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Postgraduate Medicine
Lloyd-Luke (Medical Books)
Second Edition, 1971

With D. S. Hopton

Practical Hints for Housemen
Lloyd-Luke (Medical Books), 1970

The Clinical Significance of the Essential Biological Metals

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Foreword

There are several reasons why the appearance of this book is timely: there has been a recent burgeoning of new analytical techniques for measuring metals which makes it possible to determine them rapidly and with considerable accuracy in even minute amounts in biological material; important new advances have been, and are still being made in our understanding of the role of metals in determining the structure, function and stability of proteins, and especially the activity of many enzymes; and there is now a growing concern over the possible deleterious effects caused by prolonged exposure to some of the metals which are being discharged into the environment as the result of industrial processes and the burning of motor fuel in urban areas.

Clinicians are therefore becoming increasingly aware of the medical significance of metals and they will be able to find most of the relevant literature in Dr Davies' monograph. Much of what has been written in the past about the role of metals in biology requires a considerable degree of appreciation of physico-chemical principles. However, Dr Davies, who is himself a clinician, has been mindful of the interests and training of clinicians and has concentrated on bringing together information dealing not only with the role of metals in nutrition, but also their relationship to various diseases and their potential use as therapeutic agents.

It is a pleasure to be asked to write this foreword to a book which will stimulate many clinicians to take a closer interest in an important field of medicine in which many new developments will take place in the near future.

ROBERT MAHLER

Professor of Medicine
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Preface

This book reviews current knowledge of the six trace metals known to be essential to man. The minute quantities of these metals present in animal tissue can now be measured accurately; I have therefore abandoned the word 'trace'. Because they are necessary for the health of man and other mammals I prefer to refer to them as 'essential biological metals'. The essential metals, such as sodium and potassium, which are required primarily for their physical and chemical properties when in solution are not included. I have dealt with those essential biological metals which are necessary mainly for enzyme function and protein synthesis.

The importance of the essential biological metals in medicine has only recently been appreciated. That they are as significant nutritionally as the vitamins has long been accepted in veterinary and agricultural practice.

The part played by zinc in wound healing, manganese in drug induced lupus erythematosus, molybdenum in dental caries and oesophageal cancer in South Africa, chromium in diabetes and copper in iron utilization serve to show how diverse are the effects of these essential nutrients in man. I have extracted from the world literature information which is likely to be of interest and value in relation to human metabolism and disease. Where I have considered that unreasonable conclusions have been drawn or questionable results obtained I have omitted these papers completely; apart from this I have not criticized the validity of what I have quoted.

I have refrained from the temptation of implying importance to any of the quoted findings in relation to their significance to medicine. Any inferences as to the importance of the quoted information to his branch of medicine must be made by the reader.

Even though I have looked at the metabolism and possible significance of the essential biological metals from a medical point of view it is inevitable that in bringing the subject up to date I have had to lean heavily on previous reviews of which that of Professor E. J. Underwood is outstanding.

I gratefully acknowledge the unfailing helpfulness, courtesy and forbearance of the staff of the Welsh National School of Medicine Library. The search for references was helped enormously by MEDLARS (Medical Literature Retrieval System); Mrs Van Aernsbergen of the Library of the Royal Society of Medicine, London, cheerfully and industriously compiled the necessary computer programme. Dr Raymond Greene of Heinemann, who suggested the project, waited patiently for three years until it was complete.

Once again, I have received from my kind and patient wife, Dr Joyce Davies, M.R.C.P., such help and encouragement that without her I would not have contemplated let alone completed the work. Only those who have laboured with journals, manuscripts, galley proofs, page proofs, indices, etc., can appreciate how much domestic peace aids such a project.

September, 1971 IEUAN DAVIES

For Joyce and Edwin

Philosophy is often much embarrassed when she encounters certain facts which she dare not doubt yet will not believe for fear of ridicule.

Immanuel Kant

Even the dogs may eat the crumbs which fall from the rich man's table; and in these days, when the rich in knowledge eat such specialized food at such separate tables, only the dogs have a chance of a balanced diet.

Sir Geoffrey Vickers
Introduction to *The Art of Judgement*

CHAPTER I

Introduction

Most of the stable elements are found in minute quantities in the human body. Those that have no biochemical activity in non-toxic amounts are present in variable amounts depending on their concentration in the local soil, food and atmosphere. Industrial, agricultural and chemical pollution also affect the quantity of inert solid elements present in the body. Most of these inert elements accumulate in the body with increasing age; many appear in the urine, faeces, shed skin and intestinal cells at different times, although there is no known homeostatic mechanism which controls their concentration in the body. However, there is sometimes an unknown mechanism controlling their absorption when they are ingested in large amounts. Occasionally one of the biologically inert metals is under some homeostatic control (e.g. vanadium). These stable elements are not constantly present in living mammalian cells and they have no certain essential enzymatic function *in vivo*, although several of the non-essential metals can act as enzyme activators *in vitro* in place of the metal activators which are known to be present in the body.

Some of the non-essential metals have biological and physiological activity when present in non-toxic amounts although they are not always present in the body, for example, cadmium, nickel and vanadium.

The essential biological metals are the inorganic counterparts of the essential biological organic nutrients, the vitamins. Unlike the vitamins they cannot be synthesized by living organisms and they must be present in the environment. Within certain limits they are present in the environment wherever there is life. There is known to be an inverse ratio between the atomic number of an element and its abundance on the surface of the earth; it is therefore not surprising that 99% of the structure of living organisms is composed of elements from the first 20 in the Periodic Table. The remaining 1% of their structure is made up by elements from 20-42 of the Periodic Table (cadmium to molybdenum); the one exception is iodine which has an atomic number of 53. Only about two-thirds of the first 42 elements are biologically necessary. Schroeder (1965a) has pointed out some fascinating evolutionary

patterns with regard to the handling of essential and non-essential elements. He suggests that the earliest forms of life were made up of the most readily available and abundant elements–sodium, potassium, calcium, silicon, magnesium and phosphorus. Copper and iron although not present in large amounts were also necessary at an early stage because of their importance for oxidative enzymes. With increasing sophistication and specialization of parts new enzyme systems became necessary which required elements with different properties from those which were most abundant. The earliest and most primitive organ of excretion was the primitive gut, this was present long before kidneys were necessary. With the requirement for new enzymes and hence new elements which were not necessarily abundant or uniformly distributed some form of control of excretion was necessary; naturally, this meant using the primitive gut (and its hepatic and pancreatic anlages)–thus it is probable that the earliest essential micro-nutrients would be excreted by the liver, pancreas or intestine. Of the known essential 'trace' metals all are excreted principally via the intestinal tract with the one exception of molybdenum; molybdenum is also different from the others in that it is usually an anion, whereas the others excreted by the gut are usually cations. The development of the kidneys was necessary with evolution from salt to fresh water in order to conserve sodium and the other essential bulk elements which had originally been present in the environment in optimum amounts. The situation is one in which the older essential elements are being controlled by the newer excretory mechanism.

The concept of essentiality

At first sight it may appear easy to decide whether a micro-nutrient is essential or not; one could argue that a set of postulates could be devised which had to be fulfilled rather like Koch's postulates for deciding whether a particular infectious disease is due to a suspected infective organism. However, there are considerable theoretical and practical difficulties in deciding if a micro-nutrient is essential. For example, essential to what–life, growth, health, or reproduction? One of the most obvious criteria would be to establish that an absence of an element resulted in a clearly recognizable deficiency syndrome. But even to this reasonable proposition there are three major snags.

1. It may be physically impossible to prepare diets and environments deficient in the element being studied.
2. The animal may possess known or unknown reserves of the element.
3. Deficiency of one element often predisposes to toxic effects from an antagonistic element when this is present in normal amounts.

In addition the requirements of the animal may change at different periods of its life, e.g. during lactation, reproduction or old age.

Other criteria of essentiality have been proposed viz.:

1. A deficiency of the element makes it impossible for the plant or animal to complete the vegetative or reproduction stages of its life cycle.
2. Such deficiency is specific to the element in question; and can be corrected or prevented *only* by supplying this element.
3. The element is directly involved in the nutrition of the plant or animal, quite apart from its possible effects in correcting some microbial or chemical condition of the external medium (Arnon and Stout, 1939, quoted by Schütte, 1964).

Another definition of essentiality might be—'An element which is necessary for optimal function of the organism'. However, this definition does not distinguish between inorganic micro-nutrients which are essential, those which are only beneficial and those whose deficiency results in a predisposition to disease before deficiency syndromes develop, for example, in animals cobalt and copper deficiency predispose to bacterial and parasitic infection; in plants trace metal deficiency is even more prone to predispose to infections, so much so, that certain infections occurring in certain plants are used as indicators of soil deficiency of a particular mineral.

In agriculture practical use is made of the fact that some plants and trees are particularly prone to develop mineral deficiency signs; for this reason they are called 'indicator' plants. Examples are: apple (boron deficiency), cabbage and soft fruit (iron deficiency), sugar beet and mangolds (manganese deficiency), cauliflower (molybdenum deficiency) and oranges and apples (zinc deficiency) (Wallace, 1951). It is also of interest that many plants only grow where there are deposits of a high concentration of a particular mineral or metal in the soil. In other words some plants require, as essential, amounts of a biological metal which would be toxic to others. With regard to criteria for deciding whether a metal is essential for human requirements, Schroeder and Balassa (1966) have suggested a number of factors which should be considered:

1. The metal must be more or less ubiquitous, available to and absorbed by animals and plants.
2. The chemical nature of the metal must be compatible with some physiological function.
3. The atomic number should fall within those known to be essential to health.
4. The metal must cross placental and mammary barriers in order to supply the foetus and neonate.
5. The concentration of the metal in the tissues must remain constant or decline with increasing age.
6. The metal must be of low toxicity when administered in the form in which it occurs naturally.

7. A homeostatic controlling mechanism must be present.

Function of the essential biological metals

By far the most important function of the essential biological metals is their necessity for a large number of enzymes and their content in ribonucleic acid. Their association with enzymes is of two types, they may be an integral part of the enzyme protein molecule (metallo-enzymes) or they may be 'activators' of the enzyme (metal ion activated enzymes). Some of the distinguishing features of metallo-enzymes and metal ion activated enzymes are shown in Table I.

TABLE I

Characteristics of metallo-enzymes and metal ion activated enzymes
(Modified from Parisi and Vallee, 1969)

Metallo-enzyme	*Metal ion activated enzyme*
Firm binding of metal to protein e.g. zinc and alkaline phosphatase.	Weak binding of metal to protein e.g. manganese and leucine amino peptidase.
Metal is retained by the enzyme during purification.	Metal is lost during purification of the enzyme.
Ratio of metal to protein rises during purification, and reaches a fixed limit when purification is achieved. The metal content can be used as a guide to the degree of purification.	
Numerical identity between the number of metal atoms and the number of substrate or co-enzyme binding sites can often be demonstrated.	
Specifically bound intrinsic metal can be differentiated from non-specifically bound extrinsic metal by selective dialysis.	Specifically and non-specifically bound metal cannot be distinguished.
Metal content and enzyme activity are directly related. Removal of the intrinsic metal results in a proportionate loss of activity.	Enzymatic activity and proportion of metal present are not directly related.
Enzyme activity is not increased by adding more of the metal once the necessary amount of metal for optimum function is present.	Enzyme is activated by the addition of a variety of metal ions.

TABLE II

*MAMMALIAN METALLO-ENZYMES**

Metal	*Enzyme*	*Source*	*Reference*
Zinc	Carbonic anhydrase	erythrocytes	Keilin and Mann, 1940 Rickli and Edsall, 1962 Duff and Coleman, 1966
	Carboxypeptidase	pancreas	Vallee and Neurath, 1954
	Alcohol dehydrogenase	liver	Vallee and Hoch, 1957 Vallee, 1955
	Glutamic dehydrogenase	liver	Vallee *et al.*, 1955
	D glyceraldehyde 3-phosphate dehydrogenase	muscle	Kaleti *et al.*, 1962
	Lactic dehydrogenase	muscle	Vallee and Wacker, 1956
	Malic dehydrogenase	heart	Harrison, 1963
	Alkaline phosphatase	kidney	Mathies, 1958
		leukocytes	Trubowitz *et al.*, 1961
	Metallothionine	liver	Pulido *et al.*, 1966
		kidney	Kägi *et al.*, 1960
	Procarboxypeptidase A	pancreas	Keller *et al.*, 1956
	Procarboxypeptidase	pancreas	Wintersberger *et al.*, 1962
Copper	Cytochrome oxidase	heart	Tzagoloff, 1965
	Ceruloplasmin	plasma	Kasper and Deutsch, 1963
	Uricase	liver and kidney	Mahler *et al.*, 1956
	Dopamine hydroxylase	adrenal	Friedman and Kaufman, 1965
	Amine oxidase	plasma	Yamada and Yasunobu, 1962
	Diamine oxidase	kidney	Mondovi *et al.*, 1967
	Tyrosinase	skin liver	Brooks *et al.*, 1966
Manganese	Pyruvate carboxylase	liver	Scrutton *et al.*, 1966
	Arginase	liver	Hirsh-Kolb and Greenberg, 1968
Molybdenum	Xanthine oxidase	liver	Rajagopalan and Handler, 1967
Cobalt	Methylmalonyl-GA mutase	liver	Barker, 1967
(Vitamin B_{12})	Homocysteine transferase	liver	Taylor and Weissbach, 1969
	Methyl tetrahydrofolate oxido reductase	liver	
	Ribonucleotide reductase	liver	Blakley, 1966
Chromium	Exact enzyme not known		Stickland, 1949

* Throughout the book the nomenclature of enzymes is that recommended by the International Union of Biochemistry on the Nomenclature and Classification of Enzymes in Volume 13 (Second Edition, 1965) *Comprehensive Biochemistry* (Editors M. Florkin and E. H. Stotz), Elsevier, Amsterdam, New York and London.

TABLE III

MAMMALIAN METAL ION ACTIVATED ENZYME

Zinc	Glycylglycine dipeptidase
	Arginase
	Dehydropeptidase
	Alanyl and leucyl glycine dipeptidase
	Tripeptidase
	Glycyl L-leucine dipeptidase
	Carnosinase
	Amino peptidase
	Histidine deaminase
	Lecithinase
	Enolase
	Oxalo decarboxylase
	Dihydrocrotase
	L-mannosidase
Copper *Cobalt*	probably only involved with metallo-enzyme
Manganese	Polymerases
	Galactotransferase
	Arginase
	Phosphoenol pyruvate carboxylase
Molybdenum	Xanthine oxidase
	Aldehyde oxidase
Chromium	Phosphoglucomutase

Reasons why the nutritional significance of the essential biological metals has been comparatively neglected

The nutritional and metabolic significance of the essential biological metals has been only slowly appreciated. Some of the reasons for this are:

1. The minute quantities involved.
2. The difficulty of producing specific deficiency diets.
3. The metal stores which animals have and their ability under natural circumstances to wander to non-deficient pastures.
4. The relative infrequency of naturally occurring deficiency syndromes compared with the frequency of vitamin deficiencies.
5. The remarkable ability of animals to find plants or other sources of missing trace metals (e.g. zinc troughs, copper vessels or molybdenum-containing objects).
6. The highly variable soil content of trace metals in neighbouring districts for natural reasons, e.g. glaciation, leaching of soil, periodic flooding or rarely (as in New Zealand) earthquakes.

7. An unwillingness to consider that deficiencies might exist because of commercial considerations. Once accepted that trace metal deficiency could occur it must follow that soils being depleted by animals and crops would need to have their trace metal content replaced regularly.
8. Inability to measure accurately the trace metal content of enzymes.
9. Complicated factors affecting the availability of trace metal from the soil, e.g. soil pH and the type of fertilizer being used. (see p. 8).
10. Complicated interaction between the trace metals affecting availability, absorption and tissue metabolism (v.i.).
11. Possibly the very frequency in everyday life of certain metals e.g. copper, chromium and zinc make it seem improbable that nutritional deficiency of these metals could exist.

The importance of interactions between the essential biological metals

The theoretical situations in which chemical and biochemical interactions between the essential biological metals may occur are:

1. In the soil preventing uptake by plants or in food being consumed by animals.
2. Within the gastro-intestinal tract.
3. At the site of metabolic action.
4. At some other situation in the body, e.g. (a) promoting excess storage in a non-metabolic form; (b) promoting release from storage; (c) combining to form a non-metabolizable compound such as occurs with copper and molybdenum; (d) altering binding sites on carrying protein.
5. At the excretory pathway, promoting abnormal excretion (either reduced or excessive).

Examples of biological metal interactions

Calcium and zinc

Excess calcium in the diet induces signs of zinc deficiency when zinc intake is normal. The interaction probably involves mechanism 1, 3 and 5 (Forbes, 1960).

Iron and copper

Copper is essential for some aspects of iron utilization, release from iron states, haem synthesis and for the iron-containing enzyme cytochrome oxidase mechanisms (Matrone, 1960).

Zinc and iron

Excess zinc causes iron deficiency anaemia and excess liver concentration of iron; it also reduces the iron content of ferritin (Settlemire and Matrone, 1967). Mechanisms 4a, c and d.

Zinc and cadmium

Excess cadmium causes some of the features of zinc deficiency (Lease, 1968). Mechanisms probably 2 and 4d.

Molybdenum and fluoride

Molybdenum increases absorption of fluoride from the stomach (Crane, 1960). Mechanism 2.

Manganese and magnesium

There is a complicated interrelationship between manganese and magnesium (Matrone, 1960). Manganese in excess leads to a reduction in magnesium. Mechanism unknown.

Copper and molybdenum

Excess molybdenum may produce features of copper deficiency; in addition excess molybdenum may be used to treat copper poisoning. Mechanism 4c (Miller and Engel, 1960).

Zinc and copper

High zinc intake produces features of copper deficiency. Mechanisms probably 2, 3 and 5. In addition the effects of zinc toxicity can be reversed by copper. Copper absorption is reduced by zinc (Van Campen and Scaife, 1967).

The importance of fertilizers (Schütte, 1964)

The essential biological metals have complicated interactions when ingested and absorbed by animals; in addition there are often complicated interactions in the soil. It is the essential mineral which is the least abundant which regulates the crop yield. It is no good redressing zinc deficiency in the soil if by so doing relative copper deficiency results.

Some examples of the adverse effects on crop yields using the common fertilizers are:

Lime (increases soil pH):	decreases availability of manganese and zinc: increases availability of molybdenum possibly leading to molybdenum-induced copper deficiency.
Nitrogenous fertilizers:	increases requirement of all essential metals but decreases availability of copper (leading to copper deficiency in grazing animals).
Phosphates:	decreases availability of zinc, increases availability of molybdenum (leading to molybdenosis)
Potassium (potash):	decreases boron deficiency and results in boron deficiency in crops.

The complicated interrelationships between the trace metals in the soil have not been sufficiently emphasized. This was demonstrated on a large scale in Holland after the Second World War when intensive fertilization schemes were introduced and there was a marked fall in crop productivity and decline in animal health. The intensive application of fertilizers led to a relative deficiency of copper, manganese and cobalt.

The importance of geochemical surveys

Trace metal analysis of representative soil samples and the incorporation of the findings onto geographical maps to produce geochemical maps has given invaluable information. In agriculture areas of mineral deficiency and excess have been identified and appropriate detailed studies have been instituted and the ground treated accordingly. Geochemical maps are likely to help in identifying areas of unusual incidence of human disease, for example, the high incidence of stomach carcinoma in particular soil areas of North Wales. At the present time a complete geochemical atlas of England and Wales is being prepared. One sample per square mile is being taken from stream sediments of tributary drainage. Stream sediments correspond closely to a composite sample of the products of weathering derived from the rocks and soil upstream from the sampling site (Webb *et al.*, 1968).

Water supply and soil mineral content and cancer mortality

In 1947 Stocks noticed that the four London boroughs supplied largely by well water had lower cancer mortalities than most of the boroughs supplied by river water (Stocks, 1947). Workers in the Netherlands have also shown a relationship between the chemical composition of drinking water and cancer frequency (Tromp, 1954).

Studies from North Wales in which the soil composition of the island of Anglesey was compared with cancer mortality showed a correlation between cancer of the stomach and soil type (Wynne Griffiths and Davies, 1954). In further more widespread studies additional disturbing correlations have been shown between the type of soil and cancer mortality. Soil rich in zinc or cobalt is found with excessive frequency where cases of stomach cancer occurred; however, the geographical distribution of such soils appears to be unrelated to that of stomach cancer rates (Stocks and Davies, 1960). The soil zinc : copper ratio is higher in the soil of gardens at houses where a person had just died, after 10 or more years of residence, of cancer of the stomach than it was at houses where a person had died of a non-malignant cause (Stocks and Davies, 1964). Correlations have also been demonstrated between the trace metal content of smoke from areas of heavy air pollution and carcinoma of the bronchus (Stocks, 1960).

Influence of drinking water on atherosclerosis

It has been noticed in several countries that death rates from hypertensive and atherosclerotic heart disease are correlated with the use of soft water (Schroeder, 1966; Morris *et al.*, 1961; Biork *et al.*, 1965). The reasons for the correlations are not known.

The non-essential but biologically significant metals

Many metals are more or less constantly present in human tissue; they may act *in vitro* as activators of certain enzymes in place of the known metal ion activator (see Appendix 3). They may alter the metabolism of one of the essential biological metals and even when present in non-toxic amounts they may play some part in the pathogenesis of disease. If present in toxic amounts they may produce adverse reactions and disease. Some of the metals non-essential to man are occasionally essential to lower organisms; on certain rare occasions nature has performed freak and unique experiments, e.g. substituting vanadium for copper or iron in the respiratory pigment of Ascidia, a group of marine sand worms. Nevertheless, despite man's environmental involvement with these metals and their ubiquity there is no evidence that they are essential to him by whatever criteria are used to assess essentiality.

Cadmium

In the experimental animal acute and chronic administration of cadmium has been shown to produce hypertension. Furthermore this hypertension can be cured by removing the cadmium by chelation or by

replacing it with zinc (to which it is chemically closely realted) (Schroeder, 1964; Schroeder *et al.*, 1966). Cadmium has been shown to be excreted in the urine of hypertensive patients in much higher concentrations than in normal controls (Perry and Schroeder, 1955). In addition the cadmium concentration of the kidneys of patients dying from the complications of hypertension is higher than in those of patients dying from other causes (Schroeder, 1965b). Patients absorbing cadmium via the lungs do not have an excessive incidence of hypertension (Schroeder, 1967). No cadmium metallo-enzymes are known although it can act as a facultative activator in place of other activators for three known enzymes. There is no homeostatic mechanism for cadmium, nevertheless in the kidney, a protein, metallothionine, of unknown function, contains a higher concentration of cadmium than does any other metallo-protein of its constituent metal (Pulido *et al.*, 1966). This protein also contains fixed proportions of zinc. The occurrence of this cadmium-containing protein has raised the suggestion that cadmium may be shown to have an essential biological role in the future (Schroeder *et al.*, 1967).

Nickel

Nickel has been suggested to have a biological function especially as it fulfils some of the criteria for ascribing essentiality; it does not accumulate with age, it is present in the new-born, there are hepatic and intestinal barriers and it is the only metal which does not have specific biological activity between atomic numbers 22 to 30. *In vitro* it activates liver arginase (in common with cobalt, iron and manganese). There is some indirect evidence that nickel may be involved in skin and hair pigmentation in some mammals (Schroeder *et al.*, 1962).

Vanadium

Vanadium is known to be essential for some lower animals and replaces iron and copper in the respiratory pigment of some marine worms. It is also essential for some lower plants. Its main medical interest is that it is known to interfere with cholesterol synthesis. The mean serum cholesterol concentration of vanadium workers is significantly lower than that of other workers (Lewis, 1959). It does not increase catabolism of cholesterol; it acts on cholesterol synthesis in the step between mevalonic acid and squalene probably through the enzyme mevalonic kinase (Azarnoff and Curran, 1957). The reduction of plasma cholesterol by vanadium is only small and may only be temporary. Vanadium salts are too toxic for this finding to be of therapeutic value.

Significant dates in the discovery of the metabolic significance of the essential biological metals

ZINC

1854 Zinc salts recovered from plant ash
1869 Zinc shown to be essential for the growth of *Aspergillus niger*
1919 Essential biological role for zinc postulated
1926 Zinc shown to be essential for higher plants
1934 First definite zinc deficiency syndrome demonstrated in animals
1936 Zinc shown to prolong the duration of action of protamine insulin
1940 Enzymes alkaline phosphatase and uricase shown to contain zinc
1940 More sophisticated experiments on zinc deficiency in animals
1946 Zinc first shown to be involved in iron metabolism
1950 onwards. Importance of zinc in reproduction investigated
1952 Intensive investigation into the role of zinc in the choroid and retina of the eye
1952 Further effects of zinc on physical properties of insulin demonstrated
1953 Intensive studies of the role of zinc in diabetes mellitus in animals and humans
1953 First suggestion that zinc might be involved in wound healing
1955 Porcine parakeratosis shown to be due to zinc deficiency
1955 onwards. Other zinc metallo-enzymes identified and purified
1956 Disturbances of zinc metabolism in alcoholic cirhosis demonstrated
1958 onwards. Further sophisticated zinc deficiency experiments on mammals and birds
1961 onwards. Zinc deficiency in humans described and intensively studied
1967 onwards. More convincing evidence of the role of zinc in wound healing

COPPER

1817 Copper first identified in plants
1830 Copper identified in ox blood
1847 Copper identified in the respiratory pigment of molluscs
1869 Copper identified in the pigment of bird feathers
1917 Copper shown to be essential for plants
1920 Copper postulated as being essential to life
1928 Copper shown to be essential for the growth and development in animals
1937 Copper deficiency shown to cause 'swayback' in lambs in Australia
1930-1948 Several reports that the copper content of the brain in Wilson's disease is high
1952 Copper deficiency in animals shown to induce disorders of skin, hair and pigmentation

1952-1956 Intensive studies of copper metabolism and kinetics in normals and in patients with Wilson's disease
1956 Link between excess molybdenum and copper deficiency studied
1950-1960 Many studies on the role of copper in haemopoiesis and bone formation
1950-1960 Several copper-metallo-enzymes identified and purified
1962 Investigation into the link between copper deficiency, lathyrism and cardiovascular disorders
1964 Tentative report of copper deficiency in humans

MANGANESE

1923 Manganese shown to occur in plants and animals
1924 Manganese shown to be connected with chlorophyll
1928 Manganese suspected of being an essential biological metal
1931-1936 Some manganese deficiency effects identified in birds and mammals
1937 Manganese metallo-enzymes arginase and pyruvate carboxylase identified
1940-1944 Lipotropic action of manganese identified in mammals
1944 Skeletal defects in manganese deficiency described
1956 Link between manganese and hydralazine induced lupus erythematosus identified
1957 Manganese shown to be involved in cholesterol synthesis
1967 Low dopamine levels in substantial nigra found in manganese-induced Parkinsonism
1968 Effect of manganese on gene expression discovered

MOLYBDENUM

1932 Molybdenum found to occur in all animal tissue
1938 Effects of molybdenum excess in cattle discovered
1942 Essential role for molybdenum postulated in animal metabolism
1940-1948 Antagonistic effect of molybdenum and copper noticed and investigated
1953 Xanthine oxidase found to be a molybdenum metallo-enzyme
1956 Effects of molybdenum deficiency in animals studied
1956 Anti-cariogenic effect of molybdenum noticed
1962 Link between molybdenum deficiency and oesophageal cancer in South Africa discovered

CHROMIUM

1911 Chromium found to be taken up from the soil by plants
1948 All plants and animals found to contain chromium
1949 Enzyme phosphoglucomutase found to contain chromium
1950 Affinity of erythrocytes for chromates rapidly led to the use of radioactive chromates as a label for red cells

1954 Chromium shown to decrease cholesterol synthesis in male rats
1959 Chromium found to be a component of RNA
1959 Chromium identified as the 'glucose tolerance factor'
1964 Effect of chromium in human diabetes demonstrated

COBALT

1841 Cobalt regularly found in plants
1926 Liver therapy shown to be of value in pernicious anaemia
1928-1948 Painstaking attempts to isolate the effective factor
1929 Cobalt first shown to cause polycythaemia in animals
1935 Cobalt deficiency identified as the cause of certain long recognized deficiency syndromes in cattle. Found to be effective when given by mouth but not by injection
1948 Vitamin B_{12} crystallized and cobalt identified as an essential component
1965 Cobalt in beer shown to cause cardiomyopathy

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CHAPTER II

Zinc

Introduction

Zinc was first shown to be essential for the growth of living organisms in 1869. In 1934 zinc was shown to be necessary for the growth of normal development of mammals. In 1940 carbonic anhydrase was shown to be a zinc metallo-enzyme and since then numerous zinc metallo-enzymes and zinc metal ion activated enzymes have been found. Zinc is also involved in the synthesis of ribonucleic acid. There are complicated interactions between zinc, copper and iron. Zinc is known to affect the incorporation and release of iron from ferritin.

Certain tissues and diseases have been studied more extensively with regard to the biochemistry of zinc than others. These tissues either contain more zinc than the average of the remainder of the body or else zinc metabolism is known to be abnormal in some diseases of these tissues. The best known of these disorders is post-alcoholic cirrhosis in which the level of serum zinc is usually reduced and the urine contains more zinc than normal (hyperzincuria). Lately, zinc has been shown to be beneficial in accelerating the healing of wounds. This finding was prompted by the knowledge that in animals hyperkeratosis of the skin is a specific feature of zinc deficiency suggesting that zinc is essential for the normal growth of skin. The known effect of zinc salts in prolonging the duration of action of insulin and corticotrophin has prompted extensive investigation into zinc metabolism in diabetes. Hyperzincuria occurs in diabetics even if they are not on insulin. The high tissue concentration of zinc in portions of the male genital tract, the fact that alkaline phosphatase is a zinc metallo-enzyme and the fact that gonadal atrophy is a feature of zinc deficiency in animals have led to much information concerning zinc and the male genital tract. The highest concentration of zinc in the body occurs in a layer of the choroid in the eye; this layer contains a zinc metallo-enzyme, retinene reductase. There are some interesting observations about the relationship between zinc and the function of the retina. The high zinc content of erythrocytes and leucocytes and the high concentration of carbonic anhydrase (another

zinc metallo-enzyme) in the red cells have caused disease of the blood to be investigated for disturbance of zinc metabolism. Autoradiographic studies have indicated that zinc is concentrated in areas of bone undergoing active osteogenesis.

There are definite but unexplained disturbances of zinc metabolism with abnormal handling of zinc loads in some patients with bronchial carcinoma.

Absorption and availability

The average zinc content of Western diets is 15-20 mg per day. In animals the proportion of ingested zinc which is absorbed varies markedly according to the species studied and the constituents of the diet. Most of the studies of absorption have been made using radioactive $zinc^{65}$ which has a half-life of 270 days and is mainly a β particle emitter making it particularly useful for autoradiographic studies of the main sites of zinc localization.

The proportion of ingested dietary zinc which is absorbed has been shown to be as low as 3-10% in cattle (Feaster *et al.*, 1954) and rats (Feaster *et al.*, 1955). Early work on zinc absorption in man showed absorption to be approximately 20% of intake (McCance and Widdowson, 1942). More recent work using rats suggested that the proportion of dietary zinc absorbed may be higher, probably in the region of 40% (Hoeskstra, 1964). A higher figure for absorption has been shown in man (Spencer *et al.*, 1966). These workers have shown that absorption of zinc appears to be increased in the presence of the known and specific human zinc deficiency syndrome described in Egypt and Iran when previous zinc intake has been below average (Prasad, 1966).

Following oral administration the uptake of zinc by the intestinal mucosa is rapid and occurs at a rate comparable to that of iron. Incorporation of zinc-65 into intestinal mucosa occurs maximally in the distal ileum (Pearson *et al.*, 1966). *In vitro* studies with Zn-65 using everted segments of rat small bowel have shown that the passage of zinc from intestinal mucosal wall to the interstitial fluid is much slower than is the mucosal uptake from the lumen (Pearson *et al.*, 1966). Maximal concentration of radioactive zinc in the distal ileum has suggested that this is the site of maximum absorption although following oral ingestion of radioactive zinc by humans some radioactivity can be detected in the plasma after 15 min suggesting that at least part of the absorption must occur from the stomach and duodenum (Spencer *et al.*, 1966). Peak levels occur in the blood about 4 h after ingestion.

In vitro zinc uptake is partially inhibited by sodium cyanide and nitrogen suggesting that transfer is an active enzymatic process although dinitrophenol does not depress absorption suggesting the zinc uptake is

not dependent on oxidative phosphorylation (Pearson *et al.*, 1966).

The site of maximal absorption of zinc has not yet been finally settled; there is some evidence which conflicts with the site of maximal absorption being the distal ileum. Some workers have found that like iron the site of maximum absorption and maximum localization in the gastro-intestinal tract is the duodenum (Van Campen and Mitchell, 1965; Sahagian *et al.*, 1966). If the duodenum is the true site of maximum absorption it helps to explain the known link between zinc-induced depression of iron absorption and iron utilization and the known increase in zinc turnover and increased zinc concentration in erythrocytes in iron deficiency.

Factors influencing absorption of zinc

Influence of dietary protein

Although the zinc requirements of laboratory animals are low, zinc deficiency and the resulting specific and easily recognizable deficiency syndromes occur commonly in laboratory animals fed with proteins of plant origin (soy protein). However, zinc deficiency does not develop when similar quantities of protein from animal sources are given. Plant proteins contain higher concentrations of phytic acid which forms insoluble complexes with zinc and casein. These complexes are resistant to digestion by proteolytic enzymes (O'Dell and Savage, 1960). The importance of phytate in reducing the availability of zinc has been confirmed by other workers (Oberleas *et al.*, 1966).

Type of zinc salt

The evidence from experiments measuring weight gain in zinc-depleted chicks suggests that zinc from commonly available zinc salts is readily available for absorption but that zinc from mixtures of zinc, manganese and iron salts is not readily available (Edwards, 1959).

Effects of copper on zinc availability

It has been recognized for many years that there is partial biological antagonism between zinc and copper. Additional dietary copper will improve some of the features of zinc toxicity in rats (Smith and Larson, 1946). Rats given excess dietary zinc have decreased activity of hepatic cytochrome oxidase (a copper metallo-enzyme). The activity of the cytochrome oxidase can be increased when additional excess copper is given in the diet (Van Reen, 1953). Conversely copper toxicity symptoms can be improved by giving zinc supplements (Ritchie, 1963). If a deficiency of one of the metals is present the deficiency effects can

be exaggerated by feeding only a small excess of the antagonistic metal (Van Campen, 1970). It is suggested that the antagonism occurs at the site of absorption (Van Campen, 1970). However, some workers have failed to demonstrate that an excess of copper ion in the intestinal lumen has any effect on zinc absorption (Pearson *et al.*, 1966). Antagonism between copper and zinc at the level of the intestinal mucosa would not explain the antagonism that occurs in the body once absorption has been achieved.

In zinc-deficient pigs with typical porcine parakeratosis the addition of copper to the diet partially reverses some of the effects of zinc deficiency. For complete reversal of the syndrome zinc must be added to the zinc-deficient diet (Ritchie, 1963). Excess zinc in the diet reduces the copper and iron content of the liver suggesting that some of the enzymes of the liver are common to zinc, copper and iron (Cox and Harris, 1960).

Effect of calcium on zinc availability

A diet rich in calcium aggravates and exaggerates the effects of zinc deficiency. In zinc deficiency porcine parakeratosis a diet rich in calcium produces marked changes even when zinc deficiency is not particularly severe (Luecke *et al.*, 1957; Forbes, 1960; Hoekstra, 1964). There is evidence to support an interaction between calcium and zinc at an intestinal level (Luecke, 1966). Experiments involving balance studies with rats have failed to show that high dietary calcium reduces zinc absorption; this suggests that the undoubted interaction that does occur between zinc and calcium must occur at a cellular level (Forbes and Yohe, 1960). Further support for interaction between calcium and zinc at both an intestinal and cellular level is given by the beneficial action of the chelating agent EDTA in improving zinc absorption from the bowel. Zinc absorption is probably increased because of the formation of soluble EDTA-zinc complexes in preference to the insoluble calcium-phytate-zinc complexes which are present in the absence of EDTA (Oberleas *et al.*, 1966). For the effect of zinc on bone and teeth formation see p. 35.

Effect of zinc on iron absorption

Iron-deficient animals absorb more iron, cobalt, zinc and manganese than control animals. The absorption of copper is the same in iron-deficient and non-iron-deficient animals. In addition the iron-deficient reticulocytes also take up more of the three trace metals than normal reticulocytes. There seems to be a common pathway for absorption and incorporation into erythrocytes for iron, cobalt, zinc and

manganese; the avidity of this common transport mechanism for the four metals is, in order: iron, cobalt, zinc and manganese. Nevertheless the system can be saturated with any of the metals and so preventing the absorption of iron (Forth, 1970).

Zinc toxicity in the rat produces iron deficiency anaemia (Smith and Larson, 1946). In insects zinc toxicity causes disturbances of iron and copper metabolism (Sivarna *et al.*, 1958). In the rat excess dietary zinc results in accumulation of zinc in the liver and marked loss of liver iron. Reduction of iron content is probably responsible for the resulting anaemia and depression of iron-containing enzymes (Cox and Harris, 1960).

In the human zinc deficiency syndrome the plasma zinc and the zinc metallo-enzyme, alkaline phosphatase, are generally low. There is also an increased turnover of injected radioactive zinc (Prasad, 1966; Sandstead *et al.*, 1967). Other workers have suggested that these findings might be due to coincidental iron deficiency (Coble *et al.*, 1966). Experiments with rats have confirmed that zinc turnover is increased in iron-deficient states and that the zinc level of iron-deficient erythrocytes is higher than normal (Spry and Piper, 1969). They also found that intravenous injection of iron did not affect the retention of Zn-65 and they concluded from this that absorption of zinc must involve at least one different step from the absorption of iron because injection of intravenous zinc will decrease the retention of Zn-65.

Zinc turnover is increased in iron deficiency; part of the reason is the rapid loss of duodenal mucosal cells (and consequent increased loss of zinc) into the gastro-intestinal tract which is known to occur in iron deficiency anaemia (Charlton *et al.*, 1965).

Other factors influencing zinc absorption: pH, vitamin D, pregnancy, androgens and weight loss

Lowering the pH of the intestines and the changes occuring during the final period of pregnancy both result in increased zinc absorption (Feaster *et al.*, 1955). Low vitamin D intake depresses zinc absorption (Worker and Migicovsky, 1961). Androgens markedly increase the uptake of radioactive zinc by the prostrate and presumably will increase intestinal absorption (Gunn and Gould, 1956). Zinc is known to protect against one of the toxic effects of cadmium which is rapid destruction of the seminiferous tubules of the testes. This is a known toxic effect of cadmium in humans and zinc therapy may have a part to play in protection against this unfortunate effect of cadmium toxicity. The interaction between zinc and cadmium probably occurs as a result of competition for binding sites on the serum albumin (Gunn *et al.*, 1961).

Zinc deficiency itself soon leads to appetite suppression in rats (Mills and Chester, 1970) and prolonged starvation leads to an abnormal

zincuria (Spencer and Samachson, 1970). In the zinc-deficient humans of Egypt and Iran an increase in absorption of zinc was found when zinc supplements were added to the diet (Prasad, 1966).

Zinc excretion

Zinc loss occurs from the body mainly by way of the faeces. The faecal zinc is made up of zinc from four sources viz. unabsorbed oral intake, zinc-rich intestinal juices, loss by shedding of zinc-containing intestinal mucosal cells and possibly active transfer back into the lumen of absorbed zinc. Some zinc is lost by desquamation of skin. The urine normally contains far less zinc than the stools although this is increased in certain pathological states. The main intestinal juice which contributes to zinc excretion is pancreatic juice, probably mainly due to a zinc metallo-enzyme carboxypeptidase (Miller *et al.*, 1964). The zinc content of the bile is very low.

The urine zinc is fairly constant and does not vary much with alterations of zinc intake unless this is increased enormously (McCance and Widdowson, 1942; Davies, 1971). The normal urine zinc output per 24 h is around 0.5 mg (500 μg). Normal values in one series are 342 ± 23 for males and 308 ± 26 for females (Pidduck *et al.*, 1970). The urine zinc excretion per day does not appear to vary with urine volume (Vallee *et al.*, 1957).

Following injection of radioactive zinc only about 1% of the total dose is excreted via the kidneys. One reason why so little is excreted in the urine is because of the binding of zinc by the plasma proteins (Spencer *et al.*, 1966). A markedly increased excretion of zinc in the urine occurs in proteinuria, alcoholic cirrhosis, porphyria and starvation. A small but significant zincuria also occurs in diabetics whether or not they are on insulin (Pidduck *et al.*, 1970) and in hypertensives (Schroeder, 1957). A well-documented but unexplained paradox of zinc metabolism is the fall in the high urine zinc value which occurs when zinc supplements are added to the diet, in portal cirrhosis (Vallee *et al.*, 1957) and diabetes (Davies, 1971).

Zinc excretion in the milk, espeically the colostrum, is high and unlike the urine zinc output it can be increased when zinc is added to the diet (Berfenstam, 1952).

Distribution of zinc in the body

Zinc occurs in all living cells (Underwood, 1962). It is essential for the function of many enzymes common to animals and man. In some enzymes zinc is an essential part of the molecule (metallo-enzyme); with other enzymes zinc is necessary for their proper functioning and acts as an activator for the enzyme (metal ion-activated enzyme).

The amount of zinc in the human body is in the range 1.36-2.32 g; the

amount of iron is in the range 4.2-6.1 g and the amount of copper 81-230 mg (Widdowson *et al.*, 1951). Thus, on a normal zinc intake the average person contains approximately half the amount of zinc as iron but 10 times the amount of zinc as copper.

The amount of zinc in the body of animals can be increased by feeding *high* quantities of zinc in the diet (Hoekstra *et al.*, 1956). This also applies to many of the metabolically inert elements. Where these elements are abundant plants, animals and humans existing from the products of these soils contain a higher concentration of these elements than normally. The presence of increased amounts of zinc in the body does not necessarily imply that the metabolically active zinc pool is bigger than normal. Zinc can be artificially removed from the body by chelating agents and dialysis, however about one-sixth of the total body zinc always remains, suggesting that this proportion is ireversibly bound to proteins within the tissues.

Within the serum proteins about a third of the zinc is loosely bound to imidazole group of albumin (Gurd and Wilcox, 1956). The remaining two-thirds is more firmly found to the globulins. A small amount of the plasma zinc is due to the presence of alkaline phosphatase and other zinc metallo-enzymes. Zinc deficiency leads to a profound fall in the major plasma proteins of some experimental animals (Fox and Harrison, 1966).

The zinc content of the main viscera in micromoles of zinc per gram of tissue ash is:

Kidney	49-120
Liver	34-92
Heart	31-59
Aorta	17-43
Spleen	15-29
Lung	13-27
Brain	8-17

(Perry *et al.*, 1962).

Measuring the zinc content of organs in this way does not, of course, necessarily indicate the metabolic importance of zinc for distinct portions of the organ or even for the organ as a whole. Zinc may only be a passive passenger.

The location of zinc at a subcellular level has been studied by the technique of centrifugal fractionation and is:

Clear supernatant	1.7 mg/g of nitrogen
Microsomes	1.4
Nuclei	1.1
Connective tissue	0.8
Whole liver	0.78
Mitochondria	0.35

(from Thiers and Vallee, 1957)

The zinc content of wet tissues of man (in parts per million) is:

Prostate	102
Kidney	55
Liver	55
Muscle	54
Heart	33
Pancreas	29
Ovary	22
Testis	17

(Underwood, 1962 quoting private communication of Tipton and Cooke).

Some tissues have been investigated with regard to their zinc metabolism because they contain a high concentration of zinc, other tissues and structures have been investigated not because they contain a high concentration of zinc but because uptake or turnover of radioactive zinc is particularly rapid. Tissues which have been investigated because of their high concentration of zinc are:

1. Prostate
2. Skin and its appendages
3. Choroid of the eye
4. Liver
5. Pancreas
6. Bone and teeth
7. Blood

The prostate provides a good example of a tissue whose uptake of zinc is rapid, whose concentration of zinc is high but in which the turnover rate of zinc is slow (Wakely *et al.*, 1960).

Intermediary metabolism

Zinc in the bones is relatively firmly bound, that in the hair is unavailable to exchange with the zinc pool; apart from zinc in these two situations the zinc in the body is freely exchangeable. The large pool of metabolically active body zinc is constantly changing. The zinc pool is attached to intracellular and plasma proteins. The part of the zinc pool with the slowest turnover of zinc is that within the red blood cells and muscles (Gilbert and Taylor, 1956). The organs in which the zinc turnover is most rapid vary according to the species. Injected radioactive zinc combines with the plasma proteins and is then distributed to the body zinc pool or is excreted mainly by way of the faeces. From the plasma proteins some of the radioactive zinc passes to the intracellular proteins within the red cells (and to the other blood cells) and to the large soft tissue zinc pool. The exact mode of transport from plasma

proteins to intracellular tissue proteins is not known. The rate of uptake of radioactive zinc from the plasma proteins (mainly the plasma albumin, to which zinc is more loosely bound than to the globulins) to the intracellular proteins of the erythrocytes is at first rapid (over a period of 2 h) and then more slowly reaching a maximum at about 4 h (Sastry *et al.*, 1960). The uptake of zinc from the plasma proteins to the soft tissue zinc pool is complete in about 66 h (Gilbert and Taylor, 1956).

The distribution and behaviour of labelled zinc in man

Intravenous injection of labelled zinc

From an initial high level immediately after the injection the level of radioactive zinc in the blood falls sharply so that after 30 min 90% of the dose has been removed from the blood (Spencer *et al.*, 1966). Another figure which has been obtained is 90% removal at 3 h (Dennes *et al.*, 1962). The remaining 5-10% has a half-life within the blood of approximately 75 days. From the plasma some of the labelled zinc passes to the carbonic anhydrase of the erythrocytes. After 24 h the amount of zinc in the erythrocytes is about four times that in the plasma. Approximately 10 days after the injection the quantity of labelled zinc in whole blood begins to fall. The half-life of labelled zinc within the blood after the initial fall to 5-10% of the administered dose is about 75 days; most of the labelled zinc during this time being within the erythrocytes. Although rapidly removed fro the blood after injection about 80% of the administered dose remains within the body zinc pool one month after the injection. Similar time sequences are seen when zinc-labelled plasma proteins are given showing that the plasma protein zinc is in equilibrium with the body zinc pool (Dennes *et al.*, 1962). Prior treatment with intravenous iron salts to saturate all the plasma protein iron binding sites does not interfere with the sequence of disappearance described. This suggests that zinc and iron do not share common binding sites on the plasma proteins (Dennes *et al.*, 1962).

Oral administration of labelled zinc in man

Following oral administration of labelled zinc peak levels in the plasma are reached usually after about 4 h; the plasma level declines afterwards at a rate comparable with that following intravenous administration. Some radioactivity can be detected in the plasma with 15 min of giving oral labelled zinc suggesting that at least some absorption occurs high in the gastro-intestinal tract.

With regard to uptake and decay of radioactivity in various organs after parenteral administration of labelled zinc four types of activity have been demonstrated in the guinea pig (Garcia-Amo *et al.*, 1970).

Type I carcass (skeleton and muscle)

Slow uptake with slow decay. Decay is divided into two phases; the first phase gives a half-life of 20 days and the second a half-life of 178 days.

Type II liver and viscera

Uptake is rapid. Decay is also rapid initially giving a half-life of 30 days. Later the half-life is longer.

Type III central nervous system

Uptake of radioactivity increasing over 20 days and then declines, first at a rapid rate corresponding to a half-life of 19 days, then more slowly corresponding to a half-life of 128 days.

Type IV hair

The hair slowly accumulates radioactive zinc over long periods.

Metabolism

Zinc was first shown to be essential to the growth of living organisms in 1869 by Raulin. He showed that the fungus *Aspergillus niger* failed to thrive unless a small quantity of zinc was present in the nutrient medium. In 1934 zinc was first demonstrated to be essential for the growth and normal development of animals (Bertrand and Bhattacherjee, 1935; Todd *et al.*, 1934; Stirn *et al.*, 1935). In 1940 Keilin and Mann purified and analysed the enzyme carbonic anhydrase and demonstrated that it contains 0.33% zinc. Since then zinc has been shown to be essential for numerous enzymes (reviewed by Li, 1966).

1. Alcohol dehydrogenase
2. Lactate dehydrogenase
3. Malate dehydrogenase
4. Alkaline phosphatase
5. Carbonic anhydrase
6. Metallothionine
7. Glyceraldehyde phosphate dehydrogenase
8. Glutamic dehydrogenase
9. Carboxypeptidase A and B

The molecules of all these enzymes contain zinc as an essential and integral part of the molecule. Another and much larger group of enzymes

also require zinc (and other trace metals) for them to function properly; with these enzymes zinc is not an integral part of the molecule but their biological activity is increased with the addition of optimal concentrations of zinc: These metal ion activated enzymes include (reviewed by Vallee, 1962):

1. Glycylglycine dipeptidase
2. Arginase
3. Dehydropeptidase
4. Alanyl- and leucylglycine dipeptidase
5. Tripeptidase
6. Glycyl L leucine dipeptidase
7. Carnosinase
8. Amino peptidase
9. Histidine deaminase
10. Lecithinase
11. Enolase
12. Oxalo-acetic decarboxylase
13. Di hydro crotase
14. L mannosidase

Zinc is involved in the synthesis of ribonucleic acid (RNA). Zinc deficiency can result in failure of nucleic acid synthesis (Schneider and Price, 1962). It is therefore a component of most cellular proteins.

Knowledge of the necessity for and biochemical function of zinc at a subcellular (enzymatic) level is in advance of knowledge of its function at a physiological level. The study of the zinc content of individual organs and parts of organs and of endocrine and exocrine secretions is proceeding along the more classical lines which have proved useful but painstaking in the study of other essential nutrients such as the vitamins. There is a tremendous knowledge gap between isolated facts about the necessity of zinc for certain key enzymes and a large number of apparently isolated observations obtained from zinc feeding, excretion and deficiency experiments in man, animals and plants. The situation is more complicated with the vitamins in which a more or less unique and specific deficiency syndrome occurs with lack of the particular vitamin. With the trace metals relative deficiencies occur more commonly than isolated pure ones. For example, one trace metal may reduce the biological potency of another. An excess of molybdenum leads to features of copper deficiency even though the intake of copper is normal or at least is at a level which does not result in syndromes of copper deficiency. This complicated interrelationship and mutual antagonism between an excess of one trace metal and a normal amount of another makes the interpretation of all naturally occurring and most artificially induced mineral deficiency syndromes difficult and controversial to

interpret. When three trace minerals are interrelated (which is sometimes the case) controversial discussion can easily pass to didactic argument.

A deficiency of copper in the liver reduces the concentration of iron in the liver and reduced storage of iron (Cox and Harris, 1960). Large amounts of zinc induce an iron deficiency anaemia suggesting a link between zinc and iron (Smith and Larson, 1946). However, the link is really: excess zinc causes liver copper reduction which causes reduced liver iron concentration (Van Reen, 1953; Duncan *et al.*, 1953). Nevertheless, recently it has been shown that zinc directly affects the incorporation and release of iron from liver ferritin (Settlemire and Matrone, 1967a) and that zinc has a direct effect on the erythrocytes causing them to increase in fragility (Settlemire and Matrone, 1967b).

Zinc and the liver

In 1956 Vallee and his colleagues published the most important of their observations concerning zinc metabolism in alcoholic cirrhosis. It was known that the liver contained alcohol and glutamic dehydrogenase, both of which are zinc metallo-enzymes. Vallee and his colleagues found that the concentration of zinc in the serum of 28 patients with cirrhosis was always low. The fall in serum zinc was proportional to the severity of the liver dysfunction. The group of 28 patients had an average serum zinc concentration of 66 ± 9 μg/100 ml as compared with 120 ± 19 μg/100 ml for a control group. Later work showed that many patients with post-alcoholic cirrhosis have hyperzincuria which *falls* to normal levels when zinc sulphate is administered in pharmacological doses by mouth (Vallee *et al.*, 1957). The explanation for this curious paradox is not known. In *alcoholic* cirrhosis the fall in serum zinc seems to correlate well with bromsulphthalein excretion; however, this correlation does not apply when liver damage is not due to *alcoholic* cirrhosis.

The low serum zinc in cirrhotics has been confirmed and the additional interesting observation made that not only is the serum zinc lowered but the intracellular zinc level of leucocyte of patients with cirrhosis is also below normal (Fredricks, 1960).

In animals made to consume large amounts of alcohol the hepatic zinc concentration and hepatic alcohol dehydrogenase are reduced (Sullivan, 1962; Figueroa and Klotz, 1962). The high urinary zinc excretion in human alcoholics has also been confirmed (Sullivan and Lankford, 1965). In rats made cirrhotic by alcohol feeding, the hepatic zinc content is reduced but in rats made cirrhotic by a choline-deficient diet the liver zinc is normal but the manganese level is reduced. This different effect on trace metals may explain some of the difference between the two types of cirrhosis produced experimentally by alcohol and by choline deficiency (Barak *et al.*, 1967).

An interesting but unexplained relationship exists between zinc and porphyrins. In acute intermittent porphyria and the porphyrias due to lead poisoning the abnormal porphyrins are excreted in the urine and/or faeces as zinc-porphyrin complexes.

In the congenital type of porphyria the porphyrins are excreted without being conjugated with zinc (Watson and Larson, 1947). Several steps in the synthesis of porphyrins are controlled by zinc metallo-enzymes.

Zinc in diabetes mellitus

Diabetics as a group have hyperzincuria as compared with a control group (Pidduck *et al.*, 1970). The average serum level in diabetics is within the normal range. The hyperzincuria is out of proportion to any zinc which may be administered with insulin. Many diabetics who have hyperzincuria are being controlled by diet or oral hypoglycaemic drugs.

The well-known action of zinc on the physical and chemical properties of insulin has led to extensive investigation of the pancreas of normal controls and diabetic patients to try to establish a connection between zinc metabolism and diabetes. At the pH within the pancreas (around 6) insulin can only be crystallized in the presence of zinc, cadmium, cobalt and nickel ions (Hallas-Møller *et al.*, 1952). The interrelationship between zinc and insulin is complicated. Insulin extracted from the pancreas of animals contains impurities and has a variable duration of action. More pure insulin can be obtained by adding small quantities of zinc to crude extracts of insulin–this enables crystalline insulin to be prepared; this has a longer duration of action than amorphous (impure) insulin. Although zinc is not chemically incorporated into the insulin molecule crystalline insulin is coated with zinc–the more zinc which can be made to adhere to the insulin molecule the longer the duration of action of the insulin. The amount of zinc adhering to the insulin molecule depends on the pH, the type of buffer used and on the concentration of zinc in the insulin solution. The use of an acetate buffer at the optimum pH enables the maximum amount of zinc to coat the insulin molecule thus giving maximum prolongation of insulin action. Protamine and globin when added to insulin solution alter the physical properties of the zinc-insulin complex in such a way that suspensions of globin or protamine with zinc-insulin complex are produced which further prolong the duration of action of insulin.

There is evidence that zinc is used in the β cells of the pancreas to store and release insulin as required. Degranulation of β cells and release of insulin is accompanied by loss of zinc from the β cells and regranulation is accompanied by an increase in zinc content of the β cells

as demonstrated histochemically (Logothetopoulos *et al.*, 1961). Certain compounds produced by the β cells in response to hyperglycaemia form stronger complexes with zinc than insulin does. These compounds (histidine, cysteine, citric acid, oxalo-acetic acid and organic phosphorus compounds) *in vitro* combine with zinc and may *in vivo* remove zinc from the insulin thus allowing insulin to be released from the β cells (Maske, 1957). In dogs histidine and glycine (but not leucine as in humans) cause release of insulin into the blood-stream. It has been suggested that the amino acids act in this way by chelating the zinc in the pancreas, removing it from insulin and hence allowing release of insulin from the β cells.

With regard to the peripheral action of zinc-insulin some recent work has suggested that *in vitro* zinc stimulates glucose uptake by adipose tissue independent of a similar action by insulin; in addition, zinc deficiency reduces the uptake of glucose by adipose tissue (Quarterman, 1968). Other workers have suggested that the zinc which insulin contains has a specific action which has hitherto been attributed to insulin. In animals zinc-insulin produces swelling of the mitochondria of liver cells; this action is much greater with insulin containing zinc than with amorphous insulin (which contains no zinc) (Cash, 1968).

Many workers have demonstrated differences of zinc content and handling between diabetics and control pancreatic tissue. In 1938 Scott and Fisher demonstrated that the pancreatic tissue of diabetics contained about half the concentration of zinc and less than a third of the insulin activity as control pancreatic tissue. Unfortunately subsequent investigators have found that the difference between the diabetic and control pancreatic tissue is more apparent than real. When the *fat free* glands are used, the zinc concentration of the diabetic pancreatic tissue is less than that of controls but the difference is not statistically significant (Eisenbrand, 1941).

The zinc content of isolated islet tissue has been measured in certain teleost fish in which the islets of Langerhans are anatomically distinct from the exocrine pancreas. The zinc content of (normal) isolated fish islet tissue is very high and variations in the zinc content of islet tissue correlate well with the insulin assays (Weitzel *et al.*, 1953b).

Further attempts to localize more accurately the site of maximum zinc concentration in the pancreatic islets have followed four main lines:

1. Animal studies using animals known to have anatomically distinct groups of α and β islet cells.
2. Histochemical localization of zinc within the islets.
3. Autoradiography of pancreatic tissue, i.e. administering radioactive zinc and locating the sites of maximum uptake.
4. Experimental production of diabetes.

Animal studies

The β cells of birds are contained in distinct, identifiable masses. In ducks the zinc content of such islet cell masses is approximately 10 times that of the remainder of the pancreas. The duck pancreas contains approximately 10 times the amount of glucagen as mammalian pancreatic tissue suggesting a close relationship between zinc, α cells and glucagen (Weitzel, 1956).

Autoradiography

Localization of radioactive zinc in islet tissue has been demonstrated in rats. Because β cells predominate in the rat pancreas it was suggested that zinc might be concentrated in the β cells of other species (McIsaac, 1955).

Histochemical localization

Using dithiazone as a histochemical stain for zinc one group of workers have been unable to demonstrate any difference in localization of zinc in α and β cells (Runge *et al.*, 1956).

Experimental production of diabetes

Dithiazone is a chelating agent with a special affinity for zinc; when injected into experimental animals it induces diabetes probably by inactivating insulin by its action of chelating the zinc adhering to the insulin. Because of its affinity for zinc dithiazone is localized in the islet tissue of the pancreas. Alloxan induced diabetes is believed by some workers to arise by the same mechanism (Okamoto, 1949 quoted by Lowry *et al.*, 1954). Following Alloxan induced diabetes there is considerably less uptake of radiozinc by diabetic pancreatic tissue than by that of normal animals (Lowry *et al.*, 1954).

Injection of radioactive zinc which localizes in the islet tissue does not induce diabetes although it is doubtful if the theoretical amount of radioactivity of such localized zinc would be enough to destroy the β cells (Sherrill and Wick, 1953).

The zinc content of the serum of human diabetics falls within the normal range (Davies *et al.*, 1968a; Pidduck *et al.*, 1970). Zinc deficiency in rats does not result in any alteration of the blood sugar. Human patients with zinc deficiency probably do not have abnormal pancreatic function although a few zinc deficient patients have reactive hypoglycaemia thought to be due to delayed glucose absorption plus hypopituitarism which occurs in zinc deficiency (Sandstead *et al.*, 1966).

Zinc and the skin

The zinc content of the epidermis and skin appendages is high. In view of this and the known development of porcine parakeratosis in pigs fed on zinc-free diets it seemed reasonable to look at analogous problems in humans. While studying the effects of purified β-phenyllactic acid on wound healing in rats Strain and others in 1953 noted that the zinc-contaminated commercial product accelerated the healing of wounds whereas the pure compound did not. In a further group of animal experiments using rats and a variety of zinc preparations including zinc oxide and methionine zinc this group of workers have shown that wound healing is accelerated with zinc compounds fed orally but more especially with zinc-methionine—suggesting that an amino acid carrier may be necessary for maximum utilization of the zinc (Strain *et al.*, 1960). To demonstrate that zinc is actually involved in wound healing these workers injected radioactive zinc and measured the uptake of zinc in healing wounds as compared with the uptake in adjacent skin. Zinc was concentrated in healing wounds for approximately three weeks, thereafter the zinc concentration of the wounds fell, so that after 100 days no radioactive zinc could be found in the healed wounds although it was present in adjacent tissues (Savlov *et al.*, 1962).

From human studies Pories and others have produced convincing evidence of the beneficial effects of supplementing with zinc sulphate the diet of young men having wounds arising from excision of recurrent pilonidal sinuses (Pories *et al.*, 1967).

In young bulls zinc-deficient diets cause a delay in wound healing and the wound areas are especially prone to develop the parakeratotic skin lesions characteristic of more severe zinc deficiency (Miller *et al.*, 1965).

Pories and his group have also reported interesting observations on the zinc level in the hair. They have shown that following extensive skin burns the zinc level of emerging hair falls progressively from normal values suggesting that body zinc is being diverted presumably to the healing skin (Pories and Strain, 1966).

This work has stimulated further studies into the value of zinc supplements in accelerating wound and uler healing. The beneficial effect of zinc sulphate on the healing of chronic leg ulcers was demonstrated by Husain (1969).

In 1967 Greaves and Boyde showed that the plasma zinc concentration was significantly lower in psoriasis, other dermatoses and venous leg ulceration than a group of control patients. However, Withers *et al.*. (1968) could not demonstrate a significant difference in plasma zinc in psoriasis or dermatoses although they confirmed that plasma levels are lower in the presence of chronic leg ulceration and that they are low in the presence of pressure sores in the elderly (Abbott *et al.*,

1968). In the United States Myers and Cherry (1970) have been unable to demonstrate an acceleration of healing in patients with chronic leg ulcers when given oral zinc sulphate, nor could they demonstrate a difference in the serum zinc values between these patients and controls.

In a further study Greaves and Silken (1970) found that long term administration of zinc sulphate to a group of patients with venous leg ulceration which had failed to show any signs of healing using conventional treatment resulted in a complete healing of the ulceration in 13 of 18 patients. Although plasma zinc levels were normal before and after treatment there was a significant rise in the plasma zinc with continued zinc sulphate administration. Oral zinc sulphate has been shown to be beneficial in accelerating the healing of chronic leg ulcers due to sickle cell disease (Sarjeant *et al.*, 1970). The tensile strength of wounds of zinc-deficient rats is less than that of wounds in normal rats (Sandstead and Shepard, 1968). Patients with severe burns quickly develop a low serum zinc and zincuria possibly due to release of zinc from the damaged cells (Nielsen and Jemec, 1968).

Zinc and the male genital tract

The high zinc content of the prostate and epididymus of several species including man has been confirmed. The zinc content of testicular tissue is normal. Zinc deficiency in animals gives rise to degeneration and atrophy of the genital tract which is reversible, if not too severe, when the zinc deficiency is corrected.

Hypogonadism is a feature of the syndrome of zinc deficiency syndrome of humans (Miller *et al.*, 1958).

Spermatozoa are extremely rich in zinc and probably contain the highest concentration of zinc of any human tissue with the possible exception of the choroid of the eye (Mawson and Fischer, 1953), but this high concentration is not due to carbonic anhydrase or alkaline phosphatase.

Following injection of radioactive zinc the normal prostate concentrates zinc. The zinc content of normal prostatic tissue based on chemical analysis is more than that of malignant prostatic tissue. The tissue of benign prostatic hypertrophy contains about the same amount of zinc as normal tissue (Prout *et al.*, 1959). Mawson and Fischer (1952) also found a very high concentration in normal and hypertrophied prostatic tissue but less zinc in malignant prostatic tissue (Mawson and Fischer, 1952). However, using radioactive zinc Daniel *et al.* (1956) have demonstrated that human prostatic carcinoma concentrates zinc to a high degree. This observation has led to attempts to use radioactive zinc to localize and identify carcinoma of the prostate (Barber *et al.*, 1969).

Autoradiographs of prostatic tissue suggest that zinc is especially

concentrated in the mucosal cells of the glands (Daniel *et al.*, 1957), however, when estimated chemically the amount of zinc in glandular prostatic tissue does not seem to be more than the surrounding prostate although acid phosphatase (a zinc metallo-enzyme) is concentrated in the glandular tissue (Hoare *et al.*, 1956; Kerr, 1960).

Zinc and the eye

Certain structures within the eye contain high concentrations of zinc. In particular the choroid and retina of fish and carnivores contain very high concentrations (Bowness and Norton, 1952; Bowness *et al.*, 1952; Weitzel *et al.*, 1953a). In herbivorous animals the choroid contains far less zinc. The choroid of carnivores contains a structure which is absent from that of herbivores, this is the tapetum lucidum which it is believed causes the familiar iridescence of the eyes of cats and dogs (Weitzel *et al.*, 1954 and 1955). In dogs the zinc content of the tapetum lucidum may reach 8.5% of the dried weight (Weitzel *et al.*, 1954). Injection of dithiazone which chelates zinc causes sudden blindness of producing retinal detachment–the plane of separation being the tapetum lucidum of the choroid (Weitzel *et al.*, 1955).

The retina

The zinc content of the retina is also higher than other parts of the body although not nearly as high as that of the choroid and parts of the male reproductive system. In the retina zinc is contained in a zinc metallo-enzyme, retinene reductase. The enzyme is apparently identical to alcohol dehydrogenase (Bliss, 1951) and is concerned with the reconstitution of visual purple. Alcohol dehydrogenase has been shown to be depleted in alcoholic cirrhosis (Vallee *et al.*, 1957). A proportion of patients with cirrhosis have night blindness which does not respond to the administration of vitamin A (Patek and Haig, 1939). It is thought that the explanation for this night blindness is failure by the visual purple mechanism due to a deficiency of retinene reductase (Vallee *et al.*, 1957).

Zinc content of the blood

Zinc occurs in plasma, erythrocytes and leucocytes. In the erythrocytes it is probably all in the abundant supply of carbonic anhydrase which erythrocytes contain (Vallee *et al.*, 1949; Hove *et al.*, 1940); leucocytes do not contain carbonic anhydrase. The zinc that occurs in leucocytes is bound to protein which has no enzymatic activity (Vallee *et al.*, 1954). In some species the zinc in leucocytes may be

associated with the phosphatase enzymes (Mager and Lionetti, 1954). There are characteristic changes in the zinc content of blood cells in certain haematological diseases (*vide infra*). In the plasma zinc is bound to both albumin and globulin, more firmly to the latter. There does not appear to be any specific zinc-carrying plasma protein analogous to transferrin for iron although *in vitro* zinc can combine with transferrin (Surgenor *et al.*, 1949). Diseases causing a reduction of the plasma proteins also cause a reduction of the plasma zinc level. When the plasma proteins are lowered because of proteinuria increased amounts of zinc are found in the urine (Vallee *et al.*, 1956). In the blood about 85% of the zinc is contained in the erythrocytes, 12% in the plasma and the remainder in the leucocytes (Vallee, 1959). In normal subjects there is little diurnal, sex, age or seasonal variation although for about an hour after a meal the serum level may be reduced in some patients by about 10% (Davies *et al.*, 1968a). There may be more of a seasonal variation in some disease states (Davies, 1971).

The zinc level of the plasma does not usually fall even when fairly marked zinc deficiency is present; in human zinc-deficient subjects described by Prasad *et al.* (1963) the serum zinc levels were usually in the normal range. Zinc feeding by mouth does increase the plasma level when large amounts are given. In man more than 10 times the normal dietary zinc intake has to be given before there is any significant rise in plasma levels; even with intake of this order urinary excretion of zinc only increases marginally (Davies and Maddocks, 1971). The levels are high at birth compared with adults but fall so that at one year the approximate adult value is reached (Berfenstam, 1952). Using modern techniques of analysis (atomic absorption spectrometry) the fasting normal adult should have a plasma zinc between 75 and 125 gm% (Davies *et al.*, 1968a). The levels given in earlier literature (cited by Vallee, 1962) are generally higher than this probably due to contamination by zinc of glassware.

Blood zinc in disease

Erythrocyte zinc level is elevated in chronic lymphatic leukaemia, multiple myeloma and lymphoma. The plasma zinc is decreased in pernicious anaemia, lymphoma and chronic lymphatic leukaemia (Rosner and Gorfien, 1968). Zinc content of bone marrow and peripheral blood granulocytes was measured by Szmigielski and Litwin (1965) who found that in the bone marrow zinc appears in metamyelocytes, the amount increasing with maturation. In the granulocytes of peripheral blood zinc values are about 30% higher than bone marrow. Zinc is significantly decreased in granulocytes in chronic granulocytic leukaemia, acute myeloblastic leukaemia, multiple myeloma

and Hodgkin's disease, and increased in chronic lymphocytic leukaemia and osteomyelosclerosis. These same authors have demonstrated that the zinc content of granulocytes was always reduced in the presence of neoplastic disease in all 50 cases of carcinoma which they studied; in controls the granulocyte level was always within the normal range. This interesting observation has never been followed up (Szmigielski and Litwin, 1964).

The concentration of zinc in normal leukocytes is about 25 times that in the erythrocytes (Vallee and Gibson, 1948). In a study of cases of myeloid leukaemia and of lymphatic leukaemia Gibson and colleagues (1950) found that the zinc content of the leukocytes was always lower than in normals. The zinc content of the leukocytes tends to rise with response of the white cells to successful treatment. There are considerable technical difficulties in obtaining undamaged leukocytes uncontaminated with erythrocytes and platelets from leukaemic blood. However, using an improved technique Dennes *et al.* (1961) have confirmed the earlier work that the zinc content of leukaemic leukocytes is lower than normal. The zinc content of leukocytes from subjects with lymphatic leukaemia was approximately inversely proportional to the number of leukocytes in the blood. The erythrocytes showed a slight but significant difference in the opposite direction, leukaemic erythrocytes containing more zinc than did normal human erythrocytes. The low zinc content of leukaemic leukocytes cannot be explained on the basis of the leukocytes being more immature than in normal people because in chronic lymphatic leukaemia the majority of white cells are mature leukocytes. The zinc content of erythrocytes is increased in megaloblastic anaemia (Fredricks *et al.*, 1964). These same workers confirmed the low leukocyte zinc and high erythrocyte zinc in patients with leukaemia. Intravenous injection of zinc gluconate does not increase the zinc level of leukaemic leukocytes, neither does zinc gluconate therapy influence the course of leukaemia (Gibson *et al.*, 1950).

In the erythrocytes the majority of zinc is contained in carbonic anhydrase (Vallee *et al.*, 1949). As L.D.H. is a zinc metallo-enzyme it is not difficult to understand why the plasma zinc is elevated in conditions which cause a rise in L.D.H. Zinc seems to form a higher proportion of L.D.H. 2 than other L.D.H. isoenzymes (Vessell and Bearn, 1957).

Zinc and bone

In animals it is known that a high calcium diet depresses zinc absorption (Forbes, 1960). Using radioactive zinc in experiments in humans it has not been shown that absorption, blood levels or excretion of zinc are affected over short periods by a high, normal or low calcium intake during the period of the experiment (Spencer *et al.*, 1966). Only

three patients were used in each group and these results must be regarded as equivocal.

The action of vitamin D on zinc absorption is complex: in pigs vitamin D decreases zinc absorption when zinc intake is low, and does not affect zinc absorption when zinc intake is high (Whiting and Bezean, 1958). In rats vitamin D increased the retention of oral ^{65}zinc when diets rich in zinc were given. This is not found when the diet is depleted of zinc or when ^{65}zinc is given by injection. Vitamin D increased zinc in bone but not in soft tissue (Becker and Hoekstra, 1966). Most of the fundamental work on zinc and bone has been done by Haumont in Leopoldville using Dithiazone, the zinc specific chelating agent, and autoradiographs. Haumont and McLean (1966) conclude that zinc is concentrated in bone especially in the areas of active osteogenesis and that it is more likely to be related to the organic protein of bone rather than calcium. This is in keeping with the fact that zinc is an essential component of many proteins and zinc metallo-enzyme, including carbonic anhydrase and alkaline phosphatase, which are abundantly present in the organic matrix of bone in areas of active osteogenesis. Zinc is actively concentrated in healing fractures (Calhoun and Smith, 1968).

Zinc and pregnancy

Zinc absorption is increased in the later stages of pregnancy possibly due to pH changes in the intestine or possibly related to the elaboration of large amounts of hormones in which zinc metallo-enzymes are involved.

The foetus contains more zinc than it does copper or iron and the foetal liver contains a greater concentration than the maternal liver.

In the dog and rat zinc crosses the placenta readily and does so in increasing amounts towards the end of pregnancy (Feaster *et al.*, 1955). In the rabbit transfer of radioactive zinc from mother to foetus rises sharply in mid pregnancy suggesting either maturation of a foetal zinc receptor organ or a sudden increase in placental permeability to zinc (Terry *et al.*, 1960). The zinc content of erythrocytes of the foetus and neonate is only about a quarter of the normal adult level; this corresponds with the low level of carbonic anhydrase in the foetal erythrocyte (Berfenstam, 1952).

In human plasma zinc concentration is usually reduced in the third trimester (Halsted *et al.*, 1968).

Zinc in neurological disorders

Certain areas of the brain are more susceptible to carbon monoxide poisoning than others. One such area is very rich in zinc. This has been

demonstrated by histochemical methods and autoradiography (McLardy, 1962a, b). A raised urinary zinc occurs in a variety of neurological conditions including diabetic neuropathy, alcoholism, peripheral neuropathy and porphyria (Peters, 1961).

Zinc in malabsorption

Following descriptions of zinc deficiency syndromes in animals and the known beneficial effect of zinc salts in promoting healing of skin ulceration and wounds, several clinicians have been watching for signs suggestive of zinc deficiency in malabsorption syndromes. Zinc salts have been used to supplement other nutrients in cases of malabsorption with beneficial results (MacMahon *et al.*, 1968).

Zinc metabolism following surgery

Significant and marked zincuria occurs following major surgery causing depletion of body zinc stores. Zinc deficiency and its consequences of poor wound healing can occur in severe trauma. It is suggested by some workers that supplementary zinc should be given with other dietary supplements to such patients (Henzel *et al.*, 1967).

Zinc and atheroma

The zinc level of the blood has been reported lower than controls in a group of atherosclerotic subjects (Volkov, 1963). Serum zinc levels are depressed following myocardial infarction in which levels of lactic dehydrogenase (a zinc metallo-enzyme) are raised. The apparent paradox between a lowered serum zinc at a time when *activity* (as opposed to *concentration*) of a rich zinc metallo-enzyme is increased is explained by the fact that zinc metallo-enzymes may be inhibited by a high concentration of the very metal they require for their proper function. A lowering of the serum zinc would thus allow the enzymatic activity of serum L.D.H. to increase. The mechanism of the removal of the zinc is not known (Wacker *et al.*, 1956). The zinc level of the hair of patients with proven atherosclerosis is reduced (Strain and Pories, 1966). The zinc level of some tissue including the aorta in the presence of atherosclerosis is reduced (Volkov, 1963). In hypertension the zinc level of vessels is generally normal in dogs (Fischer *et al.*, 1968).

Zinc and neoplasia

Studies on the connection between zinc and malignancy are numerous; low zinc concentrations have been reported in leukaemic cells

(Gibson *et al.*, 1950; Dennes *et al.*, 1961). Addink and Frank (1959) found that leukocyte zinc was reduced in all 31 patients who had a carcinoma, and Vikbladh (1951) has also reported low serum zinc values in the presence of neoplasia. The serum values are reduced in Hodgkin's disease and other lymphomas (Szmigielski and Litwin, 1965).

Zinc is suggested as an aetiological agent in oesophageal cancer in East Africa where Malawi Gin contains about 5.2 mg zinc per litre (McGlashan, 1967).

Certain areas of the world where the incidence of stomach carcinoma is higher than average (North Wales and Japan) have a high zinc concentration in the soil (Stocks and Davies, 1964). The plasma zinc has been shown to be reduced in bronchogenic carcinoma but normal in other carcinoma (Davies *et al.*, 1968b). These findings have been confirmed (Morgan, 1969). Morgan also found that the level of cadmium was high in the serum of her carcinoma patients. There is a known association between zinc and the carcinogenic effects of cadmium. Zinc has been shown to reduce the teratogenic effects of cadmium (Ferm and Carpenter, 1967). Studies on the tumour levels of zinc have produced conflicting results. Liver metastases have been reported to contain very low concentrations of zinc which the zinc content of the uninvolved hepatic tissue increases (Olsen *et al.*, 1954 and Bergel *et al.*, 1957). The zinc content of experimentally induced skin tumours in mice is lower than normal tissue (Carruthers and Suntzeff, 1945). Although zinc concentrations in tumour tissues may be low zinc *turnover* and uptake by some tumours is increased (Heath and Liquier-Milward, 1950). Experiments using mice afflicted with melanoma indicate a high zinc content but slow zinc turnover of the tumour but a generalized disturbance of zinc homeostasis (Prasad *et al.*, 1969).

Zinc and haemodialysis

Plasma of patients undergoing haemodialysis actively takes up zinc from the dialysis fluid so that zinc deficiency is not likely during haemodialysis. The source of zinc in the dialysis fluid may be the zinc oxide tape around the dialysis coil. Copper is also actively taken into the plasma from the dialysis fluid. Increased concentrations of zinc in the plasma may protect against the toxic effects of excess copper (Blomfield *et al.*, 1969).

Zinc deficiency

In 1922 Bertrand and Benson showed that zinc-deficient diets reduced the life expectancy of rats. Since then zinc deficiency syndromes have been identified and studied when occurring naturally and when induced

in experimental animals. The main features have been described in rats, mice, birds, pigs and man. In new-born mice zinc deficiency results in disturbance of ossification and abnormalities in the skin (Nishimura, 1953). In the rat there is reduced growth, skin and fur abnormalities, abnormal glucose tolerance, hyperkeratosis of the skin and oesophagus, testicular atrophy, disturbances of protein synthesis and reduced carbonic anhydrase activity (Vallee, 1962). In birds the effects of zinc deficiency are in general similar (Underwood, 1962).

Porcine parakeratosis

The best known and most commercially important zinc deficiency syndrome in animals is porcine parakeratosis, a naturally occurring disease of pigs, characterized by dermatitis, diarrhoea, weight loss and death (Kernkamp and Ferrin, 1953). In 1955 a similar disease was artificially induced in pigs by feeding zinc-deficient diets (Tucker and Salmon, 1955). Increased calcium in the diet accelerates the appearance of the condition although radioactive zinc studies have shown that absorption of zinc from the gastro-intestinal tract is normal. The main features of the syndrome are weight loss, and severe hyperkeratosis of the skin and oesophagus.

The pathology of zinc deficiency in experimental animals has recently been reviewed (Follis, 1966). The effects of zinc deficiency in animals have been extensively studied.

Zinc deficiency causes oesophageal parakeratosis and testicular atrophy in the rat. Zinc repletion may cause complete reversal of the oesophageal lesion to normal if given soon enough. No reversal of testicular atrophy occurs with zinc repletion once the histological features are developed (Barney *et al.*, 1968).

Zinc deficiency in animals is also known to give rise to a histologically specific parakeratotic skin reaction. The susceptibility of skin and mucous membrane to the changes of zinc deficiency is variable; for example the buccal mucosa is much more susceptible than the palatal mucosa. The reason for these regional differences is not known (Alvares and Meyer, 1968).

Zinc deficiency during pregnancy in the rat leads to congenital malformations in the foetus (Hurley and Swenerton, 1966). Experiments in which pregnant rats were fed on a normal zinc diet until day 1 of gestation indicate that the mother does not contain enough zinc stores for the normal development of the foetus (Apgar, 1968).

Zinc is necessary to the production of healthy offspring in many species. Zinc-deficient maternal diets in pigs have been reported to cause reduced growth, skin parakeratosis, prematurity, reduced zinc metallo-enzymes in the blood and liver, and reduced plasma proteins in the offspring (Miller *et al.*, 1968).

Zinc deficiency is profoundly teratogenic and leads to multiple system anomalies. The appearances of the foetal oesophagus when the maternal rat has been zinc deficient are similar to those seen in the adult zinc deficiency oesophagus (Diamond and Hurley, 1970).

Zinc deficiency in young birds

Zinc deficiency in young chicks causes shortening and thickening of the bones with specific and characteristic abnormalities of the epiphyseal plate. The effects of zinc deficiency on the epiphyseal plate can be reduced but not abolished by the coincidental administration of histamine and indomethacin.

Zinc deficiency causes a marked reduction in the epiphyseal plate of the zinc metallo-enzyme alkaline phosphatase. At this site alkaline phosphatase is responsible for normal resorption of cartilage. Lack of alkaline phosphatase causes cartilaginous overgrowth (Westmoreland and Hoekstra, 1969).

Metabolic disturbance in zinc deficiency

Zinc deficiency in animals reduces the activity of the following zinc metallo-enzymes at certain sites: alcohol and glutamic dehydrogenase, alkaline phosphatase, catalase, xanthine oxidase, aspartic and alanine amino transferases. The activity of non-zinc enzymes at these sites is not affected by zinc deficiency (Kfoury *et al.*, 1968).

Zinc deficiency in rats causes a deficiency of pancreatic carboxypeptidase-A which can be reversed with zinc repletion.

Zinc deficiency also causes reduced incorporation of labelled methionine into tissue proteins. This effect is due to zinc deficiency *per se* rather than to reduced food intake. Furthermore a block at methionine level is suggested by the fact that when labelled methionine is given a far greater amount of the radioactive C^{14} is produced as expired labelled CO_2 than in normals (Hsu *et al.*, 1969). By the same experimental method it has been found that zinc is necessary for the oxidation of the amino acid lysine (Theuer and Hoekstra, 1966).

There is reduced protein and RNA synthesis in zinc deficiency (O'Neal *et al.*, 1970). Other workers have suggested that in zinc deficiency there is an increase in RNA and protein catabolism rather than decreased synthesis (Macapinlac *et al.*, 1968).

In animals one of the most important effects of zinc deficiency is loss of appetite and failure of growth. The administration of zinc supplement causes a sudden and dramatic increase in appetite (Humphries and Quarterman, 1968). Loss of appetite due to zinc deficiency leads to an

increase in free fatty acids in the plasma. Loss of appetite due to other causes usually causes a fall in plasma free fatty acids (Quarterman, Mills and Humphries, 1966).

Zinc deficiency in animals causes three other interesting changes, one is reduced motility of the intestinal tract, another is an increased bacterial count from the tongue mucosa of sheep and the third is marked ability to choose diets which contain zinc (Quarterman, 1968). Weight loss in animals fed zinc-deficient diets is probably due to a combination of loss of appetite and less efficient utilization of food once it has been absorbed. Zinc deficiency leads to a fall in plasma amino acids and reduced incorporation of radioactive labelled protein into cells (Quarterman, 1968).

Zinc deficiency causes a rise in plasma free fatty acids due to loss of adipose tissue but has no effect on plasma insulin. One curious observation is that zinc-deficient rats are more resistent to hypoglycaemia caused by insulin (Quarterman *et al.*, 1966).

Cadmium is recognized as a metabolic antagonist of zinc. In excess in the diet it causes an aberration in the plasma proteins similar to that produced by zinc deficiency (Jacobs, 1969).

Zinc deficiency in humans (Prasad, 1966)

Zinc deficiency syndromes have been described in humans. In 1961 American workers described a group of patients who they believed suffered from zinc deficiency. The syndrome consists of iron deficiency type anaemia, hepatosplenomegaly, short stature and hypogonadism. Their diet consisted mainly of wheat flour and almost no animal protein: they all gave a history of geophagia. They were at first treated with a hospital diet and commercial grade ferrous sulphate and responded well. There was a decrease in the hepatosplenomegaly and anaemia; their pubic hair started to grow and their genitalia increased in size. The level of serum alkaline phosphatase increased with the clinical improvement induced by the treatment. At this point Prasad and his colleagues considered that the clinical improvement of their patients might not have been due to iron replacement alone. They felt that possibly some of the features could be due to deficiency and inadvertent replacement of some other essential nutrient. Certain features led them to postulate that zinc deficiency may have coexisted with iron deficiency. The main reasons for suggesting this were:

1. The increase in alkaline phosphatase with treatment. This is known to occur when zinc-deficient animals are given zinc supplements.
2. Testicular atrophy is not a feature of iron deficiency.
3. Retardation of skeletal maturation is not normally a feature of iron deficiency.

However, the speculation remained unsubstantiated until 1963 when the same group of workers studied a similar group of patients from Egypt. The clinical features of this new group were similar except that,

1. They had less pronounced hepatosplenomegaly.
2. They nearly all had parasitic infestation.

This second group of patients was studied in remarkable detail with a view to testing the zinc deficiency hypothesis. A *prima facie* case for zinc deficiency occurring in conjunction with the clinical features was made when it was observed that:

1. Zinc concentration of plasma and red cells was reduced.
2. Radioactive zinc studies indicate that absorption and turnover of zinc were increased.
3. Urinary zinc excretion was reduced.

Investigation of the vitamin status of the patients revealed no abnormalities. Previously schistosomiasis and hookworm infestation had always been blamed for producing dwarfism and iron deficiency anaemia; however, Prasad and his colleagues showed that other factors must be involved by studying a similar group of affected patients in an isolated oasis where these parasitic infections did not occur. In the oasis the same syndrome of presumptive zinc deficiency occurred except that iron deficiency and anaemia were not prominent. The results of giving zinc supplements to the affected patients provided additional support for the zinc deficiency hypothesis. Some of the affected patients were given pure iron and others pure zinc in addition to a good nutritional diet. The results showed that zinc-supplemented patients showed increased growth rates and sexual development compared with the others. The exact mode of action of zinc in improving these patients is uncertain. Many suggestions have been made. One is that zinc exerts its action by increasing pituitary gonadotrophin (Sandstead *et al.*, 1966), another is that zinc is involved in the synthesis of RNA, protein and DNA.

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CHAPTER III

Copper

Introduction

Copper was first shown to be essential for animal nutrition in 1928. It was found that copper-deficient diets in animals resulted in anaemia (Hart *et al.*, 1928) and that the addition of copper alone to the deficient diet corrected the deficiency syndrome (Elvehjem, 1935). Since then specific and recognizable copper deficiency syndromes have been described in a large number of different animal species. The main manifestations of copper deficiency are anaemia, brittle bones, disorders of the skin, hair pigmentation and myocardial fibrosis. The best known manifestation of copper deficiency in animals is a disorder of gait in lambs occurring as a result of spinal cord demyelination known as enzootic ataxia or swayback. Copper is an essential requirement for several important copper metallo-enzymes. The best known human disease linked with copper metabolism is Wilson's disease (hepato-lenticular degeneration) in which copper is deposited in excessive quantities in the brain, skin, liver, pancreas and myocardium. There is a congenital absence of one of the copper-carrying proteins, ceruloplasmin. Naturally occurring syndromes due to ingestion of excess copper also occur in animals. These syndromes have disclosed a close link between copper and molybdenum metabolism. Disorders of copper absorption in man can occur after partial gastrectomy, in severe malabsorption and when protein is severely deficient in the diet, as in kwashiorkor. Copper deficiency in animals produces vascular defects of the aorta leading to death from aortic rupture. These defects are reversible when copper supplements are given in the diet. Copper is involved in iron metabolism and is necessary for several of the steps of iron utilization. At the intestinal mucosal level there are complicated interactions between several of the essential biological metals.

Absorption

The average Western diet contains 3-5 mg of copper. Studies with radioactive copper in animals indicate that 5-10% of ingested copper can

be absorbed (Bowland *et al.*, 1961). Studies in man have given very diverse figures for the amount of copper normally absorbed from an average diet. As little as 1% of the dietary intake was absorbed in one series (Cartwright *et al.*, 1954). Following an oral dose of labelled copper the concentration of isotope rises to a peak 1-2 h after absorption, following this a sharp drop occurs when copper is taken up and stored by the liver. This is followed by a slow secondary rise after 48-72 h as radioactive copper is incorporated into newly synthesized ceruloplasmin. The height of the first peak reflects the amount of copper absorbed. The size of the second peak reflects the rate of synthesis of ceruloplasmin and the size of the copper pool. If it is assumed that the height of the second peak is related to the amount of copper absorbed the average absorption of an oral dose of copper is 40% (Sternlieb, 1967). This assumption can be criticized on theoretical grounds and may not be valid. In Wilson's disease the amount of copper absorbed is probably within the normal range (Gitlin *et al.*, 1960). Although in Wilson's disease the initial peak of radioactivity is higher than in normals this is probably due to delay in uptake by the damaged liver and not to increased absorption. Some animals (e.g. sheep) are unable to regulate the amount of copper absorbed from the diet so that they readily develop copper toxicity syndromes if fed diets containing excess copper. Other animals do not develop copper toxicity signs on a copper-rich diet nearly so easily. This may be because they are more resistant to higher levels of blood and tissue copper or because absorption and/or urinary excretion of copper can be controlled. There is no evidence to suggest that the proportion of ingested copper which is absorbed can be increased by prolonged feeding of copper-depleted diets. The site of maximum absorption of copper seems to be different depending on the animal species investigated. In dogs the main site is the upper jejunum (Sachs *et al.*, 1943), in pigs the colon is the main site (Bowland *et al.*, 1961). Studies in man indicate that the upper small intestine is the main site (Bearn and Kunkel, 1955; Earl *et al.*, 1954). This is in keeping with the known fact that copper absorption is increased by an acid pH.

The transport of copper through the intestinal wall probably takes place as a complex between copper and amino acids. There is evidence that absorption is best with monomeric, acidic, essential amino acids of L configuration and least good with trimeric, basic, non-essential amino acids of D configuration (Kirchgessner and Grassman, 1970). Many metals are known to interfere with the absorption of copper including zinc, iron, cadmium, mercury and silver (Hill *et al.*, 1964; Van Campen, 1966; Van Campen and Scaife, 1967). These findings have suggested that the absorption of copper is by an active transport system. Whether the antagonistic metal blocks protein binding sites or inhibits, in some other way, the transporting system is not known. Some workers have suggested

that absorption is not an active process but is controlled by the diffusion characteristics of the individual antagonizing metals (Sahagian *et al.*, 1967). More recent work has suggested that copper^{64} given by mouth is firmly and specifically bound to a protein within the duodenal mucosa of molecular weight approximately 10,000. Zinc and cadmium act as copper antagonists by preferential binding to this duodenal mucosal protein and displacing copper from it (Starcher, 1969). Other factors are also known to influence the absorption of copper.

Form of copper in the diet

The more soluble copper salts are, in general, more easily absorbed than the less soluble (Lassiter and Bell, 1960). Copper combined with organic compounds is probably more suitable than inorganic salts (Mills, 1956) although copper-porphyrin will not correct copper deficiency induced anaemia in rats (Schultz *et al.*, 1936).

Effect of calcium and phosphate

Calcium salts depress copper absorption (Tompsett, 1940), possibly because they result in a more alkaline medium in the upper small intestines–this would discourage the formation of acidic amino acids which are known to promote copper absorption (Kirchgessner and Grassman, 1970).

Effects of other biological metals

Zinc and cadmium

Copper toxicity can be reduced by the addition of extra zinc or iron to the diet (Suttle and Mills, 1966). Animals fed a diet deficient in zinc readily develop zinc deficiency signs when a slight excess of copper is added to the diet and similarly animals fed a mildly copper-deficient diet readily develop copper deficiency signs when a small excess of zinc is added to the diet. There is now evidence that at least some of the antagonism between copper and zinc is in the absorption of both metals from the small intestine. It has been found that the absorption of radioactive zinc or copper is depressed by the addition of the antagonistic metal. This suggests a common binding site within the intestinal mucosa (Van Campen, 1970).

It is also known that zinc interferes with the absorption of copper from the intestine when administered with the copper but not when given parenterally (Van Campen and Scaife, 1967). The reverse is also now known to be true, namely that copper interferes with the absorption of zinc at an intestinal mucosal cell level (Van Campen, 1964).

Molybdenum

Cattle grazing on molybdenum-rich soil develop signs suggestive of copper deficiency and if excess copper is added to the soil the signs disappear. It has been shown experimentally that a high molybdenum intake reduces copper retention and the copper content of the tissues (Dick, 1956). The evidence also indicates that increasing the sulphate content of the diet exaggerates the molybdenum-induced reduction of organ copper content.

Molybdenum can also precipitate copper deficiency signs (Arthur, 1965). These findings could be explained if copper and molybdenum combined in the body and that this copper-molybdenum compound is less metabolically available than either biological metal alone. Molybdenum toxicity would be relieved because the administered copper would combine with the excess molybdenum.

Copper deficiency signs would be induced when molybdenum is given because the molybdenum would combine with what little copper was available. Recent experimental work has identified the hitherto hypothetical copper-molybdenum compound and has shown that it is the same as a rare naturally occuring copper-molybdenum mineral known as lindgrenite (Dowdy *et al.*, 1969).

Dietary molybdenum and sulphate are both known to adversely affect the storage and availability of copper. It is now known that both molybdenum and sulphate reduce the uptake of radioactive copper by the liver and reduce its incorporation into ceruloplasmin as well as affecting copper absorption from the gastro-intestinal tract (Marcilese *et al.*, 1969).

Role of lymphatics in copper absorption

Radioactive copper given by mouth is absorbed rapidly; this has led to the suggestion that the lymphatic system is involved in its dissemination from the mucosal cells of the upper gastro-intestinal tract. However, the amount of copper recovered from the thoracic duct of patients who have had thoracic duct cannulation for the relief of ascites is very low (Sternlieb *et al.*, 1967).

Excretion

Most of the copper ingested is excreted by way of the faeces. Copper from the copper pool in the body is mainly excreted in the bile, a small amount is excreted via the intestinal juice and urine. In biliary obstruction the amount excreted by the latter two routes is increased so that in animals (and probably in man) biliary obstruction does not cause a rise in plasma copper (Mahoney *et al.*, 1955). The urinary excretion of

copper is more closely related to the serum copper than copper bound to the globulin ceruloplasmin (Jensen and Kamin, 1957). There is a considerable diurnal variation in the urinary excretion of copper (both the rate and urinary concentration of copper vary). Copper excretion in the urine is greatest from 12 noon to 4.0 p.m. (Butler and Newman, 1956). This parallels the diurnal changes in plasma copper. The normal urinary copper excretion in adults varies from 3.9-29.6 μg per 24 h (Butler and Newman, 1956). Urinary copper excretion is increased in the presence of proteinuria. In the nephrotic syndrome 60-80% of the urinary copper is excreted bound to ceruloplasmin and the remainder to albumin (Markowitz *et al.*, 1955).

Copper transport

Copper is transported in the blood by albumin and an α 2 globulin, ceruloplasmin. Injection of radioactive copper into the blood causes a rapid increase in plasma radioactivity as the copper is quickly bound to albumin, this is followed by a decline in radioactivity as radioactive copper attached to albumin is distributed to the copper proteins in the liver and elsewhere in the body this decline is followed by a further rise in plasma radioactivity as copper incorporated into ceruloplasmin is released from the liver into the blood (Bearn and Kunkel, 1955). After equilibration has occurred copper bound to ceruloplasmin constitutes about 95% of that in the plasma, this copper is firmly bound. The remaining 5% is loosely bound to albumin (Gubler *et al.*, 1953). Ceruloplasmin possesses enzymatic activity as an oxidase. The physiological function of ceruloplasmin as an enzyme is not known (Holmberg and Laurell, 1951). In the whole blood copper is more or less equally distributed between plasma and red cells (Cartwright, 1950). In the red cells most of the copper is contained in a protein of unknown function, erythrocuprein.

The distribution of copper in the body

The tissue and blood copper content is influenced by several factors. On a normal diet the average human body contains 100-150 mg of copper. Young and newborn animals contain a higher tissue concentration of copper than older animals. The organs and tissues containing the highest concentration of copper in man are hair, skin, liver, muscle, and lung. The amounts in the normal brain, CSF, heart and endocrine glands are normally very low. Of the total of 100-150 mg in the human body approximately 65 mg are found in the muscles, 23 mg in the bones and 18 mg in the liver (Chou and Adolph, 1935). In general

the copper content of the tissues reflects the dietary intake of copper except that some tissues and organs respond less quickly than others to a change in the dietary intake (Underwood, 1962). However, there is also a species variability in the susceptibility of organs to become depleted of copper when animals are fed a copper-deficient diet. In most species the copper content of the brain is less sensitive than that of other organs to changes in dietary copper. However, sheep and lambs fed on copper-deficient soils readily develop brain depletion of copper leading to ataxia (Howell and Davidson, 1959).

The copper content of the iris, choroid and retinal pigments of the eyes of most species is very high (Bowness and Morton, 1952). In these sites copper is bound to proteins but unlike zinc it has never been demonstrated what function it performs in the eye.

The copper content of the hair is very high and is higher in pigmented hair than in non-pigmented hair (Kikkawa *et al.*, 1955). Depigmentation of the hair is one of the signs of copper deficiency in many species.

Studies with radioactive copper

Radioactive copper given by mouth is absorbed very rapidly, becomes attached to albumin and is distributed to the tissues where it is bound (mainly in the liver). After several hours there is a secondary rise in blood radioactivity as radioactive copper is incorporated into newly synthesized ceruloplasmin. In Wilson's disease ceruloplasmin is absent so that this secondary rise does not occur. At the end of 20 h most of the injected radioactive copper is normally attached to ceruloplasmin. In Wilson's disease the copper remains attached loosely to the serum albumin and hence can be easily transferred to the binding sites in the tissues; however, in later stages of the disease these sites are already saturated with copper and so most of the injected radioactive copper remains attached to the albumin in the serum. Radioactive copper in the urine probably derives from copper attached to albumin from which it is detached in the glomeruli of the kidneys. Patients with Wilson's disease excrete more copper in the urine than normals (Bearn and Kunkel, 1955). An unexplained feature of urinary copper excretion is the variability of copper excretion from hour to hour in normals and patients with Wilson's disease (Earl *et al.*, 1954). Normal patients excrete less than 1% of injected copper in the urine. Following injection of radioactive copper approximately 7% of the dose (or remaining dose) is excreted in the bile per day. If excretion via the bile is restricted (by ligating the bile duct) the amount of copper excreted via the urine and intestinal juice rises so that accumulation of copper in the body does not occur (Mahoney *et al.*, 1955).

Functions of copper

Diets deficient in copper occur naturally and have been produced experimentally. Studies using copper-deficient diets indicate that copper is essential for the normal growth, development and function of many organs. The main disorders of function in copper deficiency are anaemia, vascular abnormalities, depigmentation of hair and wool, difficulty in parturition, abnormalities of bone formation, myocardial fibrosis, demyelination and disturbances of gastro-intestinal function.

Copper is an essential constituent of several proteins, metallo-enzymes and some naturally occurring pigments. The function of the enzymes and proteins which contain copper as an essential constituent has not been elucidated in every case.

In the majority of enzymes which contain copper the copper acts as a cofactor (metal ion activated enzymes). Other metals such as molybdenum may be essential cofactors for the same enzymes. It has been demonstrated *in vitro* that there are optimum proportions for copper and molybdenum in the induction of xanthine oxidase (Kovalsky and Vorotnitskaya, 1970).

The main proteins for which copper is essential are:

Cerebrocuprein I
Erythrocuprein
Haemocuprein
Hepatocuprein
Mitochondro cuprein

The main enzymes for which copper is essential in mammals are:

Ceruloplasmin (an oxidase)
Tyrosinase (production of melanin)
Amine oxidase (metabolism of catecholamines)
Cytochrome C oxidase (respiratory enzyme)
Uricase (oxidation of uric acid)
Dopamine B-hydroxylase (an adrenal enzyme)

The main copper-containing proteins

1. Ceruloplasmin

This is an α globulin. It can act as an enzyme, acting as an oxidase towards serotonin, adrenalin and ascorbic acid *in vitro*. The physiological role of ceruloplasmin as an enzyme is not known. Like haemoglobin and transferrin ceruloplasmin is heterogeneous, i.e. it can exist in several different forms. In the case of haemoglobin the heterogeneity can be controlled genetically. Its ability to carry oxygen varies according to

which form of the molecule is present. In the case of transferrin all the variants are the same with regard to their ability to transport iron.

2. Tyrosinase

Tyrosinase is similar in structure to ceruloplasmin. Tyrosinase is also a copper protein, has oxidase activity, is possibly heterogeneous and like ceruloplasmin can be deficient as an inherited defect (deficiency of tyrosinase results in lack of pigmentation, i.e. albinism). Wilson's disease (deficiency of ceruloplasmin) and albinism (deficiency of tyrosinase) have never been known to occur in the same individual. Only a proportion of the copper contained in tyrosinase and ascorbic acid oxidase will exchange with radioactive copper suggesting that within these enzymes copper exists in two different forms—non-exchangeable and active and inactive and exchangeable (Dressler and Dawson, 1960).

3. Amine oxidase

This enzyme, which is concerned in the destruction of histamine and other important physiological amines such as serotonin and noradrenalin, is a copper-containing enzyme. Its level in copper-deficient animals is absent or extremely low.

4. Cerebrocuprein

Cerebrocuprein is found in the brain of man and animals; it has no enzyme activity and its function is unknown.

5. Erythrocuprein

Erythrocuprein is contained entirely within the erythrocytes and accounts for at least 80% of the copper content of the red cells; it is quite distinct from ceruloplasmin. The amounts of erythrocuprein are normal in Wilson's disease; like cerebrocuprein its function is quite unknown.

6. Cytochrome C oxidase

This enzyme is essential for aerobic respiration. In addition to copper it contains haem (and therefore iron). The cytochrome C level of organs is reduced in many instances of dietary copper deficiency.

7. Hepatocupreins

The form and functions of all the copper-containing hepatic proteins have not been identified although copper is known to be a component of uricase which catalyses the oxidation of uric acid.

Disorders causing change in blood copper level
(Adelstein and Vallee, 1962)

Disorders of human metabolism associated with hypocupraemia

I. Inability to synthesize ceruloplasmin
   a. Conditioned copper deficiency
   b. Failure to absorb copper
      (1) Sprue
      (2) Coeliac disease
   c. Inability to synthesize other copper-carrying proteins
      (1) Newborn
      (2) Hepatolenticular degeneration
      (3) Kwashiorkor
      (4) Idiopathic hypoproteinaemia
II. Excessive loss or destruction of ceruloplasmin
   a. Loss through excretion
      (1) Nephrotic syndrome
      (2) Protein-losing enteropathy
   b. Accelerated catabolism

Disorders of human metabolism associated with hypercupraemia

Pregnancy
Viral and bacterial infection
Myocardial infarction
Lupus erythematosus
Haemochromatosis
Biliary cirrhosis
Hodgkin's disease
Leukaemia
Malignant disease
Aplastic anaemias
Thyrotoxicosis
Schizophrenia
Alcoholism

The biochemical effects of copper deficiency are mainly a reduction of copper-containing enzymes or sometimes an increase in enzymes in which the copper ion acts as an inhibitor.

Relationship to disease

Haemopoiesis

Anaemia is a constant feature of copper deficiency in most animals. The type of anaemia shows considerable species variation; some animals

develop a haematological picture indistinguishable from that of iron deficiency anaemia (Lahey *et al.*, 1952), in others it is normochromic and normocytic with a reduced survival time of red cells (Bush *et al.*, 1956). In some animals there is defective maturation of red cells although the haemoglobin content of the normoblasts in the presence of copper deficiency anaemia is normal (Baxter and Van Wyk, 1953), suggesting that copper is involved with the maturation of the cell membrane (Prankerd, 1961).

Immature red cells in copper deficiency are usually more liable to be destroyed by the normal haemolytic processes of the body; although if copper-deficient red cells are transfused into an animal which is not copper deficient the survival of the red cells is prolonged to normal presumably because the copper-deficient red cells are able to absorb and utilize the copper from the blood of the copper-replete normal animal (Bush *et al.*, 1956).

At least 80% of the copper in red cells is contained in the copper protein—erythrocuprein. The function of erythrocuprein is not known. The link between copper deficiency and the defect in red cell maturation is probably the copper-containing enzyme cytochrome oxidase which is known to be reduced in the bone marrow of copper-depleted animals (Schultz, 1941). Copper appears to be essential for the proper maturation of red cells and for the continued survival of red cells within the circulation. In addition, copper plays a part in the incorporation of iron into haem in the synthesis of haemoglobin and also in other aspects of the metabolism of iron.

Role of copper in the synthesis of haemoglobin and metabolism of iron

The appearance of copper deficiency anaemia is frequently hypochromic and microcytic suggesting that iron deficiency is responsible and that the haemoglobin content of red cells is reduced. It was originally believed that copper deficiency interferes with the absorption and transport of iron. Studies using radioactive iron indicate that the transport and mobilization of iron in copper-deficient animals is not impaired and is in fact greater than normal; in addition to which the life span of red cells of copper-deficient animals is reduced suggesting that the turnover of iron in copper-deficient animals is actually increased (Bush *et al.*, 1956). It seems that copper deficiency can give rise to iron deficiency anaemia while iron is available in normal amounts. This paradoxical situation has been clarified by some recent work by Lee and others (1968). Iron deficiency results in reduced haemoglobin formation because iron is an essential part of the oxygen-carrying pigment haem; deficiency of iron means reduced haem synthesis. Haem consists of

protoporphyrin plus iron in the ferrous or ferric form. Haem is joined to a protein, globin, to form haemoglobin. If copper deficiency gives rise to a situation resembling iron deficiency anaemia (when iron is abundantly available) it suggests that copper is involved in,

1. The synthesis of porphyrins
2. The incorporation of iron onto the porphyrins
3. The synthesis of globin.

Lee *et al.* (1968) have produced evidence that the biosynthesis of the pigment haem is normal and that the enzymes involved in haem synthesis in copper deficiency are present in normal amounts and are not, therefore, copper dependent. It is concluded that copper is essential for the synthesis of globin or is involved in some other way in iron metabolism.

In copper deficiency there is a reduction in tissue concentration of copper metallo-enzymes cytochrome oxidase and xanthine oxidase (Matrone, 1960). The administration of copper to copper-deficient animals causes a reduction in the iron content of the liver and an increase in the iron contained in haemoglobin. Animals deficient in copper and iron have more iron stored in the liver than animals which are only deficient in iron. These findings have led to the hypothesis that copper is necessary for the release of iron from storage sites (Marston and Allen, 1967).

Copper and the function of blood vessels

Copper-deficient animals develop anaemia, nevertheless copper deficiency causes a high mortality although the anaemia is not severe. In many species it has been shown that death in copper deficiency is due to rupture of the ascending aorta or of aortic aneurysms (Shields *et al.*, 1962), so-called 'angiorhexis'. In the copper-deficient animals the elastin of the aorta is reduced and the cells of the tunica media of the aorta are immature. The effects of copper deficiency can be accentuated by the addition of ascorbic acid (Hunt and Carlton, 1965).

It has been confirmed that the mechanical properties of blood vessels from copper-depleted animals are altered and that the solubility and elastin content of the vessels is reduced (Coulson and Carnes, 1962). There is a rapid reversal of these findings if copper supplements are given in the diet. Two other conditions cause similar defects of elastin of the major blood vessels. These are Marfan's syndrome and lathyrism.

Nature of the elastin defect in copper deficiency

The elastin content of the main vessels of copper-deficient animals is approximately half of that of controls (Starcher *et al.*, 1964). The elastin

of the blood vessels from copper-deficient animals contains four times as much of the amino acid lysine as controls. Elastin is formed by condensation of four lysine residues (Partridge *et al.*, 1964). For the condensation to take place oxidative deamination has to occur; amine oxidase which brings about deamination is known to be a copper-containing enzyme. It is postulated that deficiency of copper leads to a deficiency of amine oxidase with accumulation of lysine and failure of elastin formation. Copper deficiency causes an absence of liver mitochondrial amine oxidase which can be restored when copper supplements are given. Amine oxidase activity is absent from the aortae of copper-deficient chicks. These findings have been corroborated by the observation that when amine oxidase is added to tissue cultures of aortic strips obtained from copper-depleted birds lysine is converted into elastin. Radioactive lysine is not incorporated into elastin in copper-deficient animals (Hill, 1969).

In addition to mechanical alterations (Coulson *et al.*, 1965) there are also morphological differences in the aortae of copper-deficient animals (Shields *et al.*, 1962). The defects in amount and solubility of the elastin tissue of copper-deficient aortae is accompanied by a threefold increase in the acid mucopolysaccharide ground substance. This increase mainly affects chondroitin sulphate B (chondroitin sulphate A and C and heparitin sulphate are present in relatively normal amounts). The increase in chondroitin sulphate B in the aorta of copper deficiency is believed to be secondary to the defect in elastin (Coulson and Linker, 1968). It is thought that mucopolysaccharides do not themselves affect the stability or function of either the elastin or collagen fibres.

Copper and the skeleton

In the aortae of copper-depleted animals it has not been possible to demonstrate morphological, mechanical or biochemical defects of the collagen but only of elastin. This is largely due to the technical difficulties of obtaining the collagen separate from the mucopolysaccharide ground substance and elastin. However, there is some evidence that the solubility of aortic collagen in copper deficiency is increased and that the solubility of collagen from the tendons of copper-depleted animals is also increased (Savage *et al.*, 1966). It has been shown that in copper deficiency the solubility of bone collagen is increased and the bone content of the copper metallo-enzyme cytochrome oxidase is decreased (Rucker *et al.*, 1969).

In the elastin of copper-deficient aortae the defect is believed to be a decrease in intramolecular cross links involving deamination of some of the amino groups of the lysine molecules which normally leads to the subsequent condensation of lysine molecules to form the protein chains

of elastin. Collagen also contains intramolecular cross links which are believed to be similar to those of elastin (Rucker *et al.*, 1969). Copper deficiency in birds and animals leads to severe defects of bone which are thought to be due to defects of bone collagen synthesis due to failure of condensation of lysine such as occurs in the aortae of copper-deficient animals.

The link between copper deficiency and lathyrism

Lathyrism is a disease of man and animals resulting from excessive ingestion of the sweet pea of the genus *Lathyrus.* In man lathyrism is predominantly a disease of the nervous system and is caused by ingestion of *Lathyrus sativus, L.cicera* and *L. clymenum.* It is now mainly confined to India although outbreaks occurred in Europe in the nineteenth century and in prisoner of war camps in the Second World War. The disease was recognized by Hippocrates; it can affect domestic animals. In experimental animals the particular *Lathyrus* species toxic to man and domestic animals have no deleterious effects; however, another species of *Lathyrus* (*L.odoratus*) is toxic to the rat.

Clinically recognizable lathyrism in man is a neurological disorder characterized by an acute onset of weakness and paraplegia (neuro-lathyrism) (Weaver and Spittell, 1964). Spondylosis, kypho-scoliosis and other skeletal abnormalities are described in the affected cases (Paissios and Demopoulos, 1962). The copper status of patients affected with lathyrism has not been investigated.

In the experimental animal lathyrism produces many different abnormalities, for example scoliosis and hernias (Geiger *et al.*, 1933), neurological disorders and dissecting aneurysm (Ponseti and Baird, 1952). The high incidence of dissecting aneurysm and skeletal deformities especially kyphoscoliosis in animal lathyrism has been confirmed (osteolathyrism) (Bean and Ponseti, 1955). A high incidence of spinal deformities in hypertensive patients with dissecting aneurysm has been found in humans as compared with age-matched control hypertensive patients (Bean and Ponseti, 1955).

There is a reduction of tensile strength of about 50% in the aortae and skin of experimental animals with lathyrism (sometimes called odoratism because it is *L. odoratus* which is the toxic species of *Lathyrus* in the rat) (Fry *et al.*, 1962).

The toxin in *L.odoratus* is β amino propionitrile. The pathological features of osteolathyrism and copper deficiency in animals are very similar. In experiments using young turkeys the histology of the aortae was hardly distinguishable between those from copper-deficient birds and those from birds given β amino propionitrile (Savage *et al.*, 1966). Several workers have shown how the known lathyrogens can interact

with copper interfering with its availability to essential copper-containing enzymes involved in the condensation of lysine (Dasler *et al.*, 1961; Levene, 1961; Albert, 1950). Dissecting aneurysm and skeletal abnormalities have been reported in many different species by several workers (Kennedy and Kennedy, 1962; Menzies and Mills, 1957; Muraffo and Malmois, 196; McCallum, 1965).

The histological and electron microscopic features of the aorta are similar in lathyrism and copper deficiency in the rat and resemble those of Marfan's syndrome in the human. Radio-isotope studies of the aortae of rats affected with lathyrism indicate failure of formation of chondroitin sulphates A and C complexes with proteins (Fry *et al.*, 1962). This is a similar defect to that seen in copper-deficient animals.

D penicillamine and lathyrism

D penicillamine (β,β dimethyl cysteine) is known to produce an increase in soluble collagen in the skin in humans and in animals (Harris and Sjoerdjma, 1966). In man penicillamine is used in the treatment of Wilson's disease and it has been suggested that it is also of value in scleroderma and rheumatoid arthritis because of its effect of increasing the solubility of collagen. When used in humans approximately one-third of patients develop marked loss of taste acuity; this may return to normal when copper supplements are given at the same time (Henkin *et al.*. 1967). The whole syndrome of osteolathyrism has been produced in the experimental animal by feeding D penicillamine; in addition, the deleterious effect of penicillamine can be completely or partially reversed by feeding copper (Keiser *et al.*, 1968). The exact mode of action of penicillamine in producing similar defects to βamino propionitrile (the toxin of lathyrism) is debatable. D pencillamine is believed to form reversible complexes with the aldehyde group of polypeptide-bound lysine preventing its condensation by cross linking (Nimni, 1968). This leads to accumulation of soluble aldehyde-rich collagen. β amino propionitrile appears to produce lathyrism by blocking the conversion of amino groups of lysine to aldehyde groups which is necessary for cross linking of lysine to form collagen. There is some evidence that D penicillamine also acts on amine oxidase. D penicillamine induced osteolathyrism also produces an increase in urinary hydroxyproline excretion. Hydroxyproline is normally formed by proline hydroxylase from proline. Thus, penicillamine acts on at least three points in the synthesis of collagen viz.

1. It forms reversible complexes with the aldehyde group of lysine.
2. It acts on proline-hydroxyproline activity.
3. It also acts on amine oxidase activity.

Lathyrism seems to be mainly due to blocking of conversion of lysine amino groups to aldehyde groups by amine oxidase. Because copper reverses the effects of D penicillamine it is reasonable to presume that D penicillamine acts by producing copper chelation *in vivo* at the sites where copper is essential for the activity of copper enzymes. Some workers have produced evidence that penicillamine acts independently of its action of copper chelation, probably by its action in producing stable sulphur linkage with the aldehyde groups (Jaffe *et al.*, 1968).

Copper and the skeleton

Spontaneous fractures are common in animals feeding off copper-deficient soils or who are given artificially depleted copper diets. The bone defect is similar to that of scurvy (Follis *et al.*, 1955). The defect seems to affect osteoblasts only; chondroblasts and calcification of cartilage are normal.

Copper and neurological function

Swayback in the lambs of ewes grazing copper-deficient pastures has been reported many times from Australia; the disease can be prevented by giving copper supplements to the pregnant ewes (Underwood, 1962). The situation is complicated by the fact that in England and Scotland a correlation could not be found between a copper-deficient diet and the development of swayback in lambs. Under experimental conditions high intake of molybdenum and sulphate can produce copper deficiency and swayback but in field work swayback is sometimes seen when both the copper and molybdenum intake are in the normal range. Experimental copper deficiency causes a reduction in the copper metallo-enzyme cytochrome oxidase. Assay of this enzyme can be used to assess copper nutritional status (Poole, 1970).

The pathology of swayback in lambs includes characteristic diffuse symmetrical degeneration of the white matter of the cerebral cortex and secondary degeneration of the motor tracts of the spinal cord. Two views of the pathogenesis of swayback exist: one view is that the disease is primarily a demyelinating disease (Innes and Shearer, 1940; Everson *et al.*, 1968). The other view is that the lesions result from venous stasis, oedema and perivascular necrosis (Spais *et al.*, 1961). It has recently been suggested that the lesions result from severe tissue hypoxia (Carlton and Kelly, 1969).

The myelin changes in swayback are secondary to axonal degeneration and are Wallerian in type under some circumstances (Concilla and Barlow, 1968). It has been suggested that the changes of swayback resemble those of multiple sclerosis (Campbell, 1967) but the changes of primary axonal degeneration make this unlikely.

Histochemical studies of the nerves in swayback due to copper deficiency show a reduction of the copper metallo-enzymes cytochrome oxidase, succinic dehydrogenase, thiamine pyrophosphatase. Acid phosphatase is also reduced (Barlow, 1969).

In 1947 Campbell *et al.*, reported that four out of seven workers investigating swayback in sheep developed multiple sclerosis, which has been confirmed at autopsy in all cases. However, Campbell feels that 'swayback and copper deficiency cannot be indirectly implicated' (Campbell, 1967).

Multiple sclerosis

A relation between the incidence of multiple sclerosis and the type of soil, climate, cosmic radiation, geomagnetic latitude, dietary fat intake, potato intake, brussels sprout and gooseberry intake have all been seriously suggested as important aetiological factors (McAlpine *et al.*, 1965). Considerable effort has been devoted to proving or disproving the hypothesis that multiple sclerosis is either itself a communicable disease or is closely related to a communicable disease. This hypothesis was given further impetus by the now presumed devastating and incredible coincidence that four of seven research workers at Cambridge investigating swayback in sheep developed proven and by now fatal multiple sclerosis (Campbell, 1967).

Swayback has been shown to be produced by copper deficiency and can be prevented by adequate copper supplements; nevertheless an infective agent may still be partly responsible for the disease. It is postulated that copper deficiency predisposes to infection with the hypothetical infecting agent causing swayback in sheep (Campbell, 1967); this infective agent is postulated as being transmitted to man in whom it causes multiple sclerosis. This is an ingenious hypothesis and suffers from the advantage that it is difficult to refute entirely. Multiple sclerosis is rare in Western Australia, where both sheep and copper deficiency are common. Multiple sclerosis has not been reported in any other workers investigating swayback. In Iceland multiple sclerosis and swayback are common; however, the distribution of the cases of multiple sclerosis and those of swayback in sheep is in no way similar (Gudmundsson and Gudmundsson, 1962).

Investigations of the trace metal content of the water supply of patients with multiple sclerosis and controls show no difference between the two groups (Sutherland, 1956). However, this is not surprising if multiple sclerosis is fairly evenly distributed among the normal population outside the tropics. There is an awe-inspiring similarity between the incidence of multiple sclerosis and areas where glaciation has played an important part in providing parent material for the soil (Warren, 1959).

There is yet no proven connection between multiple sclerosis and any known natural phenomenon or deficiency. The reported coincidences are tantalizing.

Depigmentation of the hair (Underwood, 1962)

Depigmentation of the hair occurs in animals fed on copper-deficient soils; in some animals it is the earliest sign of copper deficiency. If the period of copper deficiency is short dark-haired animals develop a localized band of depigmentation in each hair. Depigmentation is probably due to copper deficiency inhibiting the melanin-forming copper-containing enzyme tyrosinase.

In the skin copper activates the dopa-tyrosinase system (Lorincz, 1954). It it is also an essential activator of the changes which take place in keratinization of wool. The copper content of normal skin has been investigated (Molokhia and Portnoy, 1969).

Copper and keratin formation

Copper deficiency causes a deterioration in the ability of the hair of sheep to 'crimp' or crinkle. In addition the tensile strength and elasticity of the wool is reduced (Underwood, 1962). The ability of hair to crimp or curl depends on the number of disulphide (i.e. –S–S–) linkages between adjacent loops of the helix of amino acids of which keratin is composed. Straight (copper-deficient wool) has fewer of these disulphide coil to coil links than normal and the helix straightens out. In addition in copper deficiency the amino acid arrangement in the polypeptide chains of the keratin molecules of the hair is altered suggesting that copper is involved in two aspects of hair formation, one being the links between adjacent coils of the helix of amino acid molecules and the other in the arrangement of amino acids themselves in the helix.

Copper and myocardial fibrosis in cattle (Underwood, 1962)

Copper deficiency can give rise to pathological fibrosis in the myocardium of cattle although it is suggested that factors other than simple copper deficiency may be responsible.

Copper and reproduction

Naturally occurring deficiency is associated with lowered fertility in animals. Artificially induced copper deficiency has also been demonstrated to reduce fertility in rats; infertility is due to maternal resorption of the conceptus (Hall and Howell, 1969).

Copper and composition and distribution of fat

The addition of copper to the diet of pigs increases their growth rate and has been found to alter the consistency and amount of fat. It is suggested that the addition of copper to the diet alters the distribution of fatty acids within the triglycerides. The significance of these findings is not known (Moore *et al.*, 1969).

Copper deficiency in humans

Copper deficiency has been recorded in humans. Four infants who had been fed exclusively on milk developed malnutrition and were rehabilitated with high calorie diets which were deficient in copper. They developed severe anaemia, neutropenia, scurvy-like bone changes and hypocupraemia. Copper supplements reversed these features. It is suggested that diarrhoea and malabsorption may be complicated by copper deficiency more frequently than is appreciated (Cordano *et al.*, 1964).

Absorption of copper in malabsorption

Absorption of copper may be defective following partial gastrectomy (Kovtunjak, 1968) as well as in kwashiorkor (Macdonald and Warren, 1961) and in malabsorption (Sternlieb and Janowitz, 1964).

Copper contamination in exchange transfusions

Where disposable apparatus for exchange transfusions of infants is not used and stopcocks are used instead slight copper contamination of the babies being transfused can occur (Blomfield, 1969). Chronic copper poisoning causes intravascular haemolysis, in addition copper enhances the agglutination of red cells. Copper may compete with bilirubin for albumin binding sites thereby increasing the toxicity of bilirubin. These three effects of copper may be deleterious to the newborn baby undergoing exchange transfusion (Blomfield, 1969).

Water standing in copper vessels and then boiled can absorb sufficient copper to produce acute toxic symptoms which are nausea, vomiting and diarrhoea (Nicholas, 1968). Copper vessels and tanks used for storing and heating water for human consumption should be plated with tin. Copper poisoning is particularly likely to occur when the food is highly acid, e.g. stewed apples (Ross, 1955). Copper poisoning has been reported due to cocktails being shaken in a copper cocktail shaker for which the inner silver lining had worn off (Wyllie, 1967). In 1967 in 27% of all notified cases of food poisoning in England and Wales no cause was found. It is

suggested that contamination of food and drinking water by copper containers may be implicated more frequently than is generally realized (Nicholas, 1968). People living in remote areas where the domestic water is acidic and where the water may linger in copper pipes are especially prone to copper toxicity. Cold water which has been stored in such pipes may contain up to 7.6 p.p.m. of copper. This is greatly in excess of the recommended upper limit (Paine, 1968).

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CHAPTER IV

Manganese

Introduction

Manganese is found in high concentrations in mitochondria; there are two manganese metallo-enzymes and a specific manganese-carrying protein in the blood as well as a good homeostatic mechanism. Manganese is involved in cholesterol synthesis. Manganese deficiency in animals is well known, the main features are neurological and skeletal deformities. Studies of chronic manganese intoxication have thrown light on the mechanism of action of L Dopa in Parkinsonism. Manganese-activated enzymes include polymerases and galacto transferases which are involved in mucopolysaccharide synthesis; disturbances of manganese metabolism have been shown in hydralazine-induced lupus erythematosus and rheumatoid arthritis. Manganese absorption and deposition in the liver are increased in haemochromatosis and other forms of liver damage. Manganese deficiency in breeding animals gives rise to congenital abnormalities in the offspring. There is evidence that alterations in manganese concentrations can alter the expression of harmful genes.

Absorption

Only a small percentage of ingested manganese is absorbed from the gastro-intestinal tract, less than 5% is normally absorbed (Greenberg *et al.*, 1943). Absorption cannot be increased by increasing the dietary intake (Kent and McCance, 1941); however, retention of radioactive manganese is greater in the liver of manganese-deficient pullets than in the liver of normal birds (Hill, 1965) and retention in other organs is increased following repletion with radioactive manganese in manganese-depleted chicks (Settle *et al.*, 1969). The increased uptake of radioactive manganese in depleted animals suggests increased gastro-intestinal absorption in the presence of manganese depletion although with the techniques available it is not possible to quantitate this. In birds manganese deficiency syndromes can be accentuated by feeding diets which contain high proportions of calcium, phosphate and

carbonates. These substances absorb manganese salts rendering them insoluble (Schaible and Bandemer, 1942). Under some circumstances in animals calcium salts can promote the absorption of manganese (Lassiter *et al.*, 1970). Different manganese salts with differing solubilities seem to be absorbed equally well (or poorly). The site within the gastro-intestinal tract at which manganese is absorbed is not known.

Distribution

Manganese is not preferentially concentrated by any organ although the manganese concentration within different organs and tissues varies widely. The concentration within any one organ remains remarkably constant. One of the paradoxes of manganese metabolism is that the manganese pool within the body (about 12-20 mg in humans) is highly mobile but the individual tissue concentration remains constant suggesting the existence of a very efficient homeostatic mechanism which has not yet been identified.

The organs which contain the highest concentration of manganese are the bones, liver, pancreas, kidney and pituitary gland (Underwood, 1962). Liver arginase and pyruvate carboxylase are the only known manganese metallo-enzymes; however, manganese is an essential cofactor for many enzymes (metal ion activated enzymes), for example polymerase and galacto transferase. Manganese is believed to be essential for mitochondrial function, following intraperitoneal injection of physiological doses of Mn^{56} manganese rapidly disappears from the blood and is distributed to those organs which have a high concentration of mitochondria (liver, kidney and pancreas). Cell fractionation studies confirm the localization of manganese in the mitochondria. The known association between mitochondrial enzymes and enzymes related to the respiration of cells has suggested that manganese is a respiratory enzyme cofactor (Maynard and Cotzias, 1955). Further studies with radioactive manganese indicate that the manganese pool within the body can exchange within an hour. Within a minute of complete mixing 70% of the dose of radioactive manganese has left the blood; excretion from the body is much slower than this, suggesting that manganese is rapidly concentrated; this concentration occurs mainly with the mitochondria of the hepatic cells. Manganese excretion into the bile is negligible during the first hour (Borg and Cotzias, 1958).

Much of the difficulty in investigating manganese metabolism stems from the fact that *in vitro* studies with manganese can often be reproduced by using other metals similar to manganese (chromium or iron), however *in vivo* the same metals cannot be used to elute manganese from the manganese pool, suggesting that manganese exists in the body in a different form to which it is used in the *in vitro*

experiments. It is suggested that in the body manganese exists in the unstable trivalent state (Cotzias and Greenhough, 1958). Analysis of the subcellular fraction of liver cells indicates that there is a remarkably constant inverse relationship between the amounts of two closely related metals, manganese and iron, in the fractions (Thiers and Vallee, 1957).

Manganese is excreted almost entirely by way of the bile; only a small proportion is excreted in the pancreatic juice.

Interesting circumstantial evidence has been produced to suggest that manganese like zinc but unlike cadmium is an essential trace metal. The absorption, distribution and excretion of radioactive zinc are variable depending on the previous level of zinc intake (Cotzias *et al.*, 1962). Cadmium (which is not an essential trace metal) does not affect the absorption, distribution or excretion of radioactive cadmium (Cotzias *et al.*, 1961). In experiments with manganese-deficient animals excretion of injected radioactive manganese occurs despite the deficiency state; when animals were fed large doses of stable manganese by mouth the organ uptake of radioactive manganese increased in proportion to the amount taken by mouth. These findings suggest that variations in manganese excretion are more important than variation in absorption in regulating tissue levels. In addition radioactive manganese is distributed into two main compartments, one with a slow rate of exchange which is mainly the bones, and one with a rapid rate of exchange which is mainly the liver (Britton and Cotzias, 1966). The size and rate of turnover of the slow compartments is related linearly to the amount of manganese in the diet. Attempts to demonstrate that the manganese concentration of different organs is under adrenal or ACTH control proved negative (Hughes *et al.*, 1966).

Excretion of manganese is almost entirely by way of the bile although some of the metal can be excreted by other intestinal juices especially in conditions of manganese overload or when biliary obstruction is present. An enterohepatic circulation of manganese has been demonstrated (Papavasiliou *et al.*, 1966). The disappearance of radioactive manganese from the body of normal subjects is a curve having two exponential components. An average of 70% is eliminated by the slow pathway which has a half-life of 39 days. The remainder is eliminated by the fast pathway which has a half-life of about four days. Increasing the manganese intake causes a more rapid elimination of injected radioactive manganese (Mahoney and Small, 1968).

Transport of manganese in the blood

The fact that *in vitro* experiments with manganese could be reproduced using two closely related metals of the same group in the periodic table (iron and chromium) and the fact that the same metals

could not affect manganese metabolism and turnover *in vivo* led to the suggestion that there is a specific manganese binding protein in the body and that manganese exists in the body at a different valency and in an unstable form. When bound to its specific transporting β globulin (transferrin) iron also exists in an unstable form with a valency of three. A specific β globulin which carries only manganese has been identified and is called transmanganin. Evidence exists that on this protein manganese has a valency of three. Transferrin will not take up any manganese although it is avid for iron. Transmanganin is probably responsible for the rapid transport of manganese to the intracellular mitochondria in which it accumulates preferentially (Cotzias and Bertinchamps, 1961).

Manganese in the blood was believed to be more or less equally distributed between plasma and cells (Bowen, 1956). More recent work has suggested that only a small amount of manganese is incorporated into erythrocytes (Mahoney and Small, 1968). High manganese intake in animals can increase the serum manganese which results in a marked depression of magnesium to such an extent that the affected animals suffer from tetany (Blakemore *et al.*, 1937).

Functions of manganese in the body

Necessity for arginase, alkaline phosphatase and other enzymes

Arginase and alkaline phosphatase activity in the liver of manganese-deficient animals are significantly reduced (Shills and McCollum, 1943). The arginase activity can be improved by adding manganese (or other divalent metals) *in vitro*. However, there are two snags in considering arginase either as a manganese metallo-enzyme or that manganese is an essential specific cofactor. The one is the beneficial effect of other divalent metals and the other is the fact that birds do not excrete nitrogen by means of the arginine-urea cycle, hence they have little or no arginase but they do develop similar deficiency syndromes as animals when fed manganese-deficient diets.

The rapid accumulation of injected radioactive manganese in the mitochondria has led to the suggestion that manganese is a necessary cofactor for certain respiratory enzymes (Maynard and Cotzias, 1955).

Manganese is also critical to enzymes involved in mucopolysaccharide synthesis (Robinson *et al.*, 1966). Defective mucopolysaccharide synthesis is known to occur in manganese-deficient animals (Leach and Muenster, 1962). The most certain manganese metallo-enzyme is pyruvate carboxylase which is involved in the metabolism of pyruvate (Scrulton *et al.*, 1966).

Cholesterol synthesis

Manganese is excreted via the bile probably combined with bilirubin.

In biliary cirrhosis the manganese content of the liver is elevated (Worwood *et al.*, 1968). Manganese is believed to be an essential cofactor for one (or more) of the enzymes involved in cholesterol synthesis (Amdur *et al.*, 1957). There is some evidence that manganese increases cholesterol synthesis in the rat liver *in vitro* (Curran, 1955). This has led to attempts at reducing cholesterol by administering a chelating agent EDTA in the calcium phase in an attempt to reduce the availability of trace metals (including manganese and cadmium) believed to be essential for the production of cholesterol (Schroeder, 1956).

However, the reverse has also been claimed, namely that high manganese levels may be beneficial because there is experimental evidence that manganese supplements to the diets of manganese-deficient animals result in a marked reduction in fat deposition (Plumlee *et al.*, 1956).

Hydralazine lupus erythematosus (Alarcon-Segovia *et al.*, 1967)

Hydralazine (apresoline) is a drug which was previously used extensively in the treatment of hypertension and is still used occasionally. In common with a number of other drugs (including procainamide) long continued use of hydralazine can produce a syndrome identical to idiopathic lupus erythematosus. The same syndrome can be reproduced in dogs given hydralazine in the diet. As well as being an anti-hypertensive drug hydralazine also acts as a chelating agent; birds given hydralazine develop a syndrome identical to the known manganese deficiency syndrome. In birds and dogs the syndrome can be completely prevented if manganese salts are given before the hydralazine. Manganese salts inhibit the formation of human LE cells *in vitro*. Patients suffering from hydralazine-induced lupus erythematosus and the naturally occurring disease have benefited from the administration of manganese salts (Comens, 1956). Recent review of the hydralazine lupus syndrome has shown that it is not due to an allergy to the drug (Alarcon-Segovia *et al.*, 1967).

While investigating the hydralazine syndrome it was found that there was a slow turnover of radioactive manganese in patients who had the joint manifestation of the syndrome which closely resembles the joint changes of rheumatoid arthritis. Further reasons for suspecting that there could be a link between rheumatoid arthritis and disturbed manganese metabolism were that in manganese deficiency there is defective mucopolysaccharide synthesis and that in rheumatoid arthritis there are also abnormalities of mucoprotein and mucopolysaccharide metabolism. It has been demonstrated that radioactive manganese turnover in the body is slower than normal in active rheumatoid arthritis—this turnover returns to normal when the disease is in either spontaneous or therapeutic remission (Cotzias *et al.*, 1968b).

Manganese and neurological disorder

Manganese deficiency in animals is known to give rise to ataxia and convulsions. Manganese intoxication in humans can give rise to Parkinsonism, extrapyramidal syndromes, hallucinations and psychomotor instability (Cummings, 1965).

In addition *in vitro* manganese interacts with phenothiazines to give compounds similar to those present in normal melanin. Manganese is known to accumulate in liver cells stimulated by dopamine and other biogenic amines (Papavasiliou *et al.*, 1968).

Cotzias and his colleagues thought that the decreased melanin in the substantia nigra might be fundamental in the causation of Parkinsonism and tried to increase the pigmentation in Parkinsonism by giving melanophore stimulating hormone. Contrary to expectation this treatment worsened the Parkinsonism although skin pigmentation increased (Cotzias *et al.*, 1967). They suggested that amino acid precursors of both melanin and neurotransmitter catecholamines had been shifted from the brain to the skin.

Feeding of phenylalanine also worsened the Parkinsonism, suggesting that there was a defect in metabolism of phenylalanine; the known low concentrations of dopamine suggested that the block was just before dopamine formation (Cotzias, 1969). Dopamine is metabolized by an enzyme dopa decarboxylase. Feeding large amounts of L Dopa saturates the enzyme dopa decarboxylase preventing breakdown of any endogenous dopamine formed. Clinical trials with L Dopa in Parkinsonism have shown marked improvement first in hypokinesia, then rigidity and finally tremor. L Dopa is used because the racemic mixture D.L. Dopa may induce leucopenia. L. Dopa also improves the Parkinsonian signs of patients with chronic manganism. Further links between manganese, dopamine and phenothiazines are that manganese and phenothiazine are known to accumulate in melanin granules (Cotzias *et al.*, 1964; Blois, 1965).

Phenothiazine drugs form co-ordinate complexes with manganese but not with any of the other similar metals (Borg and Cotzias, 1960). It has been noted that many of the features of manganese toxicity are similar to those of phenothiazine toxicity (Cotzias, 1962). It is possible that phenothiazines act as concentrators and carriers of body manganese and that they cause their action on the brain by increasing local concentration of manganese.

Manganese, Parkinsonism and L Dopa

The neurological features of idiopathic Parkinsonism, chronic manganism, Wilson's disease and phenothiazine administration may be similar. All are characterized by disturbance of the extrapyramidal

system. The improvement that occurs in Wilson's disease with copper chelating agents led Cotzias and his colleagues to investigate chronic manganism to establish whether manganese chelating agents would be similarly beneficial in treating the extrapyramidal signs. However, it was found that high levels of tissue manganese were found in healthy manganese workers whereas former manganese miners crippled with the signs of manganism had normal tissue levels of manganese. Thus neurological damage induced by excess manganese remained after manganese had been removed from the tissues (Cotzias *et al.*, 1968a). The two known biochemical abnormalities in the brain in Parkinsonism are decreased melanin concentration in the normally deeply pigmented substantia nigra and decreased concentrations of dopamine (3,4-dihydroxy-phenylalanine). Dopamine is also reduced temporarily by reserpine when it causes Parkinsonism (Carlsson *et al.*, 1958). Dopamine is a common precursor of both melanin and neurotransmitter catecholamines. These findings suggested a link between the reduced melanin concentration and neurological dysfunction.

Manganese and intrahepatic cholestasis

Extrahepatic biliary obstruction causes characteristic changes in the microvilli and Golgi apparatus of hepatic cells. Manganese overload causes identical histological changes and results in cholestasis. Although manganese toxicity can produce hepatocellular damage cholestasis is not a feature of this type of damage. The cholestasis of manganese overload is likely to be due to a defect in the transport of bilirubin (Witzelben *et al.*, 1968). The liver cell content of manganese is elevated in biliary cirrhosis of the liver (Worwood *et al.*, 1968).

Effect of manganese on catecholamine function

Recent evidence has suggested that catecholamines may produce contraction by a mechanism independent of membrane depolarization. It is known that calcium is involved in smooth muscle contraction and that manganese ions inhibit selectively calcium permeability in selected tissues. Manganese has been shown to inhibit smooth muscle contraction in response to angiotensin and noradrenalin. This effect can be offset by increasing the concentration of calcium (Sullivan and Briggs, 1968).

Effect of manganese on atrial contraction

Manganese has a negative inotropic action on the isolated rabbit left atrium. It blocks the uptake of radioactive calcium by the muscle cells and also acts competitively against ouabain which is known to exert

some of its action by promoting the uptake of calcium by the muscle cells (Sabatini-Smith and Holland, 1969).

Manganese and the blood

Iron deficiency anaemia leads to increased absorption of iron, cobalt and manganese. This suggests a common pathway for their absorption (Pollack *et al.*, 1965). Large doses of parenteral iron given to animals do not lead to cirrhosis (Brown *et al.*, 1959), whereas both manganese and cobalt produce cirrhosis when given parenterally although the cirrhosis of manganese toxicity resembles biliary cirrhosis. Nevertheless it is possible that the cirrhosis of haemochromatosis is not due to the excessive absorption and deposition of iron in the liver but is due to the excessive absorption of cobalt and manganese which accompanies the excessive iron absorption. Confirmation of this interesting hypothesis has been suggested by the finding of increased concentrations of manganese in the livers of patients dying from haemochromatosis (Altstatt *et al.*, 1967). By saturating the absorption systems with manganese or cobalt it is possible that iron absorption would be affected. Diets high in manganese do lead to deficient iron absorption in animals (Hartman *et al.*, 1955).

Increasing the iron content of the manganese-rich diet overcomes the depressing effect of the high manganese diet on haemoglobin formation (Matrone *et al.*, 1959). This suggests that manganese does not act only by saturating the binding sites, if this were the way it acts increasing the ingested iron should not increase the iron absorption. Another suggested mechanism of manganese-iron antagonism is that manganese shifts the ionic state of iron from the ferrous to the ferric form making it less easy to absorb (Somers and Shive, 1942). Radioactive manganese is quickly and irreversibly incorporated into erythrocytes (Mahoney and Sargent, 1967). Within the cells the evidence suggests that manganese exists as a porphyrin compound, suggesting that it may have a role in the porphyrin metabolism of the cells (Borg and Cotzias, 1958).

Manganese and the skeleton

Manganese deficiency in animals and birds gives rise to recognizable and specific deficiency syndromes which are remarkably similar regardless of species. The effects on the bones are to produce thickening and shortening, resulting in slipping of the tendons off the joints–'slipped tendon' disease or perosis. The abnormalities are preventable by supplementing the diet with manganese alone. The skeletal deformities characteristic of manganese deficiency are (Underwood, 1962):

1. Reduction in length, density and breaking strength of the bone.
2. Arrest of skeletal maturation due to suppression of endochondral osteogenesis.
3. No osteoporosis or bone resorption such as occurs in rickets.
4. Vitamin D utilization is not affected.
5. Chondrogenesis as well as osteogenesis are affected.

These results do not suggest that manganese deficiency has a direct primary effect on calcification. Alkaline phosphatase levels have been variously reported as reduced or normal in manganese deficiency (Underwood, 1962).

In birds the manganese content of the egg shells is reduced. The pattern of deformities of the skeleton suggests the defect is mainly in cartilage development and suggests a relationship between manganese and mesenchymal connective tissue, the three most important components of which are connective tissue cells, extracellular fibres and extracellular amorphous ground substance. The principal compounds in the amorphous ground substances are hyaluronic acid, chondroitin sulphate A, B and C, and heparin. The precursors of these substances are mainly hexosamine and hexuronic acid. Manganese deficiency has been shown to reduce the concentration of hexosamine and hexuronic acid (Leach and Muenster, 1962). These results have been confirmed by Tsai and Everson (1967), who also showed that there is a reduction in all components in the amorphous ground substance.

Manganese deficiency and congenital abnormalities in the guinea pig

Manganese deficiency in guinea pigs leads to a high rate of stillbirths. There are disturbances of otoliths in the ear, abnormal E.C.G. and reduced acid mucopolysaccharide in rib cartilages. The animals which survive to adult life have reduced resistance to infection, ataxia and tremor. There is also marked hypoplasia of the pancreas which persists to adult life. The pancreas contains fewer islets and the islets contain fewer β cells. Manganese supplements given in adult life increase the number of pancreatic islets. Glucose tolerance is impaired in the few offspring who survive when manganese deficiency has been present in the mother (Shrader and Everson, 1968).

Manganese concentration in the skin

Manganese is known to be an essential cofactor for the enzyme arginase which in the skin resides mainly in the prickle cell layer of the epidermis (Molokhia and Portnoy, 1969). In psoriasis which causes thickening of the prickle cell layer arginase activity (and presumably

manganese requirement) is greatly increased. Arginase activity is probably associated with the production of a specific keratinous protein (Crounse and Rothberg, 1961). The mean concentration of manganese in the epidermis of human skin is remarkably constant, suggesting an active homeostatic mechanism which is presumably only present for an essential substance.

Manganese and gene manipulation

The action of genes may differ according to differences in the environment in which they find themselves. In other words it should, theoretically, be possible in rare instances by altering the environment of a gene to prevent a congenital defect due to the presence of a gene normally responsible for the defect; such a finding could have awesome implications (Erway *et al.*, 1970).

Manganese deficiency in pregnant mice leads to offspring which suffer from ataxia due to a disorder of the otolith; an identical disorder can arise as a result of mutation. When homozygous mothers for the ataxic gene are given a *normal* diet most of their offspring have ataxia. If homozygous mothers for the ataxic gene are given manganese, ataxia does not occur in the offspring. In other words manganese has prevented the development of a defect in the presence of the gene for that defect. Further evidence of this interaction is that when the few non-ataxic offspring of homozygous females are bred and fed on a normal diet *their* offspring develop ataxia. This indicates that the manganese supplementation has suppressed the expression of the mutant gene.

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CHAPTER V

Molybdenum

Introduction

Molybdenum is now accepted as being an essential trace metal. It is necessary for the prosthetic group of the flavoproteins, xanthine oxidase and aldehyde dehydrogenase in mammals and nitrate reductase in plants. Deficient molybdenum intake in animals has been thought to produce xanthine renal calculi in sheep, to reduce the growth rate of experimental animals and to deplete the liver of xanthine oxidase. Apart from its effect on dental caries no specific molybdenum deficiency syndrome has been found in man, although in South Africa there is a significant correlation between molybdenum deficiency in the soil and oesophageal carcinoma.

Excess molybdenum in the diet of grazing cattle has been frequently observed and a complicated relationship between molybdenum, copper and sulphate is known to exist. The foods used in human consumption which contain large amounts of molybdenum are peas and other legumes. Excess molybdenum in the diet is known to precipitate copper deficiency syndromes in animals whose copper intake is at a low but not critical level had the molybdenum intake been normal.

Absorption

Most hexavalent molybdenum salts, whether soluble or insoluble, are rapidly absorbed from the gastro-intestinal tract. Tetravalent molybdenum salts are poorly absorbed (Fairhall *et al.*, 1945). Absorbed radioactive molybdenum is excreted mainly by way of the urine but some is also excreted by way of the bile (Comar *et al.*, 1949). The speed and extent of absorption and excretion of oral and parenterally administered molybdenum is dependent on the sulphate content of the diet. A small increase in inorganic sulphate in the diet in sheep increases the urinary excretion of molybdenum enormously. Inorganic sulphate causes a profound drop in blood molybdenum levels and has been used successfully to cure cattle suffering from molybdenosis. Sulphate is believed to prevent the renal tubular reabsorption of molybdenum (Underwood, 1962).

Food content

The molybdenum content of plants is variable and depends on the molybdenum content of the soil on which they grow. Molybdenum salts are not absorbed from acidic soils even though their molybdenum content is high. Alkalinizing the soil (with lime) increases molybdenum availability to plants. Plants generally particularly rich in molybdenum are legumes and cereals. The form of molybdenum in these plants is readily utilizable by animals (Westerfield and Richert, 1953).

Distribution

The molybdenum content of organs depends on the amount in the diet of molybdenum (Davies *et al.*, 1960), tungsten salts and inorganic sulphate (Dick, 1954 quoted by Underwood, 1962). The liver and kidney contain a higher concentration of molybdenum than other tissues in mammals. No tissue or organ seems to concentrate molybdenum preferentially. Within the plasma molybdenum is probably carried as the anion. There does not appear to be any molybdenum-carrying protein (Underwood, 1962).

Function and metabolism

The flavoenzymes xanthine oxidase and aldehyde oxidase both contain molybdenum, both these enzymes occur in animal tissues. Molybdenum is also an essential metal for certain exclusively plant enzymes (Mahler *et al.*, 1954).

Xanthine oxidase levels in rats respond to alteration in the dietary intake of molybdenum (De Renzo, 1954). However, molybdenum intake in mammals cannot be reduced to a level at which xanthine oxidase is inactive. Xanthine oxidase is essential for the metabolism of uric acid and lack of the enzymes should cause a reduction in uric acid excretion. Sodium tungstate increases the excretion of molybdenum and competitively inhibits the utilization of molybdenum in the formation of xanthine oxidase (De Renzo *et al.*, 1953). In chicks it has been possible by feeding low molybdenum diets and sodium tungstate to induce true molybdenum deficiency. This results in a fall in tissue levels of xanthine oxidase and tissue concentration of molybdenum and a rise in the urinary excretion of xanthine and hypoxanthine. In addition there is increased mortality and decreased growth of the affected birds. These findings were prevented by adding molybdenum to the feed (Higgins *et al.*, 1956). Similar findings have been reported by two other groups of workers (Leach and Norris, 1957; Reid *et al.*, 1956). There is some suggestion that the form in which molybdenum occurs in the diet affects its availability.

Sheep grazing pastures in New Zealand deficient in molybdenum have a high incidence of xanthine urinary calculi. The liver xanthine oxidase levels are also low in the affected animals. Treating the affected pastures results in disappearance of the high incidence of calculi. It is suggested that the molybdenum deficiency might be responsible by increasing the excretion of xanthine in the urine (Askew, 1958 quoted by Underwood, 1962).

Molybdenum and dental caries in humans

The incidence of dental caries was found to be lower in parts of Hungary than would be expected from the fluoride content of the water supply (Adler, 1956). On investigations it was found that the molybdenum content of the drinking water was high. Further studies from New Zealand, from the cities of Hastings and Napier, showed that incidence of caries in Napier was significantly less than in Hastings, although both towns had the same water supply. However, inhabitants of Napier consumed vegetables which had been grown in soil which was under the sea until raised by an earthquake 30 years ago. The concentration of molybdenum was much higher in this soil than in that around Hastings. The molybdenum content of the teeth of boys living in Napier was higher than in those from Hastings, although the hair content of molybdenum from boys from both cities was the same (Bate and Dyer, 1965). Other evidence suggesting a role for molybdenum deficiency in dental caries is that in Somerset the incidence of caries is high in children from areas where the cattle suffer from molybdenum deficiency (Anderson, 1966).

Many workers have given molybdenum to animals and confirmed its anti-cariogenic properties; however, in some cases the dose of molybdenum used was high (Jenkins, 1967). It is not yet established what is the effective anti-cariogenic dose of molybdenum, at what stage in tooth formation it acts or whether there is any relation between fluoride and molybdenum.

The possible sites of action of trace elements in preventing dental caries are (Büttner, 1969):

1. Enamel and dentine
   a. Influence on physical properties
   b. Mineralization either during the pre- or post-eruptive periods.
2. Influence on structure and morphology of teeth, e.g. causing hypoplasia or affecting the size of the crown.
3. Effect on saliva, e.g. physical and chemical properties or enzymes it contains.
4. Effect on dental plaques.
5. Interaction with other trace elements, e.g. affecting absorption or retention.

Molybdenum has been shown to reduce the solubility of teeth in acid and also reduces the acid output by the salivary glands. It is more likely that molybdenum acts by affecting the morphology of teeth than other mechanisms (Jenkins, 1963). The relationship between molybdenum lack and dental caries has been confirmed (Anderson, 1969). There is believed to be an additive effect between the benefits of fluoride and molybdenum. Fluorine is undoubtedly more important than molybdenum. Molybdenum is known to increase the absorption of fluoride from the stomach (Crane, 1960).

Oesophageal carcinoma and molybdenum deficiency in humans

In 1962 there was a report of a fivefold increase in oesophageal cancer in South African Bantus inhabiting the reserves of the Transkei. In about 10% of instances oesophageal carcinoma occurred in unrelated members of the same homestead. In a painstaking survey it was found that there were highly discrete localized areas where the incidence of carcinoma was extremely high (Burrell, 1962). Further work showed that the areas of high incidence were all in localities of low fertility which had lost their fertility following severe drought and storms in the early 1930s. The gardens of families with proven cases of oesophageal cancer were fully investigated and molybdenum deficiency was the dominant mineral deficiency in each one. Molybdenum deficiency in maize (the staple food) predisposes to infection of the maize with fungi which are known to be toxic to animals; one of these is *Aspergillus flavus* which is known to be carcinogenic (aflatoxin) (Burrell *et al.*, 1966). Further investigations are in progress to elucidate the mechanism of oesophageal carcinogenesis in the higher cancer areas of the Transkei.

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CHAPTER VI

Chromium

Introduction

Chromium is the latest to be added to the list of biological metals essential for man. Unlike the other essential metals it is essential only for the higher animals. Chromium deficiency syndromes have been demonstrated in rats which develop impaired glucose tolerance when fed chromium-deficient diets. In 1955 chromium was identified as the previously unidentified 'glucose tolerance factor' missing from the diet of animals with disturbed glucose tolerance due to dietary deficiencies. Chromium repletion restored glucose tolerance to normal. Chromium acts as a cofactor with insulin and experiments in humans have confirmed that it is involved in carbohydrate utilization. It also has an effect on lipid and cholesterol synthesis, amino acid utilization and nucleic acid synthesis. The main value of radioactive chromium salts in medicine has been the labelling of erythrocytes. Chromium possibly acts as cofactor for the enzyme phosphoglucomutase It is the only essential biological metal whose tissue concentration declines with increasing age.

Absorption

Trivalent chromium salts are poorly absorbed from the gastro-intestinal tract. Only about 1% of ingested chromium salts is absorbed (Visek *et al.*, 1953; Donaldson and Barreras, 1966). Absorption is less at an acid pH and in the presence of food which binds the chromium hence making it unavailable for absorption (MacKenzie *et al.*, 1958). Chromium, when a component of organic compounds, is probably better absorbed than the inorganic salts because it has been found that the daily excretion of chromium is greater than could be accounted for by 1% absorption of the amount of inorganic chromium ingested (Donalson and Barreras, 1966; Schroeder, 1968). The organic form in which chromium occurs in the diet has not yet been identified. Inorganic chromium salts seem to be better absorbed in the hexavalent form (Donaldson and Barreras, 1966). The amount of chromium absorbed seems to be independent of the nutritional state of the animal

(Mertz *et al.*, 1965). Heating food to 120°C (as in preparing food for sealing in tins) causes trivalent chromium compounds to olate, that is to form complex compounds with water and hydroxyl ions which makes them less absorbable (Schroeder, 1968a).

Transport

Physiological amounts of chromium are transported in the blood attached to transferrin (Hopkins and Schwarta, 1964). Larger amounts of chromium are attached to and transported by other plasma proteins. The fate and distribution of chromium depend very much on the form in which it is given (Visek *et al.*, 1953). Trivalent inorganic chromium (chromate) is the only form of chromium which is taken up by red cells. Transferrin has two distinct metal binding sites and forms complexes with trivalent chromium, manganese and cobalt as well as iron and copper (Aisen *et al.*, 1969). Transferrin binds trivalent chromium in a way similar to that in which it binds iron so that trivalent chromium competes with iron for the binding sites. On the other hand hexavalent chromium is absorbed preferentially by the erythrocytes although probably not in the hexavalent form (Gray and Sterling, 1950). Other forms of chromium are either excreted rapidly by the kidneys or taken up rapidly by various organs. The reticulo-endothelial system particularly takes up chromium compounds, especially if they are in colloidal form, by phagocytosis of the particles (Mertz, 1969).

Excretion

Chromium is excreted mainly by way of the urine; some is also excreted by the intestines. The proportion excreted by each route is questionable but probably at least 80% of injected chromium is excreted by the kidney (Hopkins and Majarj, 1967) although considerable tubular reabsorption also occurs (Collins *et al.*, 1961). Tubular reabsorption probably accounts for the high concentration of chromium normally found in the kidney. The turnover rate and retention of chromium is not affected by nutritional status unlike the situation that obtains with some other essential biological metals (Mertz *et al.*, 1965).

Tissue distribution and concentration

The tissue concentration of chromium has been extensively studied in man. There is a marked fall in tissue concentration with increasing age, the neonate having much higher tissue levels than the adult. Only kidney and liver maintain the neonatal concentration of chromium into early adult life. There is a marked difference in human tissue concentration

depending on the locality in which the subjects lived from whom the samples were collected. In some parts of the world the kidney chromium level is nearly a hundred times that of the average concentration from kidneys obtained from the U.S.A. The significance of these differences is not known. The tissues containing the highest concentration are, at birth, lung, heart, kidney, spleen, aorta, liver; and at age 30-40, lung, aorta, pancreas, and testes, kidney, liver and spleen. There are marked variations in tissue concentration depending on age. Only the lung shows an increased chromium concentration with increasing age. Extremely high levels of chromium have been reported in the human brain (Voiner, 1953), particularly within the caudate nucleus (Leonov, 1957). The chromium level in the hair is normally high; the hair is a useful tissue source for assessing nutritional chromium status (Hambridge *et al.*, 1968). Too much significance is not attached to the tissue concentration of an essential trace metal: it may be only an inert passenger. The concentration of the metal in an organ does not necessarily indicate the size of the metabolic pool or the concentrating ability of the organ. Chromium is said to be the only essential biological metal which decreases in the tissues with increasing age (Schroeder *et al.*, 1962).

Concentration in food

The chromium content of an average daily diet is 7.8μg. The foods containing the highest concentration are certain condiments and spices (thyme especially), meat, parsnips, corn oil, cooked but not raw tomatoes and finally cigarette smoke (Schroeder *et al.*, 1962).

Tissue concentrating ability

Giving large doses of radioactive chromium by injection it has been found that there is a rapid uptake of the chromium by the bone marrow and the membrane of red blood cells. The uptake by the marrow is not associated with the cellular elements (Kraintz and Talmage, 1952). The speed of uptake of radioactivity is related to age for most tissues, the uptake declining with increasing age. The only exceptions to this are uptake by the lungs and intestinal wall (Vittorio *et al.*, 1962). Using more physiological doses of radioactive chromium Hopkins (1965) has found that there is a rapid uptake of chromium by the testes, bone and spleen. The effect of previous diet did not affect the uptake. It is suggested that the rapid uptake by the testes means that chromium is incorporated into spermatozoa (Hopkins, 1965).

At a subcellular level chromium is concentrated in the nuclear fractions in contrast to zinc, copper and manganese (Edwards *et al.*, 1961).

Chromium pool

Despite the rapid uptake of chromium by some tissues there is evidence to suggest that the chromium pool has a slow rate of turnover and that the changes that occur in the tissue and blood concentrations with injected labelled chromium do not necessarily reflect its uptake by the main chromium pool. This may be due to the fact that chromium in biological systems exists at a different valency to that of the labelled chromium which is normally given. There is some evidence that by allowing time between injection of labelled chromium salts and the studies on its distribution a more meaningful assessment of the chromium pool can be made (Mertz, 1969).

Further difficulty in assessing the metabolically active chromium pool is shown by the fact that the chromium concentration of the foetus (especially the bones) is high and goes on increasing until birth but that no labelled chromium, regardless of valency or chemical binding, can be shown to cross the placental barrier (Visek *et al.*, 1953). This finding has important implications, namely, that specific chromium complexes are required for physiological function in the same way that cobalt is essential but only as part of vitamin B_{12}. The physiologically active chromium complex has not yet been identified.

Metabolism and relationship to disease

So far as is known chromium is not essential for any mammalian enzyme although phosphoglucomutase is activated by magnesium, and a second metal. Chromium is the metal which is most active in this respect and it is the only metal which maintains the activity of the enzyme in the absence of magnesium (Stickland, 1949). Chromium is known to be constantly present in nucleic acids (Wacker and Vallee, 1959).

In 1955 it was first shown that rats fed a low chromium diet develop progressive impairment of intravenous glucose tolerance (Mertz and Schwartz, 1955). At first the missing factor from the diet was not identified and was therefore called the 'glucose tolerance factor'. This was later identified with certainty as chromium (Schwartz and Mertz, 1959). Several investigators have confirmed that chromium deficiency results in impaired glucose tolerance; there is also evidence that different chromium compounds (in which chromium is present at different valencies) differ in their ability to restore to normal an abnormal glucose tolerance which has arisen as a result of feeding chromium-deficient diets (Mertz, 1969). There is further evidence that impaired glucose tolerance may be due to a decreased response of the chromium-deficient animal to endogenous insulin (Mertz, 1969). From *in vitro* studies on the glucose uptake in response to insulin of various organs it seems that tissue

obtained from chromium-deficient animals has a reduced uptake of glucose compared with tissues obtained from chromium-replete animals. Mertz (1969) suggests that there are at least five theoretical possibilities to account for interaction between insulin and chromium:

1. Chromium could stabilize the structure of insulin in its most effective form.
2. Chromium may inhibit tissue 'insulinase'.
3. Chromium could increase the binding of insulin to tissues.
4. Chromium could be a cofactor for a membrane transfer mechanism of insulin.
5. Chromium could facilitate the initial reaction between insulin and a specific membrane receptor site.

At a cellular level chromium undoubtedly acts as a cofactor with insulin. The interaction is most likely to be due to a complex of chromium combining with sulphydryl groups of the cell membrane and disulphide groups on insulin (Mertz, 1967).

Effects on blood lipids and atheroma

Chromium increases the hepatic uptake of cholesterol and fatty acid. Chromium fed to rats for long periods results in a fall in blood cholesterol; this effect is greater in male animals (Schroeder, 1968b). Chromium also reduces the incidence of aortic atheromatous plaques (Schwartz and Mertz, 1959).

Chromium supplements (2mg/day of chromium acetate) have been shown to cause a reduction of an average of 14.1% in serum cholesterol in half of a group of patients studied.

Effect on amino acid metabolism

There is good experimental evidence for believing that utilization of certain amino acids is impaired in chromium deficiency (Mertz, 1969). These same amino acids (glycine, serine, and methionine) are known to form chromium complexes (Rollinson *et al.*, 1967). Chromium is present in nucleic acids (Wacker and Vallee, 1959).

Effects of chromium deficiency on growth

Chromium-deficient diets result in reduced longevity and reduced growth rate in animals (Schroeder *et al.*, 1963).

Chromium and human disorders

Glucose tolerance

The known decline of tissue concentration of chromium with increasing age and the low level of dietary chromium in many foods, particularly of refined carbohydrates, has led to suggestions that chromium deficiency in humans may be more common than is generally recognized. Overheating of foods possibly produces complexes of chromium which reduce its absorption (Schroeder, 1968a).

The high content of chromium in the foetus has led to the suggestion that multiparity may be associated with chromium deficiency. It has been confirmed that the chromium content of the hair of parous women is less than a third of that of nulliparous controls (Hambridge and Rodgerson, 1969). The hair chromium level of diabetic children is also markedly reduced (Hambridge *et al.*, 1968).

There are some interesting observations on glucose tolerance in elderly patients with diabetic types of glucose tolerance tests despite adequate amounts of circulating insulin. Glucose tolerance tests performed on elderly subjects returned to normal in 10 out of 15 when supplements of 150 μg/day of chromium chloride alone were given. The time taken for improvement to occur was 1-2 months. The tests remained improved as long as chromium supplements were given but became abnormal again when chromium supplements had been stopped for about a month (Levine *et al.*, 1968). Confirmation of improvement of abnormal glucose tolerance tests with chromium repletion has been given by Hopkins and Price (1968). Failure of chromium salts to influence glucose tolerance in a double blind trial has also been reported (Sherman *et al.*, 1968). The improvement in glucose tolerance with chromium supplements is so small that it is unlikely to have any practical value in the treating of diabetic patients (Glinsmann and Mertz, 1966). The reported observations are, nevertheless, intriguing.

Changes in chromium in protein deficiency

Kwashiorkor is usually accompanied by hypoglycaemia and impairment of glucose tolerance. Two groups of workers in different countries have produced divergent results. One group (Hopkins *et al.*, 1968) studying children with protein malnutrition from Jordan and Nigeria found that glucose tolerance could be improved significantly within 18 h when chromium supplements were given. The improvement does not occur if the malnourished children were consuming drinking water with a high chromium content. Another group (Carter *et al.*, 1968) working in Egypt could not demonstrate an improvement in glucose tolerance in protein-depleted children given chromium salts although

these authors emphasize that the children they studied were consuming large amounts of chromium in their food. They also emphasize the high chromium content of many commonly used therapeutic agents.

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CHAPTER VII

Cobalt

Introduction

Cobalt is widely distributed over the earth's surface. It is present in the tissues of all mammals and is an essential metal for animals and possibly for some plants. Cobalt deficiency syndromes have been recognized and treated in animals since the 1930s. Cobalt is an essential metal for vitamin B_{12}; all the cobalt deficiency syndromes in animals can be cured by injection of small amounts of vitamin B_{12}. The only certain function of cobalt in man is as an essential component of vitamin B_{12}, nevertheless there is some evidence that cobalt may also be essential for functions other than those in which vitamin B_{12} is involved, especially since cobalt exists in the body of man and animals in forms other than as part of vitamin B_{12}. Cobalt is unique among the essential biological metals in that it is an essential component of a vitamin and that some animals are entirely dependent of the microflora of their intestinal tract to synthesize an essential vitamin using an essential metal. Cobalt in excess produces polycythaemia and has been used in the treatment of refractory anaemias; small amounts of cobalt chloride added to beer induce a severe form of cardiomyopathy.

Cobalt and vitamin B_{12}

Sheep and cattle breeders learned from experience that pastures in certain parts of the world became unsound when animals grazed them for several months. The animals developed wasting and anaemia; the only cure was to remove the animals from the unsound pasture to another area known to be sound. In the new area the appearance of the grass may be inferior but the animals recovered quickly. In 1924 it was reported that iron salts would cure the wasting disease which developed on unsound pastures. It was later shown that pure iron had no beneficial effect and attention was focused on impurities in the original iron preparation. At first copper was thought to be the essential factor, then nickel and finally cobalt was identified as the essential metal which was the only one which alone would cure the affected sheep (Underwood

and Filmer, 1935). In 1935 feeding of pure cobalt salts was used in Australia to deal with sheep on affected areas and the land was dressed with cobalt salts which were taken up by the grass and thus became available to the animal (Marston, 1952).

In 1926 Minot and Murphy established the value of raw or lightly cooked liver in the treatment of pernicious anaemia. Attempts were made to isolate from liver extracts the pure 'anti-pernicious anaemia factor'. These attempts were unsuccessful mainly because of the estremely small amounts of vitamin B_{12} in the extracts and because the only way of assessing the potency of extracts was to observe the response of patients with pernicious anaemia after they had been given the extract. In 1948 pure crystalline vitamin B_{12} was isolated simultaneously in the United States and the United Kingdom. It was shown that cobalt was an essential part of the pure crystalline extract (Smith, 1948; Rickes *et al.*, 1948).

The next development in demonstrating that cobalt could be essential when given alone rather than being only indirectly essential because it is part of the vitamin B_{12} molecule was that cobalt-deficient lambs (with signs of vitamin B_{12} deficiency) responded if cobalt was given by mouth but not if given by injection. This suggested that cobalt might act in some way in connection with the microflora of the gastro-intestinal tract. Cobalt deficiency was found to reduce the number of gut organisms and cobalt repletion caused a return to normal numbers (Keener *et al.*, 1951). It was then shown that vitamin B_{12} was synthesized by micro-organisms (Abelson and Darby, 1949) and that there is much less vitamin B_{12} in the gut of cobalt-deficient sheep than normal (Hale *et al.*, 1950). The final link was made when Smith *et al.* (1951) showed that the synthesis of vitamin B_{12} by the microflora was the reason that cobalt salts were effective when given by mouth. The higher plants contain negligible quantities of vitamin B_{12}. The ultimate source of all vitamin B_{12} is synthesis by micro-organisms. Commercially vitamin B_{12} is produced by fermentation using *Propioni-bacterium* species. Originally *Streptomyces* organisms used for producing tetracyclines were used (Smith, 1965). Minute amounts of the vitamin are found in soil and pond water. Ruminant animals obtain their B_{12} from the micro-organisms in their gut; humans and carnivores are entirely dependent on the B_{12} in the diet; they appear unable to utilize any of the B_{12} which is almost certainly synthesized in the gut (Ungley, 1955).

Vitamin B_{12} can only be absorbed when present in physiological amounts when the glycoprotein intrinsic factor is present. This protein shows some species specificity and humans given animal intrinsic factor usually develop antibodies. The mechanism by which intrinsic factor promotes the absorption of vitamin B_{12} (but not its close analogues) is

not known. There is evidence that a releasing factor is required to release B_{12} from the intrinsic factor once absorption has occurred. B_{12} is normally bound to a specific carrying protein which is an α 1-globulin (Hall and Funkler, 1968).

Metabolic role of cobalt

Since the discovery in 1948 that cobalt is an essential component of vitamin B_{12} no separate metabolic role for cobalt in mammals has been identified with complete certainty although there is suggestive circumstantial evidence that inorganic cobalt might play an essential biological role (Schroeder *et al.*, 1967). The metabolism of vitamin B_{12} has been studied extensively since its discovery. Two recent reviews have summarized present knowledge of the vitamin, one is by Smith (1965), who coincidentally with American workers succeeded in obtaining the vitamin in crystalline form and the other is by a haematologist (Chanarin, 1969). These two reviews include descriptions of absorption, distribution, excretion, metabolic role and mechanism of action of the vitamin. They are authoritative and comprehensive and should be consulted for definitive information on the actions and metabolism of vitamin B_{12}. The major metabolic defect which results from vitamin B_{12} (cobalt) deficiency is failure of propionate and methionine metabolism and failure of deoxyribonucleic acid synthesis. However, the pathology of vitamin B_{12} deficiency cannot usually be identified with failure of specific enzymes. The main enzymes requiring B_{12} are (O'Dell and Campbell, 1970):

1. Methylmalonyl-Co A mutase
2. Methyl tetrahydrofolate oxido reductase
3. Homocysteine methyltransferase
4. Ribonucleotide reductase

Claims for an essential biological role for the cobalt ion alone

The cobalt ion is believed to be an essential cofactor for some enzymes but only glycyl glycine depeptidase (Dixon and Webb, 1964) is known to occur in animals. Among enzymes in bacteria which the cobalt ion activates are catalase and pyrophosphatase (Wesley, 1952; Oginske and Rumbaugh, 1955).

Russian workers have claimed that cobalt salts have been found to enhance the immune response of animals given injections of heat-killed in intestinal organisms (Babenko, 1965 quoted by Schroeder *et al.*, 1967). Other claims that cobalt has some biological function have been made but in general these preceded the recognition of cobalt as an essential part of vitamin B_{12}.

Cobalt chloride has been shown to have a synergistic effect *in vivo* with penicillin and other antibiotics; this has important implications. Cobalt chloride is now used with penicillin in the treatment of mastitis in cows and means that less penicillin is used and therefore less gets into milk used for human consumption (Pratt *et al.*, 1948).

Cobalt salts and haemopoiesis

Pharmacological doses of inorganic cobalt salts have been shown to induce polycythaemia in many species including man. The polycythaemia is accompanied by an increase in blood volume and hyperplasia of the bone marrow. Injection of large doses of vitamin B_{12} does not produce polycythaemia. The red cell mass can be made to double by administering cobalt salts. The exact mode of action of cobalt in causing polycythaemia is debatable. There are two main hypotheses each of which is supported by some experimental evidence. The first is that cobalt acts as an enzyme poison inhibiting local oxidation enzymes in the bone marrow causing hypoxia and hypoxic stimulation of the marrow; the other is that cobalt stimulates the production of erythropoietin.

It has long been known that cobalt will increase polycythaemia which has already developed in response to change of altitude (Cohn and D'Amour, 1951). This suggests that both the postulated mechanisms of action are probably present. In favour of direct anoxic stimulation of the bone marrow is the fact that cobalt will induce erythroid hyperplasia when infused into an isolated limb (Fisher *et al.*, 1964). Cobalt in high doses is known to be a metabolic poison and interferes with cellular oxidation (Orton and Bucciero, 1948). Other workers have been unable to confirm that cobalt reduces marrow respiration and oxygen uptake (Yastrebov, 1966; Warren *et al.*, 1944). Cobalt has also been reported to increase globin synthesis and inhibit haem synthesis (Morrell *et al.*, 1958). Cobalt can reduce haem synthesis at a concentration less than that which is required to reduce marrow oxygen consumption (Laforets and Thomas, 1956).

The discovery and isolation of erythropoietin as a result of a large amount of circumstantial evidence that it must exist led to a re-investigation of cobalt-induced polycythaemia. The main evidence indicating that humoral control of erythropoiesis must exist can from the known erythrogenic effect of plasma from anaemic patients; from parabiotic animal experiments in which one animal of the pair was made hypoxic while the other demonstrated increased erythropoiesis and from a beautifully conceived experiment on a patient with a patent ductus arteriosus and pulmonary hypertension (Stohlman *et al.*, 1954). Blood oxygen saturation was normal above the diaphragm but decreased

oxygen saturations were present in blood from below the diaphragm, the sternal marrow showed increased erythropoiesis humoral factor produced somewhere below the diaphragm.

Cobalt alone among the essential biological metals is known to increase erythropoietin levels in the blood (Goldwasser *et al.*, 1958). Animals submitted to bilateral nephrectomy before being given cobalt salts only develop a small increase in erythropoietin levels (it is known that approximately 10% of erythropoietin is produced from extrarenal sources) when given cobalt salts (Jacobson *et al.*, 1957).

When cobalt is perfused into the isolated dog kidney the amount of erythropoietin in the perfusate is increased; there is also decreased renal oxygen consumption (Fisher and Langston, 1967). Cobalt increases the local lactic acid dehydrogenase iso-enzymes in the kidney indicating local cell damage (Jensen and Thorling, 1965). These findings indicate that cobalt probably increases erythropoietin production by the kidney by producing local tissue anoxia due to inhibition of cell respiratory enzymes. Cobalt salts also have a direct effect on the bone marrow.

Because of their known ability to produce polycythaemia cobalt salts have been used to treat anaemia. Most of clinical studies using cobalt were done before erythropoietin could be measured; the cobalt salts were often given in amounts which produced toxic side effects. Cobalt salts were used more or less indiscriminately to treat all refractory anaemias. It is not to be expected that cobalt salts will benefit cases of anaemia in which the erythropoietin level is already high. It is also unlikely that stimulating erythropoietin output will benefit anaemia due to primary failure of the bone marrow although there are well-documented cases of aplastic anaemia responding to treatment with cobalt salts (Voyce, 1963).

A large number of workers have described erythropoietin levels in the different types of anaemia (Krantz and Jacobson, 1971). It is clear that there are many exceptions to the general trend which emerges. In general anaemias due to haemorrhage and haemolysis have high blood and urine erythropoietin levels which stimulate the bone marrow, producing erythroid hyperplasia. Anaemias accompanied by under-production of red cells may either have low levels of erythropoietin which is partly responsible for the failure of compensating erythropoiesis or the under-production of red cells may be due to marrow damage and the increased blood erythropoietin which occurs is incapable of increasing erythropoiesis further. It is in the groups in which erythropoietin levels are low and there is under-production of red cells by the bone marrow that cobalt salts might reasonably be expected to have some therapeutic value. The anaemias of chronic infection and endocrine deficiency (pituitary and thyroid hypofunction) seem to be accompanied by a low level of erythropoietin. The anaemias of renal failure, carcinomatosis and

protein deficiency usually have a high blood erythropoietin level although in each of these situations, exceptional instances are cited in which erythropoiesis has been increased both in man and in experiment animals when erythropoietin or cobalt salts have been given. The problem is complicated by the fact that an inhibitor to erythropoietin may exist which renders erythropoietin ineffective even though it is present in amounts which are generally sufficient to stimulate erythropoiesis.

In the polycythaemias, secondary polycythaemia usually has raised erythropoietin levels whereas primary polycythaemia has erythropoietin levels which are within the normal range.

Cobalt salts and the treatment of anaemia

The known effects of cobalt salts in producing polycythaemia and increasing erythropoietin inevitably led to their use in the therapy of refractory anaemias. Their use for this purpose has now largely been abandoned in orthodox medical practice on account of their indiscriminate use when they were in vogue, the high incidence of toxic effects which inevitably ensued and because of doubts about their efficacy.

Around 1955 cobalt salts had a considerable vogue in the treatment of refractory anaemias (Lancet, 1955); in particular they seemed to be beneficial in the anaemias of prematurity and infection in children (Coles, 1955). It was, however, used in all the anaemias which proved refractory to other acknowledged haematinics. Several reports of the beneficial effects of cobalt chloride in pure red cell aplasia have appeared. The time relationships of remission and re-occurrence of relapses on ceasing treatment make it difficult to believe that the improvement has always been a coincidence (Voyce, 1963). One of the reasons for finding it difficult to prove whether cobalt salts have any beneficial action however transient or in how few patients is due to the fact that the absorption of cobalt salts is irregular and can be influenced by many factors. Factors known to *decrease* cobalt absorption are (Paley and Sussman, 1963):

1. Tagging the salt to a protein binder
2. Alkaline pH
3. Enteric coated capsules
4. Administering tablets after a meal.

Cobalt salts produce suppression of thyroid activity and can cause goitres. On the other hand cobalt deficiency and disturbance of iodine to cobalt ratios in the soil can also result in goitres in humans who live in areas where the soil contains little cobalt or the wrong proportion of

cobalt to iodine. It is suggested that cobalt is necessary for the first stage of thyroid hormogenesis. The capture of iodine by the gland may be the stage for which cobalt is required; increased amounts of I^{131} are fixed by the gland when cobalt salts are fed (Blokhina, 1970). There is some experimental evidence that the goitrogenic action of cobalt is due to interferences with uptake and transport of iodine by the thyroid gland.

Cobalt and cardiomyopathy

In 1965-1966 an epidemic of a severe form of cardiomyopathy occurred in Quebec, which had a 50% mortality. The cardiomyopathy occurred in such a localized area that a toxic cause seemed probable (*Canad. Med. Ass. J.*, 1967). After diligent searching it was discovered that all the affected patients were heavy beer drinkers and consumed only one type of beer. In 1965 various breweries started to add cobalt chloride to the beer; it had previously been shown in Scandinavia that cobalt chloride 'stabilizes' the froth on top of the beer. With the advent of newer, more powerful detergents and the inadequate rinsing of beer glasses some of the detergent remained when the glass was next used for drinking beer. The film of remaining detergent was sufficient to impair the froth of the beer. The addition of cobalt chloride to the beer 'stabilized' the froth despite the film of detergent. Similar cases of cardiomyopathy were reported from other places where cobalt salts were used for the same purpose, namely Leuven in Belgium and Nebraska and Minneapolis, U.S.A. (Kesteloot *et al.*, 1968).

Most of the affected patients had drunk beer for at least 10 years and all the cases in each area had drunk one type of beer. The cardiomyopathy was accompanied by a high incidence of polycythaemia and changes in the thyroid gland, both of which occur in cobalt toxicity. Other features of the cardiomyopathy were the high incidence of pericardial effusion and the low incidence of arrhythmias. Enzyme studies indicated that there was usually mild hepatic damage but enzymes of cardiac origin were not usually raised. In the patients who recovered the enzymes and liver function tests usually returned to normal. Electrocardiographic findings were not specific but included low voltage QRS complexes in the limb leads, P wave abnormalities, elevated ST segments in the praecordial leads, non-specific T and ST abnormalities and a paucity of arrhythmias.

The subsequent course of the survivors of the main epidemic is different from that of alcoholic cardiomyopathy in that the survivors of Quebec beer drinkers cardiomyopathy frequently regained normal cardiac status (Sullivan *et al.*, 1969).

Biopsy and autopsy studies on the hearts of patients with cobalt-induced cardiomyopathy have shown an accumulation of

glycogen (Hall and Smith, 1968), and up to 10 times the amount of cobalt as normal (Sullivan *et al.*, 1968). In cobalt cardiomyopathy the cobalt accumulates in the mitochondria. Experimental work has shown that the probable mode of action of cobalt in increasing cardiac glycogen is as follows: cobalt under aerobic conditions irreversibly chelates with the disulphide groups of lipoic acid which is an essential co-enzyme for the decaroxylation of pyruvate within the Krebs tricarboxylic acid cycle. Pyruvate and lactate accumulate and are metabolized by other routes to glycogen (Alexander, 1969).

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APPENDIX I

Concentration of essential biological metals in normal tissue (in μg/g of ash) (from Tipton and Cooke, 1963)

	Cu	Zn	Mn	Cr	Mo	Co
Adrenal	210	1600	36	10	14-15	4-5
Aorta	97	1900	11	4.5	0-4	2-4
Brain	370	820	20	0.8	0-4	0-2
Diaphragm	150	5000	17	3.7	0-4	3-5
Heart	350	2800	23	3.4	0-4	2-3
Oesophagus	140	3000	17	5.1	0-4	0-2
Duodenum	300	2500	70	3.4	1-4	1-3
Jejunum	250	2300	68	4.1	0-4	4-6
Ileum	280	3200	110	6.6	3-6	5-6
Caecum	220	3300	180	7.3	1-4	37
Sigmoid colon	230	2700	76	6.5	0-4	22
Rectum	180	3500	82	5.4	0-4	5-6
Kidney	270	4900	91	2.2	33	4-5
Larynx	59	1300	8	2.1	0-4	0-2
Liver	680	3800	130	1.5	81	4-5
Lung	130	1400	24	20	0-4	3-5
Muscle	85	4800	6	2.3	0-4	3-5
Ovary	130	1800	18	49	0-4	0-2
Omentum	190	1700	48	14	1-4	7-8
Pancreas	150	2400	110	3.7	0-4	1-3
Prostate	110	9200	19	2.2	0-4	1-3
Spleen	93	1400	11	1.3	0-4	1-3
Skin	120	1000	22	41	1-5	3-5
Stomach	230	2600	47	4.1	1-4	2-3
Testis	95	2900	19	2.4	0-4	2-4
Thyroid	100	2900	19	2.5	0-4	2-4
Trachea	65	980	14	4.7	0-4	1-3
Urinary bladder	120	3200	18	10	1-5	3-5
Uterus	110	2500	12	16	0-4	2-4

APPENDIX II

Food content of zinc, copper, manganese and chromium* in μg/g

Zinc (from Schroeder, 1967)

Oysters	1074
Kelloggs Cornflakes	76
Beef	56
Lamb	53
Dry cocoa	48
Egg yolk	35
Quaker oats	33
Nuts	32-45
Bread	31
Kippers	20
Haddock	17
Turnips	12
Parsley	9

Copper (from Schroeder *et al.*, 1966a)

Oysters	137
Corn oil	24
Margarine	24
Thyme	23
Black pepper	20
Dried split peas	12
Beef kidney	11
Nuts	7-23
Lamb chops	7
Sunflower oil	5
Carrots	3
Ground coffee	2
Apple	1

Manganese (from Schroeder *et al.*, 1966b)

Dried peas	12
Macaroni	10
Spinach	7
Rhubarb	4
Brussels sprouts	3
Bread	1
Cheese	1
Pork kidney	0.75
Nuts	0-35

Chromium (from Schroeder *et al.*, 1962)

Thyme	10
Black pepper	3
Corn oil	0.47
Cigarettes	0.39
Chicken breast	0.26
Butter	0.17
Tomatoes (cooked in stainless steel)	0.14
Parsnips	0.13
Lamb chop	0.12
Pork chop	0.10

* Figures for molybdenum not available

APPENDIX III

List of enzymes known to be influenced by mineral elements. Five types of relationships are shown. From Schweigart (1962).

Column I. The trace element (or mineral element) constitutes the prosthetic group.
Column II. The trace element (or mineral element) is an active part of the prosthetic group, or is incorporated into the enzyme itself.
Column III. Elements with an integrating function that is not understood as yet. Elements need not be specific and may replace each other.
Column IV. Faculative activators
Column V. Inhibitors of enzyme activity.

Enzyme	*I*	*II*	*III*	*IV*	*V*
Carbohydrases					
α-Amylase (animal)	Cl		Na, K, Li, Br, Sr, Mg, Ca, Ba	F, I	
α-Amylase (malt)		Ca, Cl			Fe, Cu
Lysozyme				S_2O_3, CN, S,	I, Cu
Pectin-polygalacturonidase				K, Na, Rb, Ca, Th, Al	
β-h-Glucosidase			K	S	
Hyaluronidase				K, Na, Ca, Ba, Fe, Mn, Br, I, SO_4, NO_3	F, Mg

APPENDIX III–*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Esterases					
Desoxyribonuclease			Mg, Mn, (Ca)	Ca, Ba	
Zoolipase			Ca, Mn, Cl	Pb, Mn, Na	
Phytolipase			Fe^{+++}, Al		F, PO_3, Ag, Cu, Hg^{++}
Lipoproteinlipase			W, Mo, Si		
Pectase			I, CN		
Cholinesterase		Ca	Co, Ba, Mg, Cu, Mn	S	F
Acetylcholinesterase			NaCl		
Phosphatidcholin-esterase			Mg		
Chlorophyllase			Ca	K, Na, Cl, NO_3	
Alkaline phospho-monoesterase I		Zn, Co, Mn, Mg	Ni, Fe^{++}, Ca		SH
Alkaline phospho-monoesterase II				Mg	CN
Acid phosphomono-esterase I					F, Mg
Acid phosphomono-esterase II		Co	Ca, Mg, Co, Ni		
Acid phosphomono-esterase III		Mg	Co	Ni	
Fructose-1,6-diphosphatase		Mg, Mn			Co, Ni, Zn, Cu
Phytase			Mg		

Nucleotidase		Mg		Zn, Fe^{++} Ag, Cu^{++}
Ribonuclease				F
Prostataphosphatase			Ce, La	
Tropinesterase		KCl, Na, Cl, NaBr, NaI, KCNS, $MgSO_4$, $CaCl_2$		F, CN
Phosphoprotein-phosphatase				Mo
Phosphatidic acid phosphatase		Mg		Ca, Ba, Mg, Mn
Glycocyaminase			Mn	
Arginindihydrolase			Fe	
Arylsulphatase			NO, Cl	
Desoxyribonuclease Type I (pancreas nuclease)	Mg, Mn		Ca	F
Desoxyribonuclease Type II (urine nuclease)	Mg			F
Micrococcus nuclease	Ca			F
Amidases				
Defence proteinases	S		SH	Sch-Me
Proteinases in general		K		
Papainase I (papain)	S, CN, SO_2		Ti, As, SH, S_2O_3, SO_2	Sch-Me, H_2O_2, I, SeO_2

APPENDIX III–*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Papainase II (papain)		S_2O_4			
Cathepsinase		S, CN, SH, S_2O_3, SO_2		Fe	Sch-Me, (Hg Cu)
Thrombokinase			Ca		
Bacterial endo-peptidase	Ca				
Trypsinase			Ca, Mn, NH_4, $MgSO_4$		
Rennin			Ca	Rare Earths	
Enterokinase			Ca		
Pepsinase			H, Cl		
Carosinase		Zn, Mn			PO_4, P_2O_7, F
Peptidase		Mg, Mn, Zn, Fe^{++}, Co^{++}	Zn, Co		
Dehydropeptidase I					S, CN
Folic acid conjugase				S	Hg, Ca
Yeast Polypeptidase				Zn, Cl, Br, I	
Aminotripeptidase			Me		
1-Leucin-amino-peptidase		Mg	Mn		CN, S, P_2O_7, Zn, Fe^{++}, Pb^{++}, Hg^{++}
Aminopolypeptidase		Co, Zn	Zn, Co		
1-Cysteinyl-glycin-dipeptidase				Mn, Co, Fe	
Glycyl-1-leucyl-dipeptidase II				Mn	

Glycylglycin-dipeptidase		Co, Mn		Zn
Glycyl-l-leucine-dipeptidase I		Mn	Zn, PO_4	Ca, $(Zn\text{-}PO_4)$
Prolinase I		Mn, Cd		PO_4, P_2O_7, F
Prolinase II				S, CN, Ag
Prolidase		Mn		Sn, Hg^{++}, F, Pb^{++}, Cd^{++}, P_2O_7
Tryptic carboxy-polypeptidase	Zn	Mg		
Catheptic carboxy-polypeptidase			CN	
Aminopeptidase		Mn, Mg		Zn
Histidase				Cu^{++}, Cd^{++}, Zn^{++}, KCN
DPN- and TPN-hydrolases			NO_3, NO_2, NH_4	Zn
Amino-acid acylases				Col
Arginindesimidase (yeast)		Co		Fe^{++}, I
Arginase a		Cd	Fe^{++}, Co, Ni	B, Zn, Mn
Arginase b		Mn	Cd, V	Ca, Fe, Ni
Citrullinase		AsO_5, Mg, Mn, Co, Zn	PO_4	
Asparaginase				Cu, Hg, Ag
Urease		Cd		S, CN, As, SO_3 Sch-Me, F, B
Glycocarbaminase			Mn	

APPENDIX III–*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Phosphohydrolases					
Pryophosphatase I		Mg	Mn	Al, Mg, Zn, Zr, Th, Pb, Fe, Co, La, Ce, Y, CN	Ca
Pryophosphatase II		Mg	Mn	Al, Mg, Zn, Zr, Th, Pb, Fe, Co, La, Ce, Y, CN	F
Polyphosphatases			Mg		
ATP-ase I			Mg	Ca, Na	
ATP-ase II		Mg			K, Ca
Triphosphatase		Mg, Fe, Co			
Apyrase (ATP-diphosphatase)			Ca		
ATP-Pyro-phosphatase				Cl, Br	
Oligometa-phosphatase		Mg, Mn, Co, Zn		Ca, Ba, Al, Ti, Fe, Ni, Cu, Zr, Th, Pb, La, Ce, Pr, Nd, Sm, Y	CN, F
Polymeta-phosphatase			Mn, Mg, Zn, Ca, Pb		Ag, Hg^{++}
Phosphohalogenase			Mn, Co		Hg^{++}, Cu^{++} Zn, Pb^{++}, (Mn)

Hydrolases with varying substrate specificity

C-C-Hydrolases	B_6-PO_4			
Halogenases			Cl, Br	
Iodine-tyrosine-deiodase			I	
Di-isopropylfluoro-phosphate halogenase		Ca, Mg, Co, Mn	Hg	Hg^{++}, Cu^{++}

Transglycosylases

Phosphorylase	Mg			

Transphosphatases (Kinases)

Glucose-I phosphate → amylose-trans-glucosidase			AsO_4	
Saccharase → ortho-phosphate-transglucosidase			AsO_4	
Adenylate kinase		Mg		
Creatin kinase	Mg			
Arginine kinase	Mn	Mg		
1,3-Diphospho-glycerate → ADP-Trans-phosphatase	Mg	K, NH_4		
Pyruvate kinase	K, Mg, NH_4, Rb			

APPENDIX III—*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Hexokinase		Mg			
Fructokinase		Mg	Mg, Mn	K	
Phosphofructokinase		Mg	K, NH_4		
Galactokinase		Mg, Mn			
Phosphoglucokinase		Mg, Mn			
Glucokinase		Mg			
Ribokinase (trans-ketolase)		Mg			
ATP → Nucleic-acid-trans-phophosphatases		Mg, F	PO_4		
Flavokinase			Mg, Mn		
Adenosinkinase			Mg, Mn		
Phosphoglucomutase			Mg		F
ATP → Nicotin-amide-mono-nucleotide-trans-adenylase		Mg			
ATP → FMN-Transadenylase		Mg			
ATP → DPN-Transphosphatase		Mg, Mn			
Transaminases					
Transaminase			Mg, NH_4, CO_2,		

Transglutaminase		Mn, PO_4		
Transpartase		Mn, PO_4		
Carbamyltransferase	Mg			
Transmethylases				
Transmethylase		Mg, Ca		CN, F, Ca
Transacylases				
Acyl-activating enzyme (acetyl-Co-A)	Mg	K, NH_4, Rb		Na, Cs
Cholinacetylase		PO_4, Ca, Mg, K		
Special Transferases				
Transketolase	Mg	PO_4		
Rhodanase		S_2O_3, Cu		AsO_2
Anaerobic Transhydrogenases				
Co-enzyme III		PO_4		
Co-dehydrogenase I = DPN		PO_4		
Co-dehydrogenase II = TPM		PO_4		
Aldehyde → TPN-transhydrogenase		PO_4, Ca, Ba, Mg, Mn		CN
Succinate dehydrogenase	Fe	PO_4	Ca, Al, Co, Rare Earths	

APPENDIX III—*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Formiat → DPN-Transhydrogenase			PO_4		
Formiat dehydrogenase	Cu		PO_4		
Oxalate dehydrogenase			Mg		
Choline dehydrogenase			Co		
Thiamine dehydrogenase				Mg	
α-β-unsaturated Acyl-CoA-reductase		Cu			
Fumarate reductase		Fe			
Saturase			Mn, Cl		
L-Dehydroascorbinate-reductase				PO_4, AsO_4	
Lactate dehydrogenase		Fe			
Alcohol dehydrogenase		Zn			
Nitrate reductase		Mo	PO_4	V	
Nitrite reductase		Fe	PO_4		
Hyponitrite reductase		Cu	PO_4		

Hydroxylamine reductase	Mn			
Glutathion reductase		Mg, Mn, PO_4		NaCl
Aerobic transhydrogenases				
Flavinenzyme I	Cu	PO_4, Mo, Fe		
Flavinenzyme II	Fe			
Diamine oxidase	Cu(?)	Co	PO_4	CN, Ca
Xanthine oxidase	Mo, Fe	PO_4		CN, Ca
Aldehydroxidase	Mo	NH_4, W		
Aldehydmutase (-oxidase)	Mo			
Bacterial Glucose-mannose-galactose-oxidase		Mg, Mn		
Anaerobic transelectronases				
TPN-Cytochrome c-reductase	PO_4			
DPN-Cytochrome c-reductase	Fe^{++}, PO_4		CN	Cu, Zn, Mn, Ca, Mg, PO_4
Cytochrome a, b, c,				P_2O_7, V
f, a_1, a_2, b_1	Fe^{++}			P_2O_7, V
Aerobic transelectronases				
Cytochromoxidase (Warburg's iron ferment)	Fe	Cl		

APPENDIX III—*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Phenoloxidase	Cu	PO_4, Ni, Co, V	K, Mg, Ca, Zn, Mn, Al, Fe		
Ascorbic acid oxidase	Cu				CN
Laccase	Cu	PO_4	Mn		
Uroshiol oxidase	Cu				CN, S
Ceruloplasmin		Cu		Cl, Br, NO_3	PO_4, SO_4
Uricase	Cu	Mn			CN, F
Luciferase		Mg	PO_4, Mn		P_2O_7
Hydroperoxidases					
Peroxidase		Fe^{+++}	NO_3, I		
Dioxymaleic acid oxidase		Mn, Fe^{+++}		F	
Cytochrome c-peroxidase		Fe^{+++}			
Ferro-peroxidase		Fe^{++}			
Lacto-peroxidase		Fe^{+++}			
Myclo-peroxidase		Fe^{+++}			
Indyl-3-acetic acid peroxidase		Fe, Mn			
Catalase		Fe			
Special redoxases					
Lipoxidase				V, B, Fe	

Homogentisinase		Fe^{++}		
Pyrocatechase		Fe^{++}		
Lactonizing enzyme		Mn		
Decarboxylases				
Cocarboxylase	PO_4, Mg	Mn		
Plant carboxylase	Mg	Co, Zn		
Pyruvate-oxidase factor	PO_4, Mg			
Carboxylase (pyruvate decarboxylase)	Mg, PO_4, Mn		Co, Fe, Zn, Ca, Cd	
Benzoylformate decarboxylase	PO_4			Mg
Oxylacetate-carboxylase	PO_4, Mn, Cd, Co	Mg		
Malic enzymes	Mn	Mg		
Malonate-decarboxylate system	PO_4		Mg, Mn	
Succinate decarboxylate system	PO_4, Mn			
Isocitric acid dehydrogenase	PO_4, Mn, Co	Mg		
Pyruvate dehydrogenase	Mg	Mn		
Bacterial amino-acid decarboxylases	PO_4	Me, Fe^{+++}, Mn		
Yeast decarboxylase	Mg			

APPENDIX III—*cont.*

Enzyme	*I*	*II*	*III*	*IV*	*V*
Triosephosphate-Lyases					
Aldolase (cymo hexase)		Me	Zn, Fe^{++}, Co^{++}, Cu	B	
Aspartase	Mg		Co, Cu		
Hydratases and dehydratases					
Aconitase		Fe^{++}			CN, S, F, Cu, Hg
Enolase	Mg		Mn, Zn	HPO_4, H_2, BO_2	F, Hg
Carbo-anhydratase	Zn				CN, S
Chloroplastinase		Mg			
Phosphogluconate-dehydratases		Fe, Mn, Mg			
Isomerases					
Galactowaldenase		PO_4			
D-Arabinose-isomerase				B, OH	
Glucose-1,6-phosphomutase			Mg, Mn, Co		
Glycerine-3,2-phosphomutase			Mg		
Racemases					
Alanine racemase			PO_4		

Glutaminate racemase		PO_4
C-N- and C-S-lyases and synthases		
Cystein-desulphydrase		Zn, Mn, Mg
Unclassified lyases and synthases		
Hydrogenlyases	Fe	Mn, PO_4
Hydrogenlyase	Mo	PO_4

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