebhoy, K.N., Chu, R.C., Marliss, E.B., Greenberg, G.R. & Robertson, A.B. (1977). Chromium deficiency, glucose intolerance and neuropathy reversed by chromium supplementation in a patient receiving long-term total parenteral nutrition. American Journal of Clinical Nutrition, 30, 531-538.

Johnson, R.A., Baker, S.S., Fallon, J.T., Maynard, E.P., Ruskin, J.N., Wen, L. & Cohen, H.J. (1981). An accidental case of cardiomyopathy and selenium deficiency. New England Journal of Medicine, 304, 1210-1212.

Karpel, J.T. & Peden, F.H. (1972). Copper deficiency in long-term parenteral nutrition. Journal of Paediatrics, 80, 32-36.

Karpel, J.T. & Peden, V.H. (1972). Copper deficiency in long-term parenteral nutrition. J. Paediatrics, 80, 32-36.

Kay, R.G., Tasman-Jones, J., Pybus, R., Whiting, H. & Black. (1976). A syndrome of acute zinc deficiency during total parenteral nutrition in man. Ann. Surgery, 183, 331-339.

Lane, H.W., Barroso, A.D., Dudrick, S.J. & MacFadyen, B.V. (1981). Selenium status of seven IV patients. Trace Elements in Animals and Man, Part IV, TIMA, 30-33.

Latimer, J.S., McClain, C.J. & Sharp, H.L. (1980). Clinical zinc deficiency during zinc supplemented parenteral nutrition. The Journal of Paediatrics, 97, No. 3, 434-437.

Leach, R.M. Jr. (1976). Essential and toxic elements. Trace Elements in Human Health and Disease, Vol. II, Metabolism and Functions of Manganese, 235-248. Ed. A.S.Prasad.

Lowry, S.F., Goodgaine, J.T., Smith, J.C., Maher, M.M., Makuch, R.W., Henkin, R.I. & Brennan, M.F. (1979). Abnormalities of zinc and copper during total parenteral nutrition. Ann. Surgery, 189, 120-127.

Mather, R.K. & Doisy, R.J. (1972). Effect of diabetes and diet on the distribution of tracer doses of chromium in rats. Proc. Soc. Exp. Biol. Med, 139, 836-838.

McLeod, B.A. & Robinson, M.F. (1972). Metabolic balance of manganese in young women. British Journal of Nutrition, 27, 221-227.

Mertz, W. (1979). Chromium. In Chromium Nutrition and Metabolism, 1-14, Ed. D. Shapcott & J.Hubert. Amsterdam, Elsevier/North Holland. Biomedical Press.

Mertz, W. (1969). Chromium occurrence and function in biological systems. Physiol Review, 49, 169-239.

Mertz, W. (1980). In Chromium Nutrition and Metabolism, 1-14. Ed. D.Shapcott & J.Hubert.

Mertz, W. & Reginski, E.E. (1971). Chromium metabolism, the glucose tolerance factor. In New Trace Elements in Nutrition, Chapter 7, 123. Eds. W.Mertz & W.E.Cornatzer, Dekker, New York.

Mills, C.F. (1979). Trace element deficiency and excess in animals. Chemistry in Britain, 15, No. 10, 512-520.

Peaston, R. (1973). Determination of copper and zinc in plasma and urine by atomic absorption spectrophotometry. Medical Lab. Technology, 30, 249-253

Prasad, A.S. (1978). Zinc. Trace Elements and Iron in Ibid Human Metabolism, Chapter 10, 281-346. Published in Great Britain by John Wiley and Sons.

Prasad, A.S. (1979). Zinc. Zinc in Human Nutrition, 1-81. C.R.C. Press, Florida.

Prasad, A.S. & Oberleas, D. (1974). Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissue. J. Lab. Clin. Med , 83, 842.

Prasad, A.S., Schulert, A.R., Sandstead, Miale, H.H. & Zohier,Farid. (1963). Zinc, iron and nitrogen content of sweat in normal and deficient subjects. J. Lab. Clin. Med, 62, 84-89.

Recommended Dietary Allowance. (1979). Food and Nutrition Board, 9th Edition, Nat. Acad. Sciences, Washington, D/C.

Reinhold, J.G., Faradji, B., Abadi, P. & Ismail-Beigs, F. (1976). Binding of zinc to fibre and other solids of wholemeal bread, with preliminary examination of the effects of cellulose consumption upon the metabolism of zinc, calcium, phosphorous, in man. Trace Element in Human Health and Disease, Vol 1, 163. Ed. Prasad.

Riales, R. & Albrink, M.L. (1981). Effect of chromium chloride supplementation on glucose tolerance and serum lipids including high density lipoprotein of adult men. American Journal of Clinical Nutrition, 34, 2670-2678.

Rijj, A.A., Thomson, C.D., McKenzie, J.M. & Robinson, M.F. (1979). Selenium deficiency in total parenteral nutrition. American J. Clin. Nutrition, 32, 2076-2085.

Saltman., Versieck, J., Hoste, J., Barbier, F., Vanballenberghe,L., De Rudder, J. & Cornelis, R. (1981). Serum trace element levels in hepatobiliary disease. Trace Elements in Animal and Man, TIMA, 4th edition, 534-537.

Saner, G. (1980). Chromium in Nutrition and Disease, Chapter 6, 73-95.

Sann, L., David, L., Ealy, G. & Romand-Monier, M. (1978). Copper deficiency and hypocalcaemic rickets in a small for-date infant. Acta Paediatric Scandinavia, 67, 303-307.

Schrauzer, G.N. (1976). Selenium anticarcinogenic action of an essential trace element. Industrial Health Foundation, Pittsburg, 293-299. Proc. Symp. Selenium, tellurium in the environment.

Shaw, J.C.L. (1973). Parenteral nutrition in the management of sick low birthweight infants. Pediatrics Clinics of North America, 20, 333-358.

Shenkin, A. & Wretlind, A. (1978). Parenteral nutrition. World Review Nutrition and Dietetics, 28, 1-111. Ed: G.H.Bourne. Publisher S.Karger, Basel.

Spencer, H., Rossof, B., Fieldstain, A., Colins, H. & Gusamano, E. (1965). Metabolism of Zn^{65} in man. Radiation Research, 24, 432-445.

Srouji, M.N., Balistrezi, W.F., Caleb, M.H., South, M.A. & Starr, S. (1978). Zinc deficiency during total parenteral nutrition: skin manifestations and immune incompetence in a premature infant. Journal of Paediatric Surgery, 13, No.6, 570-575.

Strobel, C.T., Byrne, W.J., Abramonts, W., Newcomer, V.J., Bleich, R. & Ament, M.E. (1978). A zinc deficiency dermatitis in patients on total parenteral nutrition. International Journal of Dermatology, 17, 575-581.

Suita, S., Ikeda, K., Nagasaki , A. & Hayashi, Y. (1978). Zinc deficiency during total parenteral nutrition in childhood. Journal of Pediatric Surgery, 13, No.1, 5-9.

Tengrup, I. & Samuelsson, H. (1977). Changes in serum zinc during and after surgical procedure. Acta Chir. Scand, 143, 195-199.

Tyrala, E.E., Brodsky, N.L. & Auerbach, V.H. (1982). Urinary copper losses in infants receiving free amino acid solutions. The American Journal of Clinical Nutrition, 35, 542-545.

Underwood. (1977). Copper. Trace Elements in Human and Animal Nutrition, 56-108. Academic Press, New York/London.

Underwood, E.J. (1977). Zinc. Trace Elements in Human and Animal Nutrition, 4th Edition, 196-242. Academic Press, New York/London.

Underwood, E.J. (1977). Selenium. In Trace Elements in Human and Animal Nutrition, 4th Edition, 302-346. Academic Press, New York.

Underwood, E.J. (1979). Trace Metals in Human and Animal Nutrition, 4th Edition, 1-12.

Utter, M.F. (1976). The biochemistry of manganese. The Medical Clinics of North America, 60, No. 4, 713-727.

Van Rijj, A.M., Thomson, C.D. MacKenzie, J.M. (1979). Selenium deficiency in total parenteral nutrition. American Journal of Clinical Nutrition, 32, 2076-2085.

Versieck, J. & Cornelis, R. (1980). Normal levels of trace elements in human blood plasma or serum. Analytica Chimica Acta. 116, 217-254.

Versieck, J. Cornelis, R. (1980). Normal levels of trace elements in human blood plasma or serum. Analytical Chimica Acta, 116, 217-254.

Wenlock, R.W., Buss, D.H. & Dixon, E.J. (1979). Trace nutrients 2^{o} manganese in British food. British Journal of Nutrition, 41, 253-260.

Young, V. (1981). Selenium, a case for its essentiality in man. New England Journal of Medicine, 304, 1228-1230.