The Influence of Trace Elements on Anemia

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THE INFLUENCE OF TRACE ELEMENTS ON ANEMIA

by

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INTRODUCTION

The fact that anemias constitute an important problem of public health has been realized only comparatively recently. When field surveys made in different countries before and after World War II had collected a great deal of information on dietary habits, food consumption levels, and nutritional status, it became more apparent that mild, moderate, and even severe anemic states existed in different communities. As was to be expected, anemia occurred more frequently in the malnourished people in the developing countries and the vulnerable groups of the population were the most affected (FAO/WHO Report, 1966).

In the early days, it was somewhat difficult to distinguish between the effects of infectious and parasitic diseases on the one hand and malnutrition on the other as contributory factors in the causation of anemia. However, a wider prevalence of anemia than that of infection and the occurrence of anemia in communities relatively free from infections and parasitic diseases clarified the basic role of malnutrition as a causative factor. When the effects of infection are superimposed on those of malnutrition, marginal deficiency may be converted into severe deficiency leading to its clinical manifestation as anemia. This statement is in agreement with that of many of the leading hematology physicians which is that anemia is a symptom of a disease or of an iron deficient diet. Other nutrients may also be involved.

Anemia is a condition in which the hemoglobin values and/or number of red blood cells are below normal. The trace elements which are considered to have influence on the formation or survival of red cells
and hemoglobin production are: iron, copper, manganese, zinc, chromium, cobalt, arsenic, cadmium, lead, molybdenum and selenium. Since iron is the central metal of the hemes, and it is used in greatest amount for hemoglobin synthesis, there is no doubt that among these elements, anemia caused by iron-deficiency is most prevalent.

Iron deficiency anemia is a medical and public health problem of prime importance, causing few deaths, but contributing seriously to the weakness, ill-health and substandard performance of millions of people. Milder degrees of iron deficiency—too mild to produce anemia—can now be detected with reasonable accuracy. A high incidence is found among children and young women; whether it impairs performance or causes symptoms is still not certain. In the United States an incidence of iron-deficiency anemia of about 10 percent has been reported in menstruating women, 10-60 percent in pregnancy when supplemental iron is withheld, and as high as 64 percent in infancy (Green, et al., 1968). Similar reports come from many other countries and substantiate the frequency of iron deficiency throughout the world. A recent collaborative study under the auspices of the FAO/WHO Report (1966) found an incidence of anemia in pregnancy in six different countries to vary from 22 to 80 percent. Of the 70 percent of iron-deficient pregnant women, there was 43 percent of them displaying anemia.

Trace elements causing changes in metabolism which adversely affect reactions involved in the metabolism of red blood cells are shown in Table 1.
### Table 1. The effect of trace elements on anemia

<table>
<thead>
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<td>Iron</td>
<td>Lower</td>
<td>High in pregnancy and infancy, up to 80%</td>
</tr>
<tr>
<td>Copper</td>
<td>Lower and high</td>
<td>No</td>
</tr>
<tr>
<td>Manganese</td>
<td>Lower and higher</td>
<td>No</td>
</tr>
<tr>
<td>Zinc</td>
<td>Lower</td>
<td>No</td>
</tr>
<tr>
<td>Chromium</td>
<td>Lower</td>
<td>A few cases</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Lower</td>
<td>A few cases</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Higher</td>
<td>No</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Higher</td>
<td>A few cases</td>
</tr>
<tr>
<td>Lead</td>
<td>Higher</td>
<td>High in children</td>
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<tr>
<td>Molybdenum</td>
<td>Higher</td>
<td>No</td>
</tr>
<tr>
<td>Selenium</td>
<td>Higher</td>
<td>No</td>
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The exact function of many of these trace elements is still unknown. Toxicity symptoms are recognized but it is possible that in the future more of the trace elements should be listed in each group, that is, within narrow limits or physiological levels, a trace element may be needed by an enzyme or coenzyme in keeping the pathway of synthesis or catabolism functioning in the body while still lower levels may cause a deficiency of that enzyme or high levels may produce a toxicity in the body. The purpose of this report is to review the literature and report on the latest findings regarding each element.
Iron Metabolism

Levels

Iron content of the normal adult was about 35 to 60 mg per kg body weight. It was composed of an essential fraction from 35 to 45 mg (in hemoglobin, myoglobin, cellular enzymes, and plasma) and a storage fraction (ferritin and hemosiderin) of from 10 to 20 mg (Moore, 1970).

The following table shows that the total body iron in normal men and women might range from less than 2 to more than 6 g, depending largely on body size, the hemoglobin level, and the amount stored.

Table 2. Estimates of total body iron in adults to emphasize the wide variations produced by body size and normal range of hemoglobin values

<table>
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<th>Male, 70 kg Hb 16g/100ml</th>
<th>Male, 100 kg Hb 18g/100ml</th>
<th>Female, 45 kg Hb 12g/100ml</th>
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<tr>
<td>*Essential iron</td>
<td></td>
<td></td>
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<td>Hb Fe</td>
<td>2.67</td>
<td>4.2</td>
<td>1.26</td>
</tr>
<tr>
<td>Functional tissue iron</td>
<td>~0.45</td>
<td>~0.64</td>
<td>~0.29</td>
</tr>
<tr>
<td>Transport iron</td>
<td>~0.005</td>
<td>~0.007</td>
<td>~0.003</td>
</tr>
<tr>
<td>Storage iron</td>
<td>0.5-1.5</td>
<td>0.5-1.5</td>
<td>0.3-1.0</td>
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<tr>
<td>Total: as low as</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>~6g</td>
<td></td>
<td>&lt; 2g</td>
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Absorption, transportation and excretion

There has been general agreement that the absorption of iron could occur at any section in the gastrointestinal tract from the stomach through the intestine (Hahn, et al., 1945, and Brown, 1963). However, absorption was greatest in the duodenum and progressively less in a descending gradient.

Barer and Fowler (1937) found that the reducing mechanisms might be supplied in the various digestive juices, or from the diet in the form of ascorbic acid, sulfhydryl compounds, and so forth. To be effective these compounds required an acid medium (pH below 5) to convert the ferric iron in foods to the soluble ferrous form, and the formation of insoluble and undissociated complexes was inhibited. This work was confirmed by Hahn, et al. (1945) and Moore (1955). Thus, the acidity of the gastric juice might exert a favorable influence on iron absorption. However, the acidity of gastric juice must be looked upon as only one of several contributory factors. Not all patients with achlorhydria became iron deficient, nor did all cases of iron deficiency have achlorhydria. Thus, achlorhydria alone would rarely lead to iron deficiency without the intervention of other factors that either increased the requirements or decreased the amount of iron available.

Since ferric iron readily formed insoluble and undissociable complexes with phosphate ions, the presence of much phosphate in the diet often materially reduced the absorption of iron. Conversely, diets lacking or very low in phosphates might lead to excess iron accumulation in the body (hemosiderosis) (Brown, 1963).

In 1943, Hahn et al. proposed a theory of the regulation of iron absorption which has been elaborated upon by Granick (1951) as the
"mucosal block" hypothesis (Figure 1). According to this theory, an acceptor was present in the intestinal mucosa that was capable of combining with iron that comes into the mucosal cells. When the acceptor was saturated with iron, no more iron could pass through the cells until some of the acceptor was made available again by removal of the iron. A protein, apoferritin, was such an acceptor which changes to ferritin when iron was accepted. Normally, there was no demonstrable apoferritin and there were only small amounts of ferritin present in the mucosa. After iron-feeding, the amount of ferritin in the upper gastrointestinal mucosa, and particularly in the duodenal mucosa was markedly increased. This disappeared gradually over a period of several days.

Figure 1. Schematic representation of the "mucosal block" hypothesis. (From Brown, 1963, p. 206)
According to Conrad and Crosby (1963) and Wheby and Crosby (1963) ingested iron was transported rapidly across mucosal cells to bloodstream or was converted to the storage form of ferritin. The iron needs of the body were supplied by the storage iron; the remainder was kept in the cells until they desquamated into the gut. Thus, the ferritin formed within the mucosa appears to control the absorption of iron.

With the use of $^{14}$C-labeled amino acids into intestinal protein and species-specific antiferritin antiserum, Bernier (1970) measured ferritin synthesis. He found more ferritin in the intestinal absorptive cells of iron-repleted than of iron-deficient animals (Bernier, 1970). He suggested that it remained uncertain whether ferritin acted to facilitate absorption or to limit absorption and enhanced excretion. Oral doses of iron produced more ferritin synthesis in the duodenum than in other parts of the gut. The quantity of ferritin synthesized was proportional to the log of the oral dose of iron (1-100 Mc moles), suggesting a relation between the mucosal uptake of iron and ferritin synthesis. Conversely, parenteral injections of iron produced more synthesis of ferritin in the terminal ileum than in the proximal small intestine. Since little iron was absorbed in the ileum, this ferritin must be held in the intestinal cell and enters the gut when the cells disintegrate. Thus, he supported the hypothesis that ferritin in the intestinal cell functions in the storage and excretion of iron rather than as the primary regulator of iron absorption.
In a study of normal and iron-deficient pregnant women, Apte and Iyenger (1970) demonstrated that dietary iron absorption is increased in the latter half of pregnancy in normal pregnant women. When using the predominantly cereal-based diets, Indian women on low incomes needed 40 mg of iron in order to meet the iron requirements of pregnancy.

In their studies, Ringelhann, Konotey-Ahulu and Dodu (1970) found that iron absorption was high measured by a fecal recovery method in young adult males living in a tropical zone, even in the absence of anemia. There was an inverse relation between the iron absorption and the hemoglobin-iron of guinea pigs. As an iron-containing porphygrin compound they found the absorption of both inorganic and hemoglobin-iron occurred primarily in the duodenum with about one-third of the quantity absorbed from the colon and small quantities from other portions of the gut of the pig. In the duodenal lumen, hemoglobin was split by proteolytic enzymes into absorbable heme and small quantities of iron. This heme entered the intestinal mucosal cells and subsequently was found in the plasma. Much of the absorbed heme entered the liver where it was probably degraded and the iron released. Liberated iron was concentrated in the intestinal mucosa where it might act to regulate the absorption of iron but not heme.

Iron absorption from food of animal origin and vegetable origin has been compared with the absorption from a meal in which both vegetable (corn and black bean) and animal food (veal and fish muscle) were combined. Findings indicate that the absorption of iron from meals consisting of veal and corn or black beans was less than that when veal was given alone. The decreased absorption was found less striking when veal was mixed with black beans. Corn did not inhibit the
absorption of fish iron. Three times as much corn or black bean iron was absorbed when combined with food from animal origin than when given alone. Amino acids in the same number and proportion as were present in 100 g of fish muscle enhanced the iron absorption from black beans (a three-fold increase). Their data appeared to indicate that the interaction of vegetable with animal food during digestion may change the pattern of iron absorption of that shown by these foods when given alone. Thus, a certain proportion of animal food should be included in the diet to enhance iron absorption from vegetable food.

After it was absorbed, iron entered the portal circulation bound to transferrin of plasma (Brown, 1963). Similarly, iron from old erythrocytes destroyed predominantly in the spleen was split from the hemoglobin molecule within cells of the reticulo-endothelial system and, after a variable stay in these cells as ferritin or hemosiderin, was released to the plasma transferrin and re-entered circulation in the portal bloodstream. Most of the transferrin-bound iron entered the liver and later was circulated to the bone marrow.

Transferrin, also known as iron-binding protein, was a beta-globulin of 90,000 molecular weight. Each molecule was capable of binding two atoms of iron in the ferric state. There were approximately 6 to 8 g of transferrin in the circulating plasma with the capability of binding about 1.25 mg of iron per g of protein; almost an equal quantity was the carrier of iron which transported the metal into sites of utilization where the iron was released; and the protein was freed to pick up more iron.

Davies, et al. (1959) proposed that the fetus had a highly effective acceptor system for assimilating iron. Maternal transferrin
transferred the iron to the placental tissue; to the fetal transferrin; and then to the fetal tissues. This pathway appeared to be a one-way street, capable of operating effectively against increased maternal requirements for iron even in maternal iron deficiency. During the last few months of pregnancy, it might account for a transfer of 3 to 4 mg of iron per day to the fetus.

Excretion of the metal was exceedingly difficult to measure, but Moore (1970) suggested that excretion was small. The major pathway of iron loss from the body was by hemorrhage, either as a result of disease, accident, blood letting, or menstrual and pregnancy losses in the female population. They suggested that urinary excretion was about 0.1 mg or less per day; in patients with proteinuria, hematuria, hemoglobinuria or iron overload, the values might be several times higher. This occurred by loss of iron carried by excreted transferrin, hemoglobin, or shed renal tubular cells. The daily fecal iron excretion was found to be about 0.3 to 0.5 mg which was from blood destruction into the alimentary canal, unabsorbed ingested or biliary iron and desquamated intestinal mucosal cells.

The loss of iron in sweat and from dermal surfaces has been estimated from 1-6 mg per day which depended on the weather and the area of skin surface (Moore, 1970).

When the above mentioned several sources of iron excretion were added together, an estimate of 0.5 to 1 mg seemed reasonable.

The pregnant woman transferred iron to the growing fetus, to the placenta and cord; the bulk of this transfer occurred during the last three months. Hemorrhage at the time of delivery has been estimated as $507 \pm 308$ ml for primipara and $280 \pm 293$ ml for multipara. Lactation
caused an additional drain of approximately 0.5 to 1 mg per day. Thus, the total iron needed for a normal pregnancy was estimated to be from 1 to 2.5 mg per day spread over a 15-month period (pregnancy 9 months, lactation 6 months).

Figure 2. The plasma iron level was the resultant of a number of factors (From Gubler, p. 89, 1956).

Functions

Iron was the central ion of the hemes (Burnham, 1969) as shown in Figure 3.

Figure 3. Structure of representative iron tetrapyrrole (heme) (From Burnham, p. 409, 1969).
The iron complexes (hemes) serve as prothetic groups of the cytochromes and hydroperoxidases including the oxygen-carrying heme proteins. Basically, they played an essential role in the transport of electrons to activate oxygen and the free energy used to meet energy requirements.

The biosynthesis of the heme was completed by the insertion of iron into protoporphyrin, as seen in Figure 4. The exact way that heme 

Figure 4. Scheme of heme biosynthesis (From Brown, p. 83, 1963).

and the protein globin were joined to form hemoglobin was not clear (Brown, 1963). However, there seemed to be enhancement of heme
synthesis from protoporphyrin in the presence of globin. The suggestion has been made that protoporphyrin and globin combine before the insertion of iron.

Some hematologic disorders resulted from abnormal iron utilization (Brown, 1963). Lead toxicity produced an anemia of different severity characterized by decreased red cell life span, increased erythropoietic activity, much of it ineffective, elevated serum iron, production of abnormal stippled cells and deranged porphyrin biosynthesis. In thalassemia patients, serum iron was high, radiocron clearance was rapid and erythropoiesis was brisk although mostly ineffective; and non-heme iron accumulated in the erythrocytes and their precursors. The outstanding defect was visible in the mitochondria by electron microscopy.

**Deficiency, overload and symptoms**

The most practical estimation for iron deficiency was the percent saturation of transferrin. When the value fell to less than 18 percent, erythropoiesis was impaired. Assuming that infection might be excluded as a cause of decreased transferrin saturation, this measurement became a useful index in identifying iron deficiency with or without anemia (Finch, 1969a).

The anemia of iron deficiency was characterized by small pale erythrocytes (microcytic), depletion of stored iron, plasma iron was lower than 50 mcg per 100 ml, the iron-binding capacity was elevated and the saturation of transferrin was lower than 18 percent (Finch, 1969b). In his earlier studies, he proposed that a decrease in marrow sideroblasts below 10 percent was an indication of the decreased supply of iron to the red cell marrow. Then, the production of red
cells by the erythroid marrow was retarded. The anemia that showed was first normocytic, normochronic and then became microcytic, hypochromic. Red cell protoporphyrin increased as an indication of the imbalance between iron supply and porphyrin synthesis.

Severe anemia developed as the speed of iron depletion increased. When hemorrhage was acute, the production of red cells could be restricted before all iron was mobilized from the reticuloendothelial cells and the anemia would be normocytic or even slightly macrocytic. In recent medical practice, about one-half of the cases of iron deficiency anemia were normocytic (Finch, 1969a).

An iron overload occurs by an excessive iron intake, abnormal activity of the iron absorption machinery, or by administration of iron parenterally. Finch (1969b) also suggested that it was very difficult to increase body iron with food, due to the effectiveness of the intestinal mucosa in opposing overload. The excess iron was deposited largely as hemosiderin in reticuloendothelial cells, or in the parenchymal cells of certain tissues. Iron accumulation in reticuloendothelial cells was not too serious, whereas parenchymal overload might be dangerous. Parenchymal accumulation lead to hemochromatosis, which was characterized by a peculiar slate-grey pigmentation of the skin, enlargement of the liver, and breakdown of liver function. Later stages of hemochromatosis were associated with fibrosis and hepatoma, the appearance of diabetes with pancreatic infiltration, and heart failure due to myocardial disease. The term hemosiderosis was used to designate an increase in iron storage without associated tissue damage. The hazard of iron overload was a limited one, and it was fortunate that iron could be so rigidly conserved in man. Otherwise it would be
extremely difficult to maintain iron balance, considering the low percentage of iron absorbed from food.

**Requirement and Recommendation**

**Requirement, prevention and treatment**

Since iron already present in the body was utilized over and over again, the amount of iron used daily for hemoglobin formation (26 to 27 mg) was far in excess of the actual daily requirement for iron in the diet (Drabkin, 1951). He proposed that the requirement represents only that iron which was lost to the body through excretory channels. Barring blood loss, the normal woman loses an average of 1 mg per day in the menses and hence had nearly double the daily iron requirement of men. During pregnancy, the loss averages 2.7 mg of iron per day which was supplied to the fetus, making a total daily loss to the mother of 3.8 mg per day. Finch (1969a) suggested the daily requirement of iron from 0.7 to 2.5 mg for the menstruating woman and 2.0 to 5.0 mg for the pregnant woman.

Since infants, children, and adolescents were expanding their blood volume and tissue by growth, their iron requirements were much greater than those indicated by the small amounts lost by excretory routes. The increment required for growth in the first 20 years of life amount to 0.3 to 0.6 mg per day. As a result of a careful study of iron losses and requirements, the Recommended Dietary Allowances (1968) for dietary iron intake by the female population in this age range was increased to 18 mg per day. The committee that evaluated iron requirement believed that the attainment of 18 mg per day could be accomplished most readily by increasing the levels of iron from
enriched cereal products. The forms of iron used for this purpose should be such as to ensure 10 percent absorption,

If women who found it impossible to obtain 18 mg of iron per day might need to take iron preparations either orally or by parenteral administration as recommended by their physicians. Moore (1970) described the use of these preparations for the iron-deficient women.

According to the Food Consumption Surveys by the U.S.D.A. in 1965 (Agriculture Research Service/U.S. Department of Agriculture, March 1969), the dietary iron intake of the female population, between 10 and 55 years of age, averaged about 11 mg per day. However, the recommended dietary allowance of 18 mg per day was recommended (Table 3).

Table 3. Recommended daily dietary allowances (1968) for iron intake

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Iron (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td></td>
</tr>
<tr>
<td>0-1/6</td>
<td>6</td>
</tr>
<tr>
<td>1/6-1/2</td>
<td>10</td>
</tr>
<tr>
<td>1/2-1</td>
<td>15</td>
</tr>
<tr>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>15</td>
</tr>
<tr>
<td>3-10</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>10-12</td>
<td>10</td>
</tr>
<tr>
<td>12-18</td>
<td>18</td>
</tr>
<tr>
<td>18-75</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>10-55</td>
<td>18</td>
</tr>
<tr>
<td>55-75+</td>
<td>10</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>18</td>
</tr>
<tr>
<td>Lactation</td>
<td>18</td>
</tr>
</tbody>
</table>

**Food sources**

Dietary iron could be divided roughly into two fractions: (1) that which could be readily converted into the ionized form by the action of dilute acids and (2) that portion which resisted ionization under these conditions. The latter fraction was composed chiefly of iron porphyrins and other complexes in which iron was firmly bound with organic molecules. These must be partially or completely broken down before the iron becomes ionizable. Thus, a food high in iron might not necessarily be a good source of biologically available iron.

The following listed food sources of iron were separated according to the iron content and their availabilities:

Rich sources: organ meats (liver, kidney, and heart), egg yolk, dried legumes, cane molasses, cocoa, shellfish and parsley.

Intermediate sources: Muscle meats, fish, poultry, nuts, green vegetables, wholemeal or enriched flour, wholemeal bread and roots.

Poor sources: milk, milk products, fats and oils, white flour and bread (unenriched), polished rice, potatoes, sago, white sugar, and fruit juices.

It appears that the recommendations of the Nutritional Research Council for females aged 10 to 55 years is a little high, and hard to obtain from ordinary foods. However, since research findings indicate 18 mg is needed for optimum health, and it is hard to cause an iron overload by dietary means, every woman should do careful planning of menus to include, if possible, the 18 mg daily.
COPPER

Metabolism

Levels

Li and Vallee (1970) proposed that the total copper content of the human body is 100 to 150 mg. The liver (10 to 15 mg), kidney, heart, hair and brain (10 mg) contained the highest concentrations of copper. Spleen, lung, muscle and bone contained intermediate amounts, while pituitary, thyroid and thymus had the lowest concentrations. During growth, the highest concentrations of copper appeared in the rapidly developing structures. Human whole blood contained about 100 mcg of copper per 100 ml distributed about equally between erythrocytes and plasma. Over 90 percent of the copper in mammalian plasma was associated with the α-globulin, ceruloplasmin, while the bulk of erythrocyte copper was presumed to be associated with erythrocuprein. A small amount of the plasma copper was bound to amino acids, such as glutamine, histidine, and threonine. They were in equilibrium with the copper which was bound to albumin. In other tissues and body fluids, the quantities of copper have not been determined.

Absorption, transportation and excretion

Dowdy (1969) presented a picture of the metabolism of copper in the human body by following oral administration of radioactive copper (Reaction 1 in Figure 5). The activity appeared very rapidly in the blood (Reaction 2). This finding suggested that, in man, copper absorption occurred in the stomach or the upper small intestine, or both.
Figure 5. Schematic representation of the major metabolic pathways of copper in the human body. (From Dowdy, 1969, p. 888)
The principal sites of copper absorption in pigs have been found to be the small intestine and colon (Reaction 3), and in rats, the stomach and to a slightly lesser extent, the duodenum (Reaction 4). Acid conditions favor copper absorption.

From the intestine, copper moved into the blood serum (Figure 5). Serum copper might be generally divided into two forms: (a) direct reacting copper (because it reacts directly in the serum with sodium diethyldithiocarbonate, a copper chelator); and (b) indirect reacting copper (because it would not react directly with the carbonate reagent). Radioactive copper given orally appeared rapidly in the direct reacting fraction and was believed to be associated with albumin. Recent evidence suggested that a small portion of the direct reacting serum copper was bound to various amino acids. Albumin bound copper was believed to be the transport fraction since copper bound to ceruloplasmin was not exchangeable with ionic copper in vivo and was released only when the protein molecule was catabolized. The amino acid bound fraction was thought to be involved with the movement of copper through membranes. Evidence supporting this contention was that amino acids added in vitro facilitate copper uptake by liver slices under aerobic conditions.

As a result of decreased serum ceruloplasmin levels, total blood copper in these patients was markedly reduced. Conversely, copper accumulated in the liver, brain, kidney, and eyes, but remained normal in heart muscle and red blood cells.

Dowdy (1969) showed that copper was transported to the various body tissues by the albumin bound serum copper fraction. The reactions which were involved are numbers 3, 5, 11, and 14 in Figure 5. Generally,
tissue copper was in equilibrium with direct reacting serum copper
(Reaction 4, 12, and 15). Some of the tissues need to be considered separately.

Copper was found in the red blood cell. The RBC copper concentra-
tion was not influenced by either total serum copper or ceruloplasmin levels. Two forms of copper have been found in the RBC: (a) erythro-
cuprein, a copper protein that accounts for about 60 percent of the
RBC copper and (b) a labile fraction known as nonerythrocuprein copper
that accounts for the remaining 40 percent. It has been proposed that
erthrocuprein was synthesized in normoblasts in the bone marrow from
whence it appeared as a constituent of the RBC (Reaction 6). The
nonerythrocuprein fraction of RBC copper may also come from normoblasts,
or it may arise from serum since RBC's incubated with radiocopper
showed activity in both copper fractions. It appeared first in the non-
erythrocuprein fraction. Dowdy (1969) believed that the erythrocuprein
copper arose from bone marrow and was in equilibrium with the non-
erythrocuprein fraction in the RBC (Reaction 7 and 8); the nonery-
throcuprein fraction was in equilibrium with direct reacting serum
copper (Reaction 9 and 10).

Although the level of copper in the kidney was low, it was excreted
in the urine (Reaction 13).

The liver received its copper from the serum albumin (Reaction
14) and was found to be the major organ for copper storage. Under
normal conditions, body copper is in a state of equilibrium. Any
"extra" copper will move to liver compartments A and B (Reactions
16 and 17), where biliary excretion and ceruloplasmin synthesis occurs,
respectively. Ceruloplasmin is emptied into the blood serum (Reaction
Although it has not been demonstrated, ceruloplasmin probably returns to the liver for catabolism (Reaction 19).

Biliary copper is emptied back into the intestine (Reaction 20) and is excreted as fecal copper (Reaction 22). In fact, the main route for excretion of absorbed copper is through the bile. Reaction 21 shows another route for excretion of absorbed copper, from the direct reacting serum fraction into the intestine. This fraction is probably available for reabsorption.

Starcher (1969) also indicated that copper absorption was greater from the duodenum than the proventriculus ventriculus in the chick. Copper-64 present in the duodenal mucosa was found to be firmly and specifically bound to protein. Approximately 5 mg of copper are consumed and excreted each day, thus maintaining copper balance. Only about 30 percent of the intake was absorbed. Of the amount absorbed, about 80 percent was excreted through the bile, and about 16 percent was emptied directly back into the intestine through the gut wall. Only about 4 percent was excreted in the urine.

According to Sternlieb and Janowitz (1964), under normal conditions almost all of the copper of the serum circulates as an integral part of the blue $\alpha_2$-globulin, ceruloplasmin, while the remainder was loosely bound to albumin. Normally, changes in the concentration of serum copper parallel those of ceruloplasmin. A decreased concentration of serum copper was most frequently the consequence of inadequate synthesis of ceruloplasmin, as in Wilson's disease; of interference with protein synthesis associated with severe malnutrition; in the rare disease, severe hepatic dysfunction; or as a result of excessive urinary or fecal losses from the body. As reported by Sternlieb and Janowitz
(1964) there was evidence that impaired absorption of copper might also result in a decreased concentration of serum ceruloplasmin. They demonstrated that significant depression of serum ceruloplasmin below the lower limit of normal values (20 mg per 100 ml) occurred in small bowel diseases. The defect in copper metabolism was due to failure to absorb copper, rather than to failure to synthesize ceruloplasmin.

Shields et al. (1961) reported that the mean value for normal human erythrocytes for 20 normal subjects was 16 mg of erythrocuprein per 100 ml of packed cells. Erythrocuprein carries 3.4 mcg of copper per mg of protein, and thus erythrocuprein contains about 60 percent of the copper in erythrocytes (Table 4).

Functions

In 1928 Hart et al. suggested that copper has many roles in the body. One of the first recognized functions was in relation to hemoglobin formation. Despite an adequate supply of iron, copper was required to prevent anemia. There were three possibilities for the role of copper in hematopoiesis; first, copper could facilitate iron absorption; second, copper could be stimulatory to the enzymes in the heme or globin biosynthetic pathway, or both; and third, copper could be involved in the mobilization of stored iron for hemoglobin synthesis.

Data concerning the role of copper in iron absorption have been conflicting. Katsova, Dikov and Voznaya (1967) reported prolonged administration of CuSO₄ (0.1 g per day) did not affect the protein fractions or the concentration of copper and iron in the blood of sheep. Schultz (1940) also reported no effect, while Chase et al. (1952), among others, has reported that copper increases iron absorption.
Table 4. Volume of packed red cells, serum copper, total erythrocYTE copper and erythrocUPREIN in normal subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Vol. packed red cells</th>
<th>Serum copper</th>
<th>Total erythro. copper</th>
<th>Erythrocuprein</th>
<th>E Cu x 100* T Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/100 ml</td>
<td>ug/100 ml</td>
<td>ug/100 ml</td>
<td>mg/100 ml</td>
<td>%</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>49 ± 2.4</td>
<td>107 ± 11.9</td>
<td>89 ± 8.5</td>
<td>16 ± 4.4</td>
<td>60 ± 20.2</td>
</tr>
<tr>
<td>Range</td>
<td>45 - 52</td>
<td>82 - 125</td>
<td>63 - 96</td>
<td>13 - 20</td>
<td>47 - 78</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>44 ± 2.5</td>
<td>127 ± 15.5</td>
<td>89 ± 12.7</td>
<td>17 ± 7.0</td>
<td>65 ± 24.0</td>
</tr>
<tr>
<td>Range</td>
<td>41 - 49</td>
<td>103 - 158</td>
<td>72 - 107</td>
<td>13 - 26</td>
<td>48 - 82</td>
</tr>
<tr>
<td>Both Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean + SD</td>
<td>47 ± 3.4</td>
<td>117 ± 17.1</td>
<td>89 ± 11.4</td>
<td>16 ± 2.9</td>
<td>62 ± 11.0</td>
</tr>
<tr>
<td>Range</td>
<td>41 - 52</td>
<td>82 - 158</td>
<td>63 - 107</td>
<td>13 - 26</td>
<td>47 - 82</td>
</tr>
</tbody>
</table>

*E Cu, erythrocuprein copper (erythrocuprein in mg x 3.4); T Cu total erythrocyte copper. (From Shields, et al., 1961, p. 2010)
Matrone (1960) suggested that copper affects iron absorption in an indirect manner. In a copper deficiency, hemoglobin synthesis was decreased and iron accumulated in body tissues. The absorptive sites were then signaled to block further iron absorption. When copper was given hemoglobin synthesis would be initiated, body iron stores would be reduced, and the absorptive sites would begin to function. Thus, the increase of iron absorption by copper was controlled by the body need (Crosby, 1968). Marston and Allen (1967) showed that copper was involved in the release of iron stores from the liver and this was probably the role of copper in hemoglobin formation.

Copper has been shown to be associated with the formation of aortic elastin (Starcher, 1964). The importance of this role of copper was that, in deficiency states, elastin was not synthesized properly and aortic rupture might occur.

Another role of copper was as a constituent of the enzyme tyrosinase. This enzyme was essential for the conversion of tyrosine to melanin, a black pigment. Melanin is responsible for skin darkening in higher animals. Maynard and Loosli (1962) observed that copper deficiency resulted in the loss of hair color, probably due to reduced tyrosinase activity and a lack of melanin.

**Deficiency, toxicity and symptoms**

Cordano, Baertl and Graham (1966) reported copper deficiency produces hypocupremia. The use of an exclusive milk diet, during recovery of 14 severely malnourished infants, produced hypocupremia in a majority of the cases, in less than 50 days. They also observed a direct relationship between total neutrophils and serum copper values, since hypocupremia goes along with neutropenia.
The copper deficient swine of Ragan et al. (1969) developed severe anemia, the morphological characteristics of which were indistinguishable from those seen in iron deficiency. The anemia did not result from the reduced activity of heme biosynthetic enzymes. However, several abnormalities in iron metabolism were observed. These included: (1) defective iron absorption, (2) an inability to transfer iron from damaged erythrocytes to plasma at a normal rate, and (3) increased deposition of iron in reticuloendothelial and hepatic parenchymal cells. These abnormalities have been shown to be due to an inability to transfer iron from cells to plasma.

In recent years, a number of clinical reports have appeared in the literature describing a syndrome characterized by microcytic hypochromic anemia, hypoproteinemia and hypocupremia (Ulstrom, Smith and Hermlich, 1956). Although this syndrome was rare, its occurrence in young infants has produced a revival of interest in the role of copper in infant nutrition.

Cartwright (1955) also described a copper deficiency anemia in swine which resembled closely the anemia associated with iron deficiency. The erythrocytes were microcytic and hypochromic, and there was normoblastic hyperplasia of the bone marrow. A marked hypoferremia develops early in the deficiency and results in levels of plasma iron comparable to those of iron deficient swine. As in a true iron deficiency, there was an increase in the iron-binding capacity of the plasma.

Copper toxicity was investigated by Braude et al. (1962), using diets containing 250 ppm of copper added to rations of wheat, barley, and oats. It improved weight gains and food efficiency in the pigs.
The same level added to a corn-soybean ration in the U.S. produced signs of severe toxicity. A Scottish report indicated that when 600 ppm of copper were added to a corn-soybean or a corn-skim milk powder ration, toxicity was much less than when the same level was added to a corn-fish meal ration.

According to Li and Vallee (1970), ingesting excess copper in fodder of sheep led to accumulations of 10 to 20 times the normal concentration of copper in the liver. The animals suffered no apparent ill effects until suddenly a large amount of excess copper was liberated into the blood stream producing a hemolytic anemia, hemoglobinuria, and jaundice. Over 60 percent of the circulating red cells might be destroyed during the crisis and atrophy of liver might occur. A similar type of hemolytic jaundice has been observed in cattle. Acute copper toxicity in man due to ingestion of copper produces nausea, vomiting, diarrhea, headache, dizziness, weakness and a metallic taste. In more severe cases tachycardia, hypertension and coma might ensue, followed by jaundice and hemolytic anemia, hemoglobinuria, uremia and death. Serum ceruloplasmin concentrations were increased as in loosely bound serum copper.

Requirement and Recommendation

Requirement and prevention

Dietary copper strongly influences the levels of copper in the blood and liver of most species fed either toxic or copper deficient diets (Milne and Weswig, 1968).

A deliberate attempt has been made to induce copper deficiency in young infants by Wilson and Lahey (1960). They found that varying
the dietary intake of copper was without effect during the first two months of life. The babies fed the low copper diet appeared normal in every respect. By six months of age, however, this group had a somewhat lower hemoglobin level, but hypocupremia was not present, nor were other manifestations of copper deficiency. Wilson and Lahey's study did not establish the minimum requirement of copper for young infants; it did document the fact that infants might thrive, for short periods of time at least, on intakes well below those currently recommended. How long they could thrive on such a diet was a most important question. The majority of infants reported in the clinical literature as suffering from hypocupremia had subsisted largely on milk for periods of six to twelve months or longer.

On a normal diet copper is not accumulated preferentially by any tissue. Gubler (1956) suggested the normal intake of man was 2.5 to 5 mg per day. This amount was adequate for the maintenance of positive copper balance. Children required about 0.05 to 0.1 mg copper per kg of body weight daily. Underwood (1962) suggested 2-3 mg per day for an adult diet. It appears that approximately 3 mg of copper per day is needed by either children or adults for a high quality diet. This amount should be easy to obtain from common foods on any well-balanced diet.

Food sources
Rich sources: organ meats (liver, kidney, heart, and brain), crustaceans and shellfish (especially oysters), nuts, dried legumes, dried vine and stone fruits, and cocoa. The copper content of these foods ranges from 20-30 ppm to as high as 400 ppm.
Intermediate sources: wholemeal flour and bread, the green leafy vegetables, eggs, muscle meats, fish, and poultry.

Poor sources: Milk, butter, cheese, white sugar, honey, margarine, non-leafy vegetables, most fresh fruits and the refined cereal products. The copper content of these foods ranges from 0.5-2 ppm.
**MANGANESE**

**Metabolism**

**Levels**

Based on presumptive evidence, manganese is listed among the trace minerals required by man. It was estimated that the adult human body contains about 10 to 20 mg of the element, and the concentrations in individual organs tend to remain relatively constant throughout life. Within cells, manganese is located both in the nucleus and in cytoplasmic organelles. However, manganese tends to be higher in tissues rich in mitochondria and more concentrated within the mitochondria than in the cytoplasm of the cell (Li and Vallee, 1970).

Bowen (1956), using thermal neutron activation analysis obtained a mean of 2.4 ± 0.8 mcg manganese per 100 ml of whole human blood and found this to be distributed about equally between the blood cells and plasma.

**Absorption, transportation and excretion**

Manganese is absorbed rather poorly from the intestinal tract. As early as 1943, Greenberg, Copp and Cuthbertson showed that only about 3-4 percent of the orally administered dose of manganese was absorbed in rats. There were also some indications from a study of normal and perotic chicks that manganese may be more efficiently absorbed under deficiency conditions. Li and Vallee (1970) reported that high dietary concentrations of calcium and phosphorus decreased absorption of the element by reducing its availability. The element was presumably transported in
plasma by binding to a beta-l-globulin which was named transmanganin. It was not entirely clear, however, that transmanganin differs from transferrin since the later protein has been shown to bind manganese and copper as well as iron.

Many papers have shown that the concentration of manganese in tissues was quite stable due to controlled excretion rather than to regulated absorption. Papavasiliou and his associates (1966) stated that manganese appeared to be almost totally excreted into the gastrointestinal tract. Several excretory routes were found for this element, all of which were linked to the gastrointestinal tract. From their own studies they found that among these routes, the bile flow seemed to participate in an enterohepatic circulation of manganese which regulated the excretion of the metal under ordinary conditions, but under conditions of overloading, auxiliary gastrointestinal routes were used. Any interference of this circulation can decrease the capacity of animals to accelerate the excretion of this metal following a metabolic load.

Since excretion of this metal was primarily fecal, there was only a little appearing in urine (Underwood, 1962). Most of the excess manganese was excreted in the feces, with the urinary output remaining within the range 0.04–0.07 mg manganese per day.

**Functions**

Manganese has been considered as an essential trace metal in the human body and has been assumed to participate indirectly in hemopoiesis. According to Borg and Cotzias (1958) many investigators indicated about 12 years ago that manganese was a firmly bound component
in the erythrocytes of humans and rabbits. This suggested that it may have a role in the prophyrin metabolism of cells. From their experiments Borg and Cotzias (1958) indicated that traces of manganese added in vitro to mature erythrocytes or their hemalysates were bound relatively weakly and primarily to the stroma, in contrast to the physiologically deposited manganese, which was incorporated into a structural component of the hemolysate. They also showed that the incorporated radiomanganese in both human and rabbit blood was isolated predominantly in the crystallized hemin. Thus, they suggested that the manganese organometallic compound was indeed a porphyrin.

Manganese has been shown to activate a large number of metal-enzyme complexes involving transferase, hydrolase, lyase, isomerase, and ligase reactions. This effect was not specific since other metals, especially magnesium, may substitute for manganese in most instances (Li and Vallee, 1970). It was also required for the enzymetic incorporation into glycoproteins of xylose and galactose. The biotin dependent pyruvate decarboxylase of chicken liver was the first manganese metalloenzyme to be identified. Manganese might replace the native zinc atoms of carboxypeptidase A of bovine pancreas and the neutral protease of B. subtilis. It has also been found to be firmly associated with ribonucleic acids (RNA). It might also play a role in protein synthesis and oxidative phosphorylation.

**Deficiency, toxicity and symptoms**

Manganese deficiency has not been obtained in man, but has in mice, rats, guinea pigs, rabbits, pigs and poultry. The deficiency was characterized by impaired growth, skeletal abnormalities, depressed and
disturbed reproductive function, and ataxia of the newborn, which were similar in all species (Li and Vallee, 1970). Bone and blood phosphatase activities were lowered.

Hartman, Matrone, and Wise (1955) indicated that in iron-depleted lambs fed manganese (1000 ppm) either as an inorganic salt in the milk diet or as a natural constituent of a roughage diet, the presence of excessive manganese depressed or retarded hemoglobin formation by interfering with the absorption of iron, the formation of hemoglobin and a combination of both. A surprising feature of their experiments was the fact that minimum levels of dietary manganese (45 ppm) for anemic lambs interfered with hemoglobin formation.

**Requirement and Recommendation**

**Requirement**

A dietary deficiency of manganese has never been recorded in man. Hence, no human daily requirement has been outlined. However, the average daily intake of manganese in adult man has been estimated to be 3 to 9 mg, and children presumably require at least 200 mcg per kg of body weight (Li and Vallee, 1970). Since many foods contain manganese, the use of a well-balanced diet containing a variety of foods should provide ample manganese for good health.

**Food sources**

The metal is widely distributed in foodstuffs, particularly in tea, wheat germ, seeds, nuts, leafy vegetables and meat. The following table lists food items in descending order of their manganese content on the fresh basis (Table 5).
Table 5. Manganese content* of groups of principal foodstuffs

<table>
<thead>
<tr>
<th>Class of food</th>
<th>No. of samples</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuts</td>
<td>10</td>
<td>6.3</td>
<td>41.7</td>
<td>22.7</td>
</tr>
<tr>
<td>Cereals and their products</td>
<td>23</td>
<td>0.5</td>
<td>91.1</td>
<td>20.2</td>
</tr>
<tr>
<td>Dried legume seeds</td>
<td>4</td>
<td>10.7</td>
<td>27.7</td>
<td>20.0</td>
</tr>
<tr>
<td>Green leafy vegetables</td>
<td>18</td>
<td>0.8</td>
<td>12.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Dried fruits</td>
<td>7</td>
<td>1.5</td>
<td>6.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Roots tubers and stalks</td>
<td>12</td>
<td>0.4</td>
<td>9.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Fresh fruits (including blueberries)</td>
<td>26</td>
<td>0.2</td>
<td>44.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Fresh fruits (excluding blueberries)</td>
<td>25</td>
<td>0.2</td>
<td>10.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Nonleafy vegetables</td>
<td>5</td>
<td>0.8</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Animal tissues</td>
<td>13</td>
<td>0.08</td>
<td>3.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Poultry and poultry products</td>
<td>6</td>
<td>0.3</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Dairy products</td>
<td>7</td>
<td>0.03</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Fish and sea foods (including oysters)</td>
<td>7</td>
<td>0.12</td>
<td>2.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Fish and sea foods (excluding oysters)</td>
<td>6</td>
<td>0.12</td>
<td>0.4</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Measured in ppm manganese (fresh edible portion). (From Underwood, 1962, p. 212)
ZINC

Metabolism

Zinc-deficiency has been found by Prasad et al. (1963) to cause anemia characterized by severe iron-deficiency anemia in Egypt and Iran. All the patients were male subjects. Female patients in villages refused to be examined and the facilities at NAMRU-3 in Cairo were such that female subjects could not be studied. Thus one cannot be certain whether this syndrome affects the females or not.

Levels

The total amount of zinc in the normal human body has been estimated to be about 2 to 3 g. This gave an over-all concentration of 20-30 ppm, which was about 10-15 times that of body copper and less than half that of total body iron. In contrast to the position with copper, the mammalian newborn did not consistently carry higher concentration of total body zinc than do mature animals of the same species (Underwood, 1962).

During the period of sexual activity of the herring, the testes of the male contained twice as much zinc as the rest of the body, while the zinc content of the ovaries was similar to that of the rest of the female body during the same period (Underwood, 1962).

Human whole blood contained about 900 mcg of zinc per 100 ml, with about 3 percent of the total zinc of blood contained in the leucocytes, 12-22 percent in the plasma, and 75-85 percent in the erythrocytes. This was about 25 times as much zinc as in the leucocytes. Plasma zinc concentrations fall markedly in rabbits and slightly in man from birth.
to maturity and were three times as high in human fetal blood plasma as in maternal blood plasma. The zinc content of the erythrocytes in newborn infants was only approximately one-quarter of the normal adult value, rising progressively over the first 12 years of life (Underwood, 1962).

Absorption, transportation and excretion

The exact site and mechanism of absorption of zinc from the intestine has not been established. This element, like iron, is poorly absorbed (Underwood, 1962). Only 3-10 percent of the dietary zinc was estimated to be absorbed by steers at both normal and high level of zinc intake. The ability of animals to absorb zinc varies with the chemical form or combination in which it was ingested.

According to some authors, high dietary levels may eventually decrease absorption of zinc in humans.

Miller et al. (1970) showed that endogenous losses were higher when levels of zinc in the diet were above normal. Thus, when excess zinc was fed, homeostatic control reduced absorption. When low zinc diets were fed, the mechanism operated through changes in both absorption and endogenous excretion via feces. A clinical zinc deficiency, independent of dietary zinc content, increased zinc absorption and decreased endogenous excretion. Probably changes at the tissue level were responsible for the homeostatic control. Zinc oxide and zinc sulfate had comparable effects on zinc levels in most tissues.

The major routes of zinc excretion were by way of the feces, chiefly via the pancreas and intestine with only a small amount via the bile (Sanstead, 1968). Fecal zinc consisted largely of unabsorbed
dietary zinc and a small portion of zinc which had been absorbed and excreted into the intestine. Urinary excretion was small, approximately 300-600 mcg per day. There was about 100 mcg of zinc present in 100 ml of sweat. Obviously, excretion via this route might be significant in hot climates.

In 1966, Miller et al. used ruminants for experiments with a single intravenous dose of zinc-65. Their results indicated that both a low zinc diet and a zinc deficiency per se caused reduced endogenous fecal excretion of zinc-65 for at least 2 weeks after the zinc entered the blood, thus contributing to hemostatis of zinc.

Functions

Zinc was a constant constituent of the serum, of the erythrocytes and of the leucocytes. In the plasma this element existed in both a firmly bound and a loosely bound fraction associated with the serum proteins, albumins and globulin and most strongly bound to the latter. In vitro studies, zinc was bound also with transferrin (Surgenor et al., 1949). Some investigators suggested that the whole of the zinc in erythrocytes exists as carbonic anhydrase.

Zinc in trace concentrations was found to be essential for growth and sexual development (Sanstead, 1968). About twenty enzymes have been identified that require zinc along with other metals. Carbonic anhydrase, a zinc compound which is important in carbon dioxide metabolism was the first metalloenzyme reported. Others include alkaline phosphatase and lactic dehydrogenase.

Zinc was also found to participate in ribonucleic acid (RNA) metabolism in a way still unknown (Sanstead, 1968). It was believed
to be an integral part of the RNA molecule and to maintain the molecule's configuration.

Zinc was also concerned in tryptophan synthesis in Neurospora and the levels of protein, Alcohol-dehydrogenase (a known zinc metalloenzyme) and tryptophan desmolase were greatly reduced during a zinc deficiency. All the findings suggested that the primary effect of a deficiency of zinc was on the synthesis of ribonucleic acid (RNA) and the inhibition of synthesis of protein and deoxyribonucleic acid (DNA) (Underwood, 1962). Theurer and Hoekstra (1966) proposed that the primary effect of zinc deficiency lies in the area of protein metabolism and not in carbohydrate or lipid metabolism. But recently, quantitative determinations of the metal content by atomic absorption analyses indicated that approximately 2 moles of cobalt and 4 moles of zinc were usually present per mole of transcarboxylase. The enzyme caused a substantial broadening of the nuclear magnetic signal arising from the methyl protons of pyruvate. The broadening effect was reversed by increasing concentration of oxalate. It was proposed that cobalt and zinc function at the active site for pyruvate (Northrop and Wood, 1969). Transcarboxylase is a biotin-containing enzyme which catalyzes the half-reaction between pyruvate and oxalacetate in a manner similar to that of manganese in pyruvate carboxylase.

**Deficiency, toxicity and symptoms**

The symptoms of zinc-deficiency affecting males in Iran were characterized by severe iron-deficiency anemia, enlargement of liver and spleen, short stature, and marked decrease of gonad secretion. There was no evidence of blood loss or hookwork infestation (Prasad,
1966). Subsequently, similar cases were found in Egypt where the nutritional status of villagers was similar to that of Iran.

Underwood (1962), zinc has been found to be relatively nontoxic to birds and mammals. Zinc poisoning has been reported in man as a result of consuming acid foods which were cooked in galvanized vessels and in young pigs after being fed on skim milk passed through galvanized pipes. The intakes necessary to produce toxicity were very high showing that a wide margin of safety existed with this element.

Growth was severely depressed with heavy mortality when 5000 ppm zinc was ingested as chloride by young rats, pigs and poultry, but only slightly depressed with little mortality when ingested as the oxide (Underwood, 1962). Intakes of 5000 or 10,000 ppm zinc, as the carbonate, produced severe anemia in young rats, in addition to subnormal growth, anorexia and heavy mortality. Levels of zinc up to 1000 ppm, either as the sulfate or the carbonate, produce no harmful effects on weanling pigs, even when fed over several weeks, but at higher levels depressed growth and appetite, arthritis and internal hemorrhages developed. Broilers and laying hens exhibit a similar tolerance to zinc at levels of 1200-1400 ppm of the diet. High zinc concentrations in the liver; subnormal concentrations of copper in the blood, liver and whole body; and subnormal concentrations of cytochrome oxidase and catalase in the liver and the heart, accompany the anemia due to a zinc deficiency.
Requirement and Recommendation

Requirement, prevention and treatment

The intake of zinc was influenced greatly by protein content of the food, by refined cereal or white flour content because of the high concentration of zinc in the germ and bran layers of the grain which are removed in the milling process. Since most people favor use of refined cereals and their products, it is possible to cause a zinc deficiency, even though at this intake the element has a wide safety margin. Thus, an adequate protein diet or a zinc-rich food should solve the problem.

The dietary zinc intake of normal adults was recommended to be about 10 to 15 mg per day (Li and Vallee, 1970), with a possibility of higher requirement in adolescent boys.

Following therapy with pharmaceutical ferrous sulfate which contained appreciable quantities of zinc and a good hospital diet, the anemia induced by zinc deficiency was corrected, the size of the liver was reduced, improved, the patients grew pubic hair, and their genitalia size increased (Prasad, 1967). The serum alkaline phosphate also increased.

The anemia which was induced by zinc toxicity could be largely overcome by dietary supplements of copper (Grant-Frost and Underwood, 1958) and completely overcome by dietary supplements of copper plus iron (Magee and Matrone, 1960).

Food sources

Zinc is widely distributed in a variety of foods. Usually, food-stuffs from animal sources, and in particular shellfish are rich in this element.
The following food sources are classified in accordance with their average zinc concentrations:

Rich sources: oysters (150-500 ppm), wheat germ and bran (80-150 ppm).

Intermediate sources: roots and tubers, white flour and bread, milk, leafy vegetables, meat, fish and eggs, whole cereals, nuts and leguminous seeds.

Poor sources: white sugar, pome, and citrus fruits (less than 1 ppm of fresh edible proteins).
Levels

Recent work indicates that chromium is an essential trace element. Feldman, Knoblock and Purdy (1966) reported the limit of detection for chromium was 10 parts per billion. Some average chromium values of albino rats were: whole blood, 24 ppb; serum, 29; liver, 130; spleen, 360; heart, 90; lung < 10; and brain 15. Thus the content of chromium in blood was extremely low when compared to values for most major nutrients. Even in the organs that contained the most chromium, the values were low indicating that it is needed only in trace amounts.

Absorption, transportation and excretion

Chromium is absorbed poorly by the intestine. Several earlier studies in which erythrocytes labeled with Na$_2$Cr$_{51}$O$_4$, ablum labeled with Cr$_{51}$Cl$_3$ or CrO$_3$ were fed to humans or experimental animals have indicated virtually total recovery of administered chromium in the feces (Donaldson and Barreras, 1966). Nevertheless, it seemed likely that some chromium was absorbed since it was found in the tissues and urine of man and other mammals. The necessity for further investigation of chromium absorption was emphasized by the consistent presence of chromium in water and soil, by the development of renal and hepatic lesions in rats which were given drinking water containing 135 ppm chromate and by the reported effects of trace quantities of chromium on cellular metabolism and enzyme
activity. Finally, red blood cells readily took up Na\textsubscript{2}Cr\textsuperscript{15}O\textsubscript{4} but not Cr\textsuperscript{51}Cl\textsubscript{3}.

In 1966 Donaldson and Barreras reported intestinal absorption of trace quantities of Cr\textsuperscript{51}Cl\textsubscript{3} and Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4} in human subjects and in rats. When these compounds were administered orally to humans or intragastrically to rats, recovery of radioactivity in the feces was virtually complete. When Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4} was placed directly in the small bowel, however, significant absorption of radioactivity was demonstrated by jejunal perfusion studies in humans and by fecal and urinary excretion tests in humans and in rats. Patients with pernicious anemia and achlorhydria absorbed significantly more orally administered Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4} than did the control subjects. Intestinal uptake of Na\textsubscript{2}Cr\textsuperscript{51}O\textsubscript{4} to the acid in gastric juice resulted in reduced intestinal uptake in vitro. Reduction of hexavalent (Cr\textsuperscript{51}O\textsubscript{4}\textsuperscript{+}) chromium ions to poorly absorbable trivalent chromium ions by acidic gastric juice was demonstrated by ion exchange column separation.

Schroeder (1968a) has reviewed the role of chromium in mammalian nutrition. The amounts of inorganic salts of chromium absorbed from the gastrointestinal tract were reported by some workers to be about 0.5 percent of the dose. It was possible that larger percentage of naturally occurring chromium complexes were absorbed from foods.

As there were about 200 mcg chromium or less in representative diets, absorption of 0.5 percent would amount to only about 1 mcg per day; thus, it would require 16-32 years for accumulation of 6-12 mg in the body if none was excreted. In the urine of normal adults, however, it has been found that 20-40 mcg per liter was excreted. This indicated that at least 10-20 percent of the ingested natural chromium was
absorbed by the intestinal tract. The organic complexes in which chromium occur in vegetable and animal foods were not known. When they are discovered they should provide efficient sources for human dietary supplementation.

It was possible that the form of chromium absorbed from foods by the intestine was the hexavalent form which tended to be reduced to trivalent chromium, unless it was firmly bound as chromates. A limited survey of the proportions of the two valence states of chromium in 12 samples of foods and vegetation revealed that 11–63 percent of the chromium was in the hexavalent form. About 11 percent of inorganic soluble hexavalent chromium was absorbed by the intestine (Schroeder, 1968a). It thus became essential to learn more about the valence state of naturally occurring chromium.

Except when supplied in inorganic forms, chromium is absorbed, transported in serum partly by sideraphilin (which carries iron as well), and stored in tissues. It is excreted via the urine. In the dog, a considerable amount of chromium was also transported in the unbound state, and was filtered by the glomerulus if it was to be excreted. Excretion was largely influenced by the dialyzed form. Some tubular reabsorption was likely. A complex of chromium and ethylenediaminetetraacetate was cleared by the sheep kidney at almost the same rate as was insulin, the ratio being 0.95 ± 0.03. Chromium combined with the beta chain of hemoglobin in red blood cells is eluted at about 1 percent per day (Schroeder, 1968).

Schroeder (1968a) reported that according to Glinsmann, Feldman and Mertz, plasma levels of fine healthy young subjects rose sharply, parallel to blood glucose levels, and fell as glucose declined. Plasma
Chromium rose from a mean control level of 28 ng per ml to 59 ng per ml. Similar values in circulating chromium have been induced in rats by injection of insulin as well as of glucose.

It was not known whether the chromium thus mobilized from tissues was bound to plasma protein or existed in the free form. As there were two forms of chromium in blood, it was possible that the increment induced by glucose or insulin was in the free or dialyzable form. If the mobilized chromium was in a free form, it should appear in the urine, at 20-30 percent of the total. Theoretical losses from a single glucose load would therefore amount to 20-30 mcg. Whereas this amount appears small, it comprised 10-15 percent of the total ingested daily.

There were other characteristics which may render natural chromium (trivalent form) unavailable for absorption by the gastrointestinal tract (Schroeder, 1968a). In an alkaline medium, trivalent chromium would form long chains with hydrophil and water molecules into complexes with a gel-like consistency and a high molecular weight (olate). This process was accelerated at 120°C. Therefore, it seemed possible that overheating foods, as in canning, might begin elation and that this process was continued in the alkaline small intestine before absorption could take place. Gastric juice inhibits absorption of the trivalent form as well as the hexavalent inorganic chromium when given in solution. Little was known about practices which free chromium in foods from its organic complexes which might lead to diminished absorption.

According to Verzhikovskaya (1969), various doses of chromium when added to the diet, above or below the physiology level, increased the sodium and potassium level in the blood serum. It did not influence
the total blood serum protein level. The amount of albumin decreased, but increased the δ-, β- and ε-globulin fractions of the blood.

**Function**

The studies of Gray and Sterling as reported by Pearson (1963) showed that the chromium-51 activity was associated with the globin rather than with the heme component of the hemoglobin molecule. In the alpha and beta chains of human hemoglobin, and also in the abnormal hemoglobins, chemical variations occur only in the protein moiety or globin. The heme groups of all these human hemoglobin variants were found to be apparently identical.

Globin from normal adult hemoglobin (Hb A) consisted of two pairs of chemically distinguishable polypeptide chains designated alpha and beta chains. Most of the abnormal hemoglobins resulted from point substitutions of single amino acids in one or another polypeptide chain. Hemoglobins S and C resulted from substitution of valine or lysine in place of a glutamic acid in the beta chain of Hb A. Hemoglobin H, and abnormal hemoglobin were unstable variants, usually occurring in certain thalassemia syndroms, and contained a tetramer of normal beta chains. This form of hemoglobin has been used in studies of site of chromium-51 binding to hemoglobin (Pearson, 1963). It was observed that chromium-51 was attached to hemoglobin largely on the beta chains which verified the findings of Pearson and Vertrees (1961). The chromium-51 tag remained with the beta-chains during dissociation and recombination.

From the data of Pearson and McFarland (1962), one patient with hemoglobin H disease (also called alpha thalassemia, which is a hypochromic microcytic anemia) also had iron deficiency and pulmonary
tuberculosis. A nearly twofold increase in the percentage of hemoglobin H was recorded, which might indicate an unusual differential effect of those processes on polypeptide chain synthesis. The markedly abnormal ferrokinetics suggested a superimposition of effects of iron deficiency, infection and thalassemia. However, the most striking change was in the red blood cell chromium-51 T 1/2 which fell from 24 days, initially, to 17 days. This decrease might be the cause of the increased percentage of hemoglobin H. In 1961 Pearson and Vertrees demonstrated that hemoglobin H bound approximately twice as much chromium-51 in the beta-chain as it did in hemoglobin A (in alpha-chain), although nothing was known about the rate of elution of chromium-51 from hemoglobin H. An unusual property of chromium-51 tagging and elution could give a falsely shortened half life time of chromium. The following table was the result for hemoglobin content and for chromium-51 and iron-59 activity by gamma-ray spectrometry in a sodium iodine well-type scintillation counter.

Table 6. The content of hemoglobin and the activity of Cr-51 and Fe-59 of a patient with hemoglobin H disease

<table>
<thead>
<tr>
<th></th>
<th>Percent of total haemoglobin</th>
<th>cc/min Fe-59</th>
<th>Percent of total Fe 59 counts</th>
<th>cc/min Cr-51</th>
<th>Percent of total Cr-51 count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin H</td>
<td>18</td>
<td>16</td>
<td>20.5</td>
<td>1.107</td>
<td>33.7</td>
</tr>
<tr>
<td>Haemoglobin A</td>
<td>82</td>
<td>62</td>
<td>79.5</td>
<td>2.178</td>
<td>66.3</td>
</tr>
</tbody>
</table>

(From Pearson and Vertrees, 1961, p. 1020)
Deficiency, symptoms and treatment

Thalassemia or Mediterranean anemia is common in certain groups of Mediterranean countries, in the Near East, in middle and south-east Asia, and in some parts of Africa. Sporadic cases have been reported in the local population of Western Europe.

There are two forms of thalassemia, alpha thalassemia and beta thalassemia. Both are characterized by hypochromic microcytic anemia. Alpha thalassemia is caused by the shortened half life of red blood cell chromium-51 T 1/2 in terms of the deficient chromium-51 in red blood cells and increased percentage of hemoglobin H (abnormal hemoglobin). This anemia or hemoglobin H occurred in association with a gene resulting in impaired alpha chain synthesis, namely, alpha thalassemia (Pearson and McFarland, 1962).

Due to the failure of the anemia, caused by the chromium-51, to bind the hemoglobin H physiologically, the anemia had no improvement following the normal treatment for deficiency anemia (Jonxis, 1961).

Requirement and Recommendation

Requirement and food sources

Schroeder (1968) recommended that about 200 mcg of chromium per day should be obtained for a normal adult diet, or about 3 mcg per kg of body weight. Rats and mice became deficient on 10 mcg per kg of body weight per day.

Since chromium was essential for carbohydrate metabolism, the chromium content of foods rich in carbohydrates should result in an adequate amount to meet the normal requirement. Moderate to marked depletion of essential trace and bulk elements occurs when grains,
especially rice and wheat (Schroeder, 1968a) are refined, due to the loss of chromium. The refined wheat flour provided only about 616 mcg per 100 Kcal, while the whole wheat flour provided 53 mcg. Chromium was found concentrated in the gluten.

Refined white sugars contained little chromium, whereas unrefined or raw sugars contained fair amounts.
The cobalt concentration of human plasma is about 60 to 80 ng per ml and that of whole blood 80-300 ng per ml (Li and Vallee, 1970). Blaszezyk (1966) determined the concentration of vitamin B₁₂ of 185 normal adults by the microbiology method using Euglena gracilis. The serum values ranged from 120 to 920 ng per ml for the 185 subjects (average 391 ng per ml).

Blaszezyk and Kroblikowska (1966) determined the level of vitamin B₁₂ in serum. They observed a significant increase in polycythemia vera while in pernicious anemia the level was significantly decreased.

According to Yusupova (1966), the blood content of cobalt as determined in healthy nonpregnant women was 19.7 mcg percent; and in a group of healthy pregnant women, 15.1. The results of analyses repeated monthly showed a steady decrease of blood cobalt values until parturition. In pregnant women with various degrees of anemia, the cobalt levels were below normal up to the first week of gravidity, then decreased parallel with the hemoglobin content. In marked anemia, the cobalt values were 5.9 mcg percent at the time of parturition. The cobalt content in placenta and umbical blood averaged 8.9 and 6.1 mcg percent, respectively. There was a slight increase of blood cobalt to 6.9 mcg percent in anemic women one week after parturition.

The concentration of total vitamin in hypoplastic and aplastic anemia in children was determined by Kalinicheva (1966). The concentration of total vitamin B₁₂ in the serum and urine for 15 anemic
children, ages 13 months to 15 years and sixty-two healthy children up to 15 years of age who served as controls. The duration of the disease was 7 months to 5 years. Two to 13 determinations were carried out in each patient. No definite correlation between severity of disease and vitamin $B_{12}$ concentration in the blood could be established. There were no vegetarians among the patients, and a relation between low vitamin $B_{12}$ content in some and peculiarities of diet could not be studied.

**Absorption, transportation and excretion**

The metabolic fate of cobalt has been studied in a wide range of species, using both stable and radioactive forms of the metal.

The synthesis of vitamin $B_{12}$ by bacteria required cobalt. In ruminants this occurred in the proximal portion of the intestine from where the vitamin is then absorbed. The total injection of 70 to 80 mcg of cobalt per day was sufficient for the bacterial formation of the vitamin required by sheep, and probably by cattle as well (Li and Vallee, 1970). In other animals and man, however, where bacterial formation of vitamin $B_{12}$ took place only in the colon, absorption was minimal. Therefore to be of nutritional value for the human, cobalt must be ingested as vitamin $B_{12}$.

Forshaw and Harwood (1966) measured the intestinal absorption of Co-57 vitamin $B_{12}$ by counting the radioactivity of 5 ml serum obtained 8 to 10 hours after the ingestion of an oral dose of 0.5 mcg of labeled vitamin $B_{12}$. The serum values obtained with Co-57 were compared with those obtained on urine with the Schilling test. Inadequate urine collection and impaired renal function were responsible for low results.
in the Schilling test in 4 of the 12 control subjects, and an incomplete urine collection in four patients with pernicious anemia. The measurement of serum radioactivity for 1000 seconds gave conclusive results, the range in the patients with malabsorption of vitamin \( B_{12} \) being between 0 and 24 counts per minute, and in the control subjects and other patients with megaloblastic anemia between 28 and 64 counts per minute. The highest serum radioactivity level in any one with pernicious anemia was 19 counts per minute. The authors felt that values for even incomplete collections were valid.

Some evidence that in man orally ingested cobalt was not predominantly excreted in the feces was presented by Harp and Scoular (1952). These workers conducted cobalt balance experiments on young women consuming high quality diets supplying an average of 6.7 mcg of cobalt daily. It was found that 73 to 97 percent of the dietary cobalt was absorbed and an average of 67 percent appeared in the urine. The daily fecal excretion of 20 of the 23 subjects was less than 1 mcg cobalt.

**Functions**

A unique nutritional situation has revealed a situation in which animals appeared to utilize cobalt solely as an integral part of vitamin \( B_{12} \) and to be directly and completely dependent upon the symbiotic of their own microorganisms for their supply of this vitamin. However, the primary metabolic defect arising in the tissues of vitamin \( B_{12} \) deficiency sheep was later shown by Marston and associates (1961) to be an inability to metabolize propionate.

Recent evidence from several sources indicated that cobalt probably played an essential part in symbiotic nitrogen fixation of
the root nodules of legumes. Significant growth responses to cobalt by clover growing under field conditions have recently been reported in Australia (Underwood, 1962).

Other functions of cobalt in humans have been explored. Experimentally, cobalt was used to replace zinc in a number of zinc enzymes where catalytic functions were modified in characteristic fashion. Cobalt activated a number of enzymes, among them a variety of phosphotransferases and lyases, although these were active in the complete absence of metals or in the presence of other metals. In pharmacology doses cobalt stimulated erythropoiesis (Li and Vallee, 1970).

Deficiency, toxicity and symptoms

Pernicious anemia is a disease which was seldom reported in children. Stevenson, Little and Langley (1957) reported a case of a seven and one-half year old Negro boy with recurrent macrocytic anemia, megaloblastic bone marrow, free hydrochloric acid in the gastric juice and vitamin B₁₂ deficiency. The anemia was due not to dietary lack of vitamin B₁₂, competitive utilization of vitamin B₁₂ by intestinal microorganisms, or an alteration in absorptive property of the intestine, but to a lack of intrinsic factor in the gastric juice and thus the patient could not absorb vitamin B₁₂. When gastric juice from the patient was administered with radioactive vitamin B₁₂ to another pernicious anemia patient there was no absorption of the vitamin. This showed that there was no intrinsic factor in the gastric juice.

An interesting finding of this study was the lack of achlorhydria in this pernicious anemia patient. There were similar findings in five of the six proved cases of pernicious anemia in childhood reported in
earlier papers. This indicated that the gastric lesions of pernicious anemia were due to vitamin $B_{12}$ deficiency (Stevenson, Little and Langley, 1957).

Another vitamin, folic acid, can replace vitamin $B_{12}$ in some forms of anemia and would prevent certain symptoms of pernicious anemia in most cases of megaloblastic anemia resulting from deficiency of folic acid or vitamin $B_{12}$. A few children have been reported with congenital megaloblastic anemia unassociated with a deficiency of either vitamin. Lampkin et al. (1969) found a previously undescribed form of congenital megaloblastic anemia occurring in two sisters. This form of anemia was diagnosed in the older sister at 7 weeks of age and in the younger at 3 weeks of age. Neither patient had arctic aciduria or hyperuricemia. There was no hematologic response to methionine, pyridoxine, or vitamin E, but did respond to folic acid or $B_{12}$.

Cox et al. (1968) found urinary excretion of propionic acid was raised in patients with megaloblastic anemia due to vitamin $B_{12}$ deficiency. Excretion was normal in three patients with folate deficiency despite the fact that two of these had low serum $B_{12}$ associated with iron deficiency. The propionic acid excretion appeared to be a less sensitive index of vitamin $B_{12}$ deficiency than that previously reported for methylmalonic acid excretion. In nine of twelve patients with pernicious anemia, urinary excretion of acetic acid was raised. The highest urinary levels occurred in patients with neurological complications. The increased urinary excretion of propionic acid probably resulted from a feedback mechanism involving inactivation of methylmalonyl isomerase in the absence of vitamin $B_{12}$ coenzyme. The cause of the raised level of urinary acetic acid was uncertain.
According to Wells et al. (1968) erythrocytetransketolase activity was raised in patients with megaloblastic anemia due to vitamin $B_{12}$ deficiency and was inversely related to hemoglobin concentration. The raised erythrocytetransketolase levels in $B_{12}$ deficiency might reflect enzyme induction due to increased availability of substrate through the hexosemonophosphate shunt.

**Requirement and Recommendation**

**Prevention and treatment**

Cobalt deficiency in ruminants can be prevented or overcome either by treatment of the soils or pastures with cobalt containing fertilizers, by direct administration of supplementary cobalt to the animals, or by injections of vitamin $B_{12}$. The choice of treatment depends upon many factors but treatment with vitamin $B_{12}$ is not of much practical significance for reasons of cost and convenience. In human beings, cobalt, as distinct from vitamin $B_{12}$, has received some attention in human medicine as a nonspecific erythropoietic stimulant in the treatment of various anemias, particularly those of nephritis and of infection (Underwood, 1962). Reports of responses to cobalt, in addition to iron, in the treatment of iron deficiency anemia in children and in human pregnancy have also appeared. The results of such treatment were frequently disappointing and serious toxic manifestations, including thyroid hyperplasia and hypofunction, might occur in some patients as a result of prolonged cobalt therapy. For these reasons use of cobalt alone occupies a very restricted place in the clinical management of human anemias.
According to Cartwright's report (1955), cobalt in doses of 100 mg per day alleviated the anemia of nephritis and infection. Its use in other anemias such as aplastic anemia or hemolytic anemia was very questionable. The toxicity of cobalt has not yet been determined.

Some investigators suggested that cobalt could be used to "potentiate" iron therapy in the treatment of iron deficiency anemia. In a panel discussion of the use of cobalt in the therapy of anemias, the various speakers had different opinions (Berman et al., 1955). Moore and associates questioned the practical value of the use of cobalt in the treatment of iron deficiency anemia. Since patients with iron deficiency anemia regularly responded to the administration of iron alone, the only effect of cobalt was to increase the rate of hemoglobin rise through the polycythemic stimulating activity of cobalt. In other words, if iron therapy alone caused the hemoglobin level to return from an initial 5 g per ml to normal in five or six weeks, cobalt plus iron might shorten that period to four weeks. The patient had, however, no greater sense of well being, might experience nausea from the cobalt, and was subjected to what essentially was a toxic or abnormal influence.

Macrocytic anemia has been reported in infants subsisting on a diet consisting solely of goats milk (Hines, 1966; and Sullivan, Luhby and Strieff, 1966). Some of these infants responded hemotologically to treatment with crude liver extract. A diet consisting solely of goat's milk was adequate only if complete absorption of the vitamin occurred. However, it was not known to what extent an infant could assimilate all of the protein bound vitamin B₁₂ in the milk. Thus,
incomplete absorption might result from a dietary deficiency of the vitamin or a deficiency of the intrinsic factor.

According to Kalinicheva (1966), his patients of hypoplastic and aplastic anemia received corticosteroids, ascorbic acid, thiamine, riboflavin, pyridoxal transfusions and vitamin B\textsubscript{12} parenterally. Following parenteral vitamin B\textsubscript{12} therapy, vitamin serum content increased, then it gradually decreased, and returned to initial levels within 2-2.5 months. Oral administration of 200 mcg of cyanocobalamin caused drastic increases in serum concentration during the first hours following its introduction. In spite of increased urinary excretion, a large part of the injected vitamin B\textsubscript{12} was retained by the organism.

Pollycove and co-workers (1956) described a patient with megaloblastic anemia attributed to dietary deficiency of vitamin B\textsubscript{12}. The patient was treated with a combination of vitamin B\textsubscript{12}, folic acid and ascorbic acid and no assessment of the patient's ability to absorb vitamin B\textsubscript{12} was made until 25 days after treatment, so that the possibility of a limiting deficiency of folate and/or vitamin C prior to therapy could not be excluded.

**Requirement and food sources**

Heyssel et al. (1966) suggested that a minimal daily dietary intake of vitamin B\textsubscript{12} of 0.6 to 1.2 mcg was adequate to maintain health and normal hemopoiesis in normal subjects operating with low body stores. Any additional needs imposed by growth, hypermetabolic states or pregnancy were not known, but it might be higher. It was valid to recognize the value of increased stores to meet periods of depletion.
Studies in two different countries each estimated that an average adult diet of good quality supplied 5-8 mcg of total cobalt daily (Underwood, 1962), but the actual intake varies appreciably with the amount and proportions of the different foods consumed as well as with their origin. Accordingly, an estimate of 3 to 5 mcg of vitamin B\textsubscript{12} as the dietary requirement for a population appears reasonable.

The green, leafy vegetables, especially spinach, are the richest and also the most variable source of this element while the dairy products and cereals are the poorest. The marked disparity between the cobalt and vitamin B\textsubscript{12} contents of human foods was apparent from a comparison of the classifications in terms of cobalt content given above and the following in terms of vitamin B\textsubscript{12} (Underwood, 1962).

Excellent sources (0.05-0.5 mcg per g dry matter): Mammalian liver and kidney, oysters and clams.

Good sources (0.05-0.5 mcg per g dry matter): Lean beef, lamb, veal, poultry meat, salt-water fish and milk.

Poor sources (in many cases no assay response): Cereal grains, leguminous seeds, green leaf vegetable, and yeast.
ARSENIC

Metabolism

Levels

Man interferes only superficially with nature’s own arsenic cycle. It occurs in trace amount in land, sea and air—and in humans. Frost (1969) reported that Bowen listed the number of atoms of arsenic in one mammalian red blood cell as about 700,000. It would take extremely sensitive methods to study arsenic metabolism in man because of the minute quantities involved.

The total amount of arsenic in the normal adult human body was estimated at 14-21 mg or 0.2-0.3 ppm by Schroeder and Balassa (1966). This element was distributed quite evenly in the body, without significant concentration in any particular organ or tissue, except in the nails and hair. The normal arsenic content of human blood ranged from 0.01 to 0.64 ppm. Arsenic content in women’s blood increased significantly during menstruation and pregnancy.

Absorption, transportation and excretion

Schroeder and Balassa (1966) indicated in their work that only two forms of arsenic existed in the environment, pentavalent and trivalent, with tissue arsenic being largely pentavalent. Only very small amounts (0.7 percent) of natural arsenic in shrimp (assumed to be pentavalent) when fed to rats was retained in tissue, whereas the same dose of sodium arsenite accumulated up to 18 percent of the amount given.
Cows fed with arsenate in small doses (1.25 mg per Kg) did not show an increase of arsenic in milk, indicating some mammary barrier and/or rapid elimination. The rat, however, accumulated the arsenate, binding it to hemoglobin and depositing it in spleen, liver, kidney and heart to a much greater extent than did cows, hamsters, guinea pigs and rabbits. Arsenite on the other hand, had a strong tendency to accumulate in the kidney and liver and in hair, nails and skin. It could be absorbed directly into the skin, and hair could incorporate it from dust. Arsenite accumulated in tissues with continued exposures up to a balance or to toxic levels, depending upon dosage.

Early work indicated that ingested inorganic arsenate was excreted from the body in the pentavalent form and was not reduced. Later work showed that arsenite tended to be oxidized slowly in the mammalian body to arsenate. Oxidation to the pentavalent form reduced toxicity. However, Schroeder and Balassa in 1966 stated that reduction by tissues of pentavalent arsenic in organic arsenicals to the trivalent form has not been clearly demonstrated. Thus, these findings confirmed the great differences in the metabolism of arsenate and arsenite.

From their studies, Lowry et al. (1942) observed that pentavalent arsenicals were excreted largely via the urine, whereas the non-physiological trivalent compounds were excreted by the intestine, some of them in bile. More recent studies by Schreiber and Brouwer (1964) demonstrated that pentavalent arsenic was much more readily excreted than trivalent arsenic. In order to understand the toxicity of arsenic in red blood cells, trivalent sodium arsenite and the pentavalents, sodium arsenate, arsenilic acid and 4-nitrophenyl arsenic acid were compared as to their effect on rat hemoglobin with the use of a Sephadex G-50 gel
filtration technique. The results suggested that the hemoglobin-arsenic bond was of the same nature in each case. The recoveries of hemoglobin from the column were significantly lowered in all cases after oral administration, especially with arsanilic acid. The hemoglobin-arsenic binding was increased up to 50 times that of control rats. After injection, only the arsanilic acid gave a lowered hemoglobin recovery. It would appear that the amount of binding of arsenic was a factor in hemoglobin destruction except in the case of arsanilic acid.

Functions

Early work indicated the arsenic had a general "tonic" effect, and was especially beneficial in the maintenance of hemoglobin and red blood cell levels. After the specific nature of copper in hemoglobin formation was recognized, the beneficial effects of arsenic lost much of their significance.

The results from Hove, Elvehjein and Hart's (1938) laboratory indicated that at the levels of 1 and 5 mcg per day of arsenic when fed to rats on a mineralized milk diet, had no effect on growth, hemoglobin levels and the red blood cell level or fragility. They also found that it had no effect on the rate of regeneration of hemoglobin or red blood cells in anemic rats when added with iron, with iron plus suboptimal copper, or with iron plus optimal copper. However, they postulated that optimal arsenic increased the length of life of red blood cells through greater resistance to hemolysis as the manner by which arsenic delayed the fall in hemoglobin. This delay was observed when iron, copper and manganese supplements were withheld from rats on a milk diet.
According to Schroeder and Balassa (1966), the pentavalent form was nontoxic in normal concentrations, at levels found normally in food, and might perform some unknown physiological functions. It was not inhibitory to most enzymes and could substitute for phosphate in some phosphorylases. Trivalent arsenic as arsenite was toxic, as it chelated with dithiol groups and inhibited those enzymes dependent there on.

**Deficiency, toxicity and symptoms**

Attempts to demonstrate an arsenic deficiency in animals have so far proved unsuccessful (Underwood, 1962).

About 80 percent of the arsenic of the blood of normal rats was concentrated in the red blood cells. During anemia there was a marked drop in both cellular and plasma arsenic. Supplementing either anemic or normal rats with increasing levels of arsenic induced sharp increases in cellular and plasma arsenic. Therefore, Hove, Elvehjein and Hart (1938) suggested that 50 ml of milk, containing 2 mcg per day, satisfied the requirements of the rats. This level was based on the assumption that arsenic was essential for the growth of rats during the rapid growth period and/or for the building of hemoglobin or red blood cells.

Buzadzhi (1967) observed that in severe arsenic poisoning in sheep there were considerable decreases in the amounts of reduced glutathione, total glutathione and sugar. Hemoglobin and residual nitrogen were increased. A gradual slow decrease of the coefficients of surface tension and viscosity occurred. Biochemical processes taking place in sheep during severe arsenic poisoning included a clearly established acidosis and a considerable change in carbohydrate and protein metabolism.
In 1967, Nanobshvili found that arsenic combined with proteins of red and white blood cells and plasmas. He suggested that arsenic was bound to albumins and globulins. In addition, it reacted with some nonprotein organic compounds.

An acute case of toxicity was described by Jindrichova and Hassmanova (1967), and was characterized by hemolytic icterus, acute renal insufficiency, and isosthenuria. There was an increase in residual nitrogen to 110 mg percent, in serum creatinine to 10 meq and of the erythrocytes to 1,980,000. The arsenic content in the urine was 1360 gamma per liter on the twelfth day following the poisoning.

In the studies of Schroeder (1968b), 455 rats of the Long-Evans strain were fed diets containing 5 ppm of each arsenic, geranium and tin from weaning until natural death. Large amounts of arsenic accumulated in the tissues, especially in the aorta and red blood cells, with no sign of toxicity.

Requirement and Recommendation

Requirement and prevention

Attempts to demonstrate the deficiency of arsenic in animals have so far failed, largely because of the ubiquity of the element. If arsenic were cumulative, it would be necessary to retain 2 mcg of arsenic per day for 30 years to accumulate 14-21 mg arsenic in the human body (Schroeder and Balassa, 1966). They suggested a daily intake of 900 mcg arsenic as arsenate would maintain a whole blood level of 0.18 ppm (normal values being 0.10-0.64 ppm), or 18 mcg per 100 ml. It might be suggested that it would decrease the toxicity of arsenic under a fresh air circumstance.
Food sources

Most human foods contained less than 0.5 ppm of arsenic and rarely exceed 1 ppm with the exceptions of bony fish which contained 3-4, oysters 5-10, and mussels as high as 120 ppm. Up to 174 ppm has been found in prawns gathered in the coastal waters of Britain and 42 ppm in shrimp from the southeastern coastal waters of the United States (Underwood, 1962).
CADMIUM

Metabolism

Levels

In most animal tissues cadmium concentrations were of the order of less than 1 mcg per g of weight of tissue. The total body concentration of cadmium in man increased with age and varies in different areas of the world (Tipton and Cook, 1963). The cadmium content of the liver and kidney was significantly higher than that of other tissues. Human kidney might contain amounts varying from 10 to 100 mcg per g wet weight.

Absorption, transportation and excretion

Recent studies suggested that both in liver and in kidney cadmium was bound specifically to metallathioncin. The protein has not yet been identified in other tissues. Cadmium is localized predominantly in the renal cortex, in regions corresponding to the proximal tubules.

The element was absorbed poorly from the gastro-intestinal tract and was excreted slowly. In man the average daily net uptake of cadmium was between 15 and 35 mcg. Urinary excretion was approximately 10 mcg per liter (Pulido, Fuwa and Vallee, 1966).

Many of the known effects of cadmium involved the blood and cardiovascular system. Jacobs, Fox and Aldrige (1969) investigated the effects of cadmium upon changes in the plasma proteins, because plasma changes could reflect metabolic alterations in various tissues of the body).
Miller, Blackmon and Martin (1968) studied metabolism in six 25-month-old goats following a single oral or *in vivo* tracer dose of cadmium-109. Whereas, more than 90 percent of the oral dose was excreted in the feces within 5 days after dosing, only 5.6 percent of the *in vivo* dose was excreted via feces during that time. The feces was the predominant route of excretion regardless of method of dosing. Following a peak level of endogenous excretion, 12 days after dosing endogenous excretion was only 7 percent of the peak. Fourteen days after *in vivo* dosing, highest concentrations were observed in the liver, kidney and spleen, in descending order. In contrast, following oral dosing, highest concentrations were found in the small intestinal wall, kidney, and liver. A much higher level of cadmium was found in the liver, heart and spleen of *in vivo* dosed animals relative to other tissues than in orally dosed ones. *In vivo* administered cadmium was apparently transported in a different form from that absorbed from the gastrointestinal tract and this materially affected cadmium metabolism.

**Functions**

Schroeder and Balassa (1965) suggested that cadmium was a toxic element and an etiological factor in essential hypertension.

Ingestion of cadmium by rats was shown to produce severe anemia (Wilson et al. 1941; Fitzhugh and Meiller, 1941). Lawford (1961) reported that the plasma of young albino rats fed small amounts of soluble cadmium salts decreased plasma, elevated plasma transferrin levels and caused precipitating anemia. These were all due to interference with iron absorption or metabolism or both.

Rabbits fed with diets containing cadmium developed hyochromic microcytic anemia, similar to that produced by iron deficiency found
by Axelsson and Piscator (1966), and Berlin and Friberg (1960). It was also observed increased phagocytosis of red blood cells and enlarged spleens (Berlin and Friberg, 1960). The red blood cells became more sensitive to change in asmatic pressure.

In their studies with Japanese quail, Jacobs et al. (1969) suggested that several mechanisms might explain the effect of cadmium in elevating the plasma level of transferrin in the quail. Transferrin functions as the iron donor in the formation of heme. During this process it was bound to reticulocytes and reticuloendthelial cells.

Jacobs et al. (1969) reported that several workers found that transferrin in the rat and rabbit was affected by the mechanisms: one specific for transferrin, depends on the oxygen supply, the other represents a nonspecific response to disturbances of plasma protein metabolism. In the case of rats made anemic by phenylhydrazine, the elevated plasma level of the transferrin was due to increased synthesis and decreased catabolism of transferrin and to its movement from the extravascular to the intravascular space. The predicted erythropoietin increases coupled with a high red cell destruction rate and ineffectual erythropoiesis no doubt would keep the animals iron-deficient and anemic.

Toxicity and symptoms

Cadmium toxicity resulted in hypochromic microcytic anemia, similar to that produced by iron deficiency. The development of this deficiency has been attributed to interference with iron absorption or metabolism or both.
Requirement and Recommendation

Requirement, prevention and treatment

Chromic ingestion of 0.1 to 10 ppm of cadmium had no discernible adverse effects on rats as judged by general health, growth rate, and pathologic examination, although large amounts of the metal were retained in the liver and kidney. Higher doses, however, caused stunted growth, accompanied by hypochromic-microcytic anemia.

Parenteral administration of iron corrected the anemia of rabbits that were made anemic by cadmium (Berlin and Friberg, 1960).

Since cadmium toxicity could be caused under the deficiency of iron, copper or zinc, the normal diet in these elements should maintain an adequate diet of cadmium.

Food sources

Most of the plant and animal tissues contained only about 1 ppm of cadmium or less (Underwood, 1962). There was little information to permit any worthwhile estimates of over-all cadmium intakes from normal human or animal dietaries or of the contributions made by special types of food.
LEAD

Metabolism

Levels

The levels of lead in the blood and urine were generally believed to reflect the degree of exposure and absorption of this metal. Data from the studies of Goldwater and Hoover (1967) indicated that the normal level of lead in the urine was about 20-65 mcg of lead per liter of urine. Level of less than 65 mcg of this metal were found in 95 percent of their samples which gave a mean of 35 mcg of lead.

They also proposed that a normal range for lead in blood was 15 to 40 mcg of lead per 100 ml of blood. Values below 50 mcg were obtained for 95 percent of their samples with a mean value of 17 mcg and a standard deviation of 11.

In general, blood lead levels of urban population were found to be slightly higher than those from rural. There was no significant difference between the sexes.

Chisolm (1965) suggested "normal values" of lead in the whole blood for different age groups (Table 7).
Table 7. Concentration of lead in whole blood of healthy persons without abnormal exposure to lead

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 6 months</td>
<td>19</td>
<td>15</td>
<td>5-31</td>
</tr>
<tr>
<td>6 months to 8+ years</td>
<td>84</td>
<td>27</td>
<td>15-40*</td>
</tr>
<tr>
<td>4 months to 14 years</td>
<td>80</td>
<td>24</td>
<td>14-42</td>
</tr>
<tr>
<td>Adult</td>
<td>30</td>
<td>27</td>
<td>15-40</td>
</tr>
</tbody>
</table>

*Middle 90 percent. (From Chisolm, 1965, p. 531)

Absorption, transportation and excretion

Goldwater and Hoover (1967) observed that lead entered the human body mainly by ingestion and inhalation. When lead was administered by mouth to 3 human subjects, Hursh and Suomela (1969) compared the 24-hour urinary excretion of the subjects. There was a wide range in their gastro-intestinal absorption of 1.3, 8.1 and 16.0 percent. They believed that the main factor in this wide range in absorption was age, and that increasing age was associated with decreased absorption.

Most of the ingested metal was excreted in the feces without being absorbed into the blood stream. The endogenous lead in normal humans was excreted by way of urine. Concern has been expressed that increased burning of gasoline by the internal combustion engine and other uses of the metal in industry were causing additional exposures to lead in our environment. Thus, the levels of lead in the blood and urine were considered to reflect the degree of absorption and exposure (Goldwater and Hoover, 1967).
Bone is the chief storage depot for lead. The rate of bone turnover has considerable influence on lead turnover in man. Since the rate of bone turnover differed between children and adults, the lead turnover rate should also be different. However, the urinary lead output of the adult was increased in response to excessive absorption. Within several weeks renal excretory capacity became saturated, whereupon the blood-lead concentration began to rise. The rate of accumulation differed with the amount of lead ingested. Chisom (1965) also found that it took twice as long for the adult to excrete a given excessive load of lead as it did for him to accumulate it.

About 90 percent of the lead in whole blood was transported attached to the red cell surface; the quantity of circulating red cells largely determined the blood's carrying capacity for lead. Thus, the rate of new absorption of this element into the body might exceed the rate of excretion and of bone deposition, with a resultant rapid rise in blood lead levels.

Functions

No suggestion of a nutritional role of lead in the human body or in any other animals was found in the literature.

However, an excessive amount of lead caused the disorder of central-nervous system (CNS), and in the heme synthesis and catabolism.

Lichtman and Feldman (1963) proposed that in the lead-poisoned erythrocyte, the activity of delta-aminolevulinic acid dehydrase, the enzyme necessary for conversion of delta-aminolevulinic acid to porphobilinogen, was decreased. In vitro porphyrin production from delta-aminolevulinic acid was markedly diminished in poisoning. And, in the
lead poisoned erythrocyte, a defect in the heme biosynthesis pathway occurred in the sequence beyond porphobilinogen.

The blocking action of lead might be exerted not only at the stage of incorporation of iron into the protoporphyrin ring but earlier, both before and after the elaboration of coproporphyrin III (Baikie, 1954). The enzyme blocking action occurred at more than one point in the synthesis of hemoglobin. A diagram of the \textit{in vivo} porphyrin synthesis in lead poisoning and iron deficiency is shown in Figure 6.

\textbf{Toxicity and symptoms}

Acute lead encephalopathy in early childhood produced irreversible injury to the brain. In clinically recognized cases of this disease, some degree of central nervous system involvement was usually demonstrated (Griggs et al., 1964).

The toxicity of lead caused a hemolytic anemia characterized with hypochromia, basophilic stippling and siderocytosis of the erythrocytes. Lead had a direct effect on mature red blood cells, which occurred independently of the effect of lead on heme biosynthesis in erythroid precursors. Also lead might affect both heme synthesis and its catabolism (Berk et al., 1970).

Rabbits with intravenous injections of toxic amounts of lead acetate, had a significant decrease in iron-59 in the liver and a distinct increase of radioactivity in the bone marrow. The mechanism of iron-binding to apoferrin was damaged, thus Sroczynski and Jonderko (1969) proposed that this was the cause of a decreased uptake of iron-59 by hepatic cells. The increase in spleen and bone marrow radioactivity was thought to result from enhanced destruction of erythrocytes and
Figure 6. Simplified scheme for the biosynthesis of heme. (From Lichtman and Feldman, 1963, p. 831)
from inhibition of iron incorporation into protoporphyrin. In an earlier study on the fecal excretion of urobilinogen of normal and lead poisoned guinea pigs, Baikie (1953) also pointed out that the poisoned animals showed a fall in hemoglobin levels with increasing excretion of urobilinogen.

Requirement and Recommendation

Requirement, prevention and treatment

The normal dietary intake of lead was suggested as 0.5 to 1.0 mg per day for adults by Chisolm (1965).

Lead toxicity was more prevalent in children than adults. In their investigation on the influences of environment of lead poisoning, Grigg et al. (1964) observed that in Cleveland, a large number of children had evidence of increased lead absorption. They also found that the oral ingestion of lead-containing material derived from the environment was the source of the lead which caused the lead poisoning. Paint containing more than 1 percent lead was considered potentially hazardous. In addition, air pollution increased the lead entering the body. Thus, the improvement of our environment is necessary to prevent lead poisoning.

Treatment of lead toxicity anemia with calcium disodium ethylenediaminetetracetic acid (EDTA) might correct heme biosynthesis in erythroid precursors, but not the hemolytic effect on mature red blood cells. Selander, Cramer and Hallberg (1966) also suggested that penicillamine might cure lead toxicity by decreasing urinary excretion of delta-aminolevulinic acid and increasing lead excretion. Fifteen patients were treated with a daily dose of 0.75-1.5 g of penicillamine
for 13-29 days. Lead concentrations in blood were lowered. A good correlation was found between blood lead and urinary aminolevulinic acid concentrations prior to and during treatment. However, exanthemata, eosinophilia, fever, and agranulocytosis were noticed as side effects in one patient.

Food sources

Most common foods, drinking water and beverages contained some lead. Air also contributed a considerable amount of lead (Goldwater and Hoover, 1966), especially in the urban area.
Diets high in molybdenum caused anemia in chick, cattle and rabbit (Davies et al., 1960; and Ferguson et al., 1943). There has been no similar clinical incidence reported in humans.

Levels

Subsequent work showed that traces of molybdenum are present in all plant and animal tissues.

Although the liver and kidneys contained higher concentrations of molybdenum than the other organs, it did not particularly accumulate in any organ or tissue. Underwood (1962) listed the concentrations of the element in some organs of three species in the following table:

<table>
<thead>
<tr>
<th>Species</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Lung</th>
<th>Brain</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult humans</td>
<td>3.2</td>
<td>1.6</td>
<td>0.20</td>
<td>0.55</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Adult rats:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal diet</td>
<td>1.8</td>
<td>1.0</td>
<td>0.52</td>
<td>0.37</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>Chicken:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal diet</td>
<td>3.6</td>
<td>4.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.14</td>
</tr>
</tbody>
</table>

*Values given in ppm Mo on dry basis. (From Underwood, 1962, p. 101)

Underwood (1956) reported that 6 mcg molybdenum per 100 ml of whole blood had been observed by some workers to be normal for sheep and
cattle grazing on pastures normal in copper and low in molybdenum. But when molybdate drenches were given to the animals, equivalent to a dietary intake of 30 ppm, the bloodmolybdenum levels rose to 60-80 mcg in young cattle and 240-340 mcg in breeding ewes (Underwood, 1962).

According to the data of Polonskaya (1966), the concentration of molybdenum in the blood of 12 normal and 37 pregnant women was determined with a colorimetric method. As the pregnancy proceeded the element concentration increased from 12.95 mcg per 100 ml blood in the 12-19 week of pregnancy to 33.85 mcg at the time of labor. The blood molybdenum content of non-pregnant controls was found to be 16.55 mcg. Since the daily molybdenum uptake was 0.26 mg, the increase in blood molybdenum concentration was probably due to a mobilization of tissue reserves.

Absorption, transportation and excretion

Molybdenum was absorbed readily from the intestinal tract. The water-soluble forms such as sodium or ammonium molydate were the most soluble. Even such insoluble compounds as MoO₃ and CaMoO₄ but not MoS₂ were well absorbed by rabbits and guinea pigs when fed in large amounts (Underwood, 1962).

Very little of an oral dose of radiomolybdenum administered to pregnant cows was transferred to the fetuses. However, placental transfer of molybdenum might occur because high concentrations have been observed in the liver of newborn lambs from ewes receiving a high molybdenum diet (Cunningham, 1950).

Molybdenum was excreted mainly in the urine. A small amount was also excreted in bile by way of the feces. The tissue molybdenum
concentration might be increased or decreased by raising or lowering dietary intake.

When 8 swine and 16 sheep were fed the same ration (Bell, Sneed and Hall, 1967), the swine excreted both *in vivo* and orally dosed radioactive molybdenum (Mo-99) in the urine. From *in vivo* dosed sheep excreted Mo-99 mostly in the urine, whereas those dosed orally excreted the Mo-99 in the feces. Whole body gamma-irradiation of 400-450 gamma showed no effect on the metabolism of molybdenum in swine and sheep. In their second experiment with 12 sheep, it was found that the peak blood levels of molybdenum in abnormally dosed sheep were 10 times the levels of orally dosed sheep. Thus, they suggested that the metabolism of molybdenum was influenced by species, route of administration and by the ration fed.

Underwood (1962) reported that sulfate limits the retention of molybdenum both by reducing intestinal absorption and increasing urinary excretion. The extent of each depended upon the previous history of the animal with respect to molybdenum and sulfate intake. Changing from a low sulfate diet to a high sulfate diet resulted in a highly significant increase in urinary molybdenum output, with little change in fecal molybdenum. The influence of sulfate on molybdenum absorption and excretion was explained as the inorganic sulfate interfered with the transport of molybdenum across membranes. If concentrations were high enough this transport was prevented. The effect was an increase in the excretion in the urine by raising the sulfate concentration in the ultrafiltrate of the kidney glomerulus. Thus, reabsorption of molybdenum was impeded or blocked through the kidney tubule.
Functions

Li and Vallee (1970) suggested that at least part of the molybdenum was bound to various metalloflavo-proteins, that is xanthine and aldehyde oxidase in animal tissues and nitrate reductase in plants and bacteria.

The element was also important for nitrogen fixation of leguminous plants and for the biologic conversion of nitrate to ammonia or amino acids by fungi and higher plants, as well as other processes in plant metabolism.

A study by Grigoryan and Brutyan (1967) involved fields which were high in molybdenum and were used for 10 sheep, 7 cows and calves. Non-protein nitrogen was higher in all animals when the diet contained 0.2-2.0 molybdenum per day. They thought that this alteration was due to disturbed purine metabolism, disturbed urea formation in the liver, and injury to the concentrating ability of the kidney.

According to the studies with chicks, Davies et al. (1960) showed that when the chick diet was increased from 500-2000 ppm of molybdenum, a small but consistent elevation of both hemoglobin values concentration and packed cell volume. At the 2000 ppm level the red cell counts were also elevated. Higher concentrations of molybdenum produced anemia. Red cell volume and red cell count of the chicks receiving 4000 ppm were below the basal group values, and hemoglobin concentration was depressed at 6000 ppm. The mechanism of this effect of molybdenum has not been elucidated.

Toxicity and symptoms

No symptoms of a deficiency of molybdenum has been reported in the literature. Using rabbits, Burke et al. (1953), produced an anemia
characterized by the toxicity of molybdenum. The total red cell volume was abnormally low in the anemic rabbits. A higher total plasma volume accounted for the normal blood volumes.

Likewise, Davies et al. (1960) reported that chicks fed a diet containing up to 4000 ppm produced anemia by depressing the hemoglobin concentration.

**Requirement and Recommendation**

**Requirement, prevention and treatment**

There is little quantitative evidence of requirement for humans, neither has there been any deficiency or toxicity cases described.

For chicks Kratzer (1952) proposed 100 ppm as the lowest molybdenum level, whereas Motzok et al. (1957) suggested that 500 ppm be used as the lowest level. In 1960, Davies also reported that chicks would not produce a growth depression if the diet molybdenum content was lower than 500 ppm.

In most species, the toxicity of molybdenum can be overcome by the addition of copper to the diet (Underwood, 1962) as a sulfate. In 1960 Davies et al. reported that sodium sulfate and ammonium sulfate both have the ability to eliminate the toxicity of molybdenum.

It could be assumed that under an adequate copper diet, 500 ppm of molybdenum per day or lower should be normal.

**Food sources**

Since molybdenum was considered as an essential element only recently, it has little practical significance in human nutrition. No comprehensive reports of the element in human foods and dietaries have appeared.
Rich sources: beans and peas (3–9 ppm).

Intermediate sources: legumes, cereal grains and some green leaf vegetables, liver, kidney and spleen.

Poor sources: fruits, berries and most root or stem vegetables

(Thr. Meulen, 1932; and Westerfeld, 1953).

It has been shown that the molybdenum concentration in plants, even the same specie, varies greatly with the soil type on which they have been grown and to some extent with the species.
SELENIUM

Metabolism

The toxicity of high intake of selenium in animals has been the subject of intensive investigation wherever high selenium in soils occurs.

Besides cattle and sheep, selenium toxicity as characterized by hemolytic anemia has been produced in rats (Halverson et al., 1970), in chicken embryos (Kury, Rev-Kury and Crosby, 1967), and duck (Rigdon, Crass and McConnell, 1953). But the possibilities of such poisoning in the human population, depending upon foods grown in the areas, has not occurred. Surveys of families living in seleniferous areas indicated that this element was being ingested in a large amount but no symptoms clearly pathognomic of selenium poisoning were observed in man.

In the intermountain area after the symptoms of a selenium toxicity were suspected and later recognized in cattle and sheep in Wyoming, soil analyses showed a high concentration of selenium. Toxic symptoms in their animals were the same as described in other parts of the world. A mild selenosis in the children in these areas of Wyoming has been indicated by the direct relationship between dental caries incidence and selenium intakes (Hadjimarkos and Bonhorst, 1943).

Toxicity symptoms of hemolytic anemia have not been observed. Within the last few years, the need of selenium in physiological amounts has been observed by other workers because of its functional relation to vitamin E.
Levels

In most species the selenium concentrations were consistently higher in the liver, kidney, heart and spleen than in other organs and sometimes reached 20-30 ppm on the dry basis at toxic intakes. The muscles and other tissues, at the same intakes, rarely exceeded 5-7 ppm. The hair and hoofs of severely affected animals had even higher levels of the element.

Absorption, transportation and excretion

The absorption of selenium appeared to depend on the solubility of its compound and on the dietary ratio to sulfur. Selenium was rapidly and efficiently absorbed from naturally seleniferous diets and from soluble salts of the element added to diets. At least part of selenium toxicity might be due to the interference with absorption of sulfur, e.g., selenate with sulfate and seleno-amino acids with the sulfur-containing amino acids both free and as protein (Underwood, 1962).

Selenium was absorbed from the gastrointestinal tract and carried mainly in the plasma from which it slowly left to enter all the tissues, including the hair, bones and erythrocytes. The only exception was fat. In most species the highest concentrations of selenium were found in liver, kidney, heart and spleen. Studies with rats, suggested that selenium was transferred in the tissues from one rapidly excreted form to another slowly excreted form. This conformed with the concept of gradual incorporation of this element into tissue proteins and implied that the rate-limiting process for the smaller rate constant was the rate of release of selenium from fixed forms in the tissues. Fixation of selenium in tissue proteins has been demonstrated in studies of
proteins from blood, liver and leucocytes (Underwood, 1962). This element was readily transmissible through the placenta to the mammalian fetus whether fed as inorganic or as organic food selenium to rats and cats. Selenium also readily passed the mammary barrier in the bovine and was transmitted with equal facility from the hen to the egg.

This metal was excreted by way of the urine, the feces and the expired air. Fecal selenium consisted largely of unabsorbed selenium, since it left the body principally in the urine after injection. The amount of ingested selenium which appeared in the feces depended on the form existing in food, the level, and duration of the intake and also upon the species. Excretion by way of the feces was greater than by the urine and by the expired air (Underwood, 1962).

Functions

Quite recently, a biological role of selenium has been identified which resulted in the classification of selenium as an essential element. Three biochemical effects now known are:

1. It is necessary in vitamin E or tocopherol metabolism.
2. In high dosages, it acts as an antagonist of sulfur metabolism.
3. In high dosages, it interferes with the formation of erythrocytes.

The studies of Desai and Scott (1965) showed that one of the biological roles of a selenium-containing compound was to act as a carrier of vitamin E. It might function in absorption, retention, prevention of destruction and perhaps transfer across cell membranes of d-a-tocopherol, thereby enhancing its biological activity in the blood and perhaps in cells throughout the body. They then suggested that selenium played an
indirect role in the prevention of muscular dystrophy, functioning perhaps through biochemical or physiological interactions with the primary protectors, vitamin H^3-tocopherol.

Li and Vallee (1970) reported research work that showed toxic amounts of this metal acted as antagonists of sulfur metabolism and inhibited some enzymes, such as succinic dehydrogenase, urease, choline oxidase, tyramine oxidase and proline oxidase.

Using duck erythrocytes, Rigdon, Crass and McConnell (1953) observed that an excessive amount of selenium interfered with some enzyme systems in the formation of erythrocytes. This interference can be counteracted by cysteine. Thus, it appeared that the selenium inhibited an enzyme system dependent on the sulfhydryl for its activity.

It might also function in maintaining the stability of biological membranes of structures such as mitochondria, microsomes and lysosomes (Tappel, 1965).

Selenium has been found in many highly purified proteins, e.g., aldolase, cytochrome c, hemoglobin, myoglobin, myosin and ribonucleoproteins.

**Deficiency, toxicity and symptoms**

Deficiencies of selenium had been shown to produce nutritional disorders in rats, chickens and turkeys. Similar diseases have also been found in livestock when fed diets low in selenilum. Chicks fed diets containing low levels of selenium and vitamin E developed a fatal disease, exudative diathesis, in which there are massive accumulations of fluid in the muscles and connective tissues (Thompson and Scott, 1969). The deficiency of this element has caused liver necrosis in
the rat and pig, nutritional muscular dystrophy in lambs, calves and chicks, a multiple necrotic degeneration of heart, liver, muscle, and kidney in the mouse.

In studying the inhibition of maturation of duck erythrocytes by sodium selenate, Ridgon, Crass and McConnell (1953) observed an anemia developed as a result of chronic selenium poisoning which did not show any morphologic evidence of injury to the erythrocytes in the peripheral blood in their white Pekin ducks.

When Kury et al. (1967) injected selenilic acid into fertilized White Plymouth Rock eggs during day 4 of the incubation, they produced anemia in a number of malformed and grossly normal 19-day-old embryos. The anemia was characterized by low red blood cell counts and hemoglobin values. The mean corpuscular hemoglobin showed a wide range of variation around the mean, especially in the grossly normal embryos. Although a higher percentage of malformed embryos were anemic, some of the grossly normal embryos had lower red blood cell counts and hemoglobin values.

In studying the cause of anemia in chronic selenite toxicity, control and selenite-fed rats were injected with iron-59. The animals soon showed anemia (Halverson et al., 1970). The hemoglobin values decreased rapidly as did the newly incorporated iron-59 in the blood at the same time. At death, the overall reduction of the hemoglobin concentration in the blood was less than the reduction of the labelled iron. During the late stage, hemoglobinuria occurred. The animals which did not show extensive loss of the iron-59 in the blood did not show anemia or die. Thus, their results indicated that the anemia is caused by hemolysis rather than by a defect in red blood cell synthesis.
Requirement and Recommendation

Requirement, prevention and treatment

The minimum dietary requirement of selenium for normal growth, health and reproductive performance in animals has not been estimated because of the minute amount needed. It is difficult to prepare a selenium deficient diet because quantity of the several chemical forms in foods vary; the physiological active form or forms as found in food have not been established; and absorption and utilization are influenced by other components of the diet (Underwood, 1962).

In recent studies, Thompson and Scott (1969) proposed that chicks were protected against exudative diathesis by supplements to semi-purified diets of either 10 ppm d-α-tocopheryl acetate or 0.04 ppm selenium. In contrast, chicks given diets prepared with crystalline amino acids and containing less than 0.005 ppm selenium had poor growth and high mortality even when the diet contained up to 200 ppm d-α-tocopheryl acetate. Using diets containing 100 ppm vitamin E, the selenium requirement was less than 0.01 ppm, whereas with 10 ppm vitamin E it was more than 0.02 ppm and with no added vitamin E, approximately 0.05 ppm. It was suggested that the exudative diathesis in chicks could be prevented by supplementing the diet with either selenium or 5-20 ppm d-α-tocopheryl acetate.

In their studies of hemolytic anemia in rats fed selenite, Halverson et al. (1970) found that the effect of the selenite treatment on the iron-59 loss from the blood was reduced when arsenite was added to the diet. The use of arsenite in the diet without selenite also had some sparing effect on the loss of iron-59. When arsenite was fed with labeled selenite, the selenium level in the liver and kidneys was
reduced. Thus, it can be seen, that the addition of arsenite to a selenite-treated diet was associated with a reduction in hemolysis and in selenium accumulation in certain organs.

Since the need of selenium is so minute, deficiencies caused by diet are unlikely to occur, especially with an adequate amount of vitamin E. Cadmium and arsenic are the protectors of selenium toxicity but they are not too wholesome nutrients. Unless the toxicity is severe, it is not recommended to use them.

Food sources

Since selenium concentrations are higher in the liver, kidney, heart and spleen, these organs are considered to be the rich sources in animal foods.

Miller and Byers (1937) divided plants into three classes according to their capacity to assimilate selenium and thus influence toxicity. These were (1) plants showing a limited tolerance and absorbing only small amounts (5 ppm or less on the dry basis) from seleniferous soils, that is, most vegetables; (2) plants which absorbed moderate amounts (up to about 30 ppm) without harm to themselves, that is, all the cereals and onions; (3) plants which absorbed selenium readily and could accumulate up to several thousand parts per million in their tissues, that is, some species of the genera Astragalus, Stanleya, Oonopsis, and Xylorrhiza in this class.
A study of characteristic rates of manganese, zinc, cadmium and mercury uptake and their transport has been made by Sahagian, Harding-Barlow and Perry (1967). They used radioisotopes of the metals and loops of rat small intestine in a new in vitro perfusion method. The relative uptake-to-transport molar ratios for zinc, mercury, cadmium and manganese were approximately 20:12:6:1, respectively. The uptake or transport of a metal was strikingly enhanced or depressed by the presence of a second metal particularly when the initial concentration of the second metal exceeded the concentration of the first. There was competition among these metals for uptake sites. Transport was controlled to a lesser degree by the diffusibility characteristics of the ions of the particular metal. In their previous communication, they reported that their regional in vitro uptakes of zinc, cadmium and mercury by intact strips of rat intestine were least by the jejunum; for manganese least by the ileum. Jejunum and ileum preparations showed the highest uptake of zinc, then mercury, cadmium, and with manganese showing the least. They found cadmium and mercury enhanced the uptake of mercury, but depressed the uptake of manganese. Cadmium was depressed by mercury. Manganese was found to have no effect on zinc or cadmium uptake, but the uptake of mercury was slightly enhanced by manganese with concentration of $10^{-4}$ to $10^{-3}$ molar. Cadmium uptake was enhanced by zinc with concentration of $10^{-5}$ to $10^{-3}$ molar but depressed at higher zinc concentration.
Lease (1968) found, at low dietary levels of cadmium and zinc, that interference might not occur. Cadmium when retained in the liver and tibiae occupied the zinc-binding sites in the blood. Even small amounts of cadmium over a long period of time, owing to lack of homeostatic control, could cause an accumulation of cadmium which could occupy the blood-binding sites of zinc and thus cause a decrease in absorption of zinc. Cadmium might also interfere with utilization of the absorbed zinc. Thus the effect of dietary cadmium on dietary zinc would depend on the absolute amounts of each, the proportions of one to the other, and the length of the feeding period.

That dietary cadmium decreased absorption and tissue concentration of zinc-65 was shown by Powell in 1967 following a single oral dosing in zinc-deficient and normal calves and goats. Cadmium had little effect on urinary excretion of zinc-65.

Zinc and selenium were found to be the protectors of cadmium toxicity (Gunn, Gould and Anderson, 1968). Selenium was the most potent, completely preventing the toxic effect of cadmium in dosage ratio 2:1. The protectors, none of them work in the same way, did not reduce the amount of cadmium necessary to produce testicular damage, but somehow inactivated the cadmium which reached the testis. Cadmium damage occurred at the vascular endothelium of the testis but zinc and selenium were capable of inactivating cadmium at the site. A selenium-cadmium complex was formed, which transported cadmium safely in an inactivated form past the vulnerable site.

A crude diet greatly increased the elimination of selenium via the expired air; arsenite markedly increased elimination via the gastrointestinal tract, whereas cadmium minimized elimination and increased
the retention of selenium in the body (Ganther and Baumann, 1962). Young male rats were used in their experiments. This excretion of selenium caused by arsenite amounted to 30 to 40 percent of the injected dose in most animals, compared with 5 to 15 percent in the absence of arsenite. Levels of selenium in the blood, liver, and carcass were decreased by arsenite, as well as the amounts in the expired air; kidney levels were increased. Urinary excretion of selenium was not affected by arsenite.

When Willingham (1970) fed a dried skim milk-glucose diet with varying levels of iron and manganese to chicks, there was an interaction between the two metals on hemoglobin concentration. Hemoglobin values were reduced with increasing manganese concentrations which could be prevented by the addition of increasing amounts of dietary iron. Using isolated intestinal segments in vivo and iron-59 as a marker, it was shown that manganese interfered with iron absorption. Fractionation of mucosal proteins showed that the manganese-54 which had been introduced into the lumen was not associated with the ferritin fraction but was eluted with a protein at pH 7.5.

In vitro studies with radioactive elements showed that the manganese interfered with cobalt uptake by rumen microorganisms.

Studies with lambs indicated that manganese source, cobalt level and source, protein source and fat level all contributed to the observed change in ration digestibility, live weight gain, fat deposition, endocrine development and zinc availability (Pfander, Beck and Preston, 1966).

Even manganese in the diet depressed hemoglobin formation in both mature rabbits and baby pigs (Matrone, Hartman and Clawson, 1959).
They observed 2000 ppm manganese in the diet depressed hemoglobin formation in both rabbit and baby pigs. The minimal level of manganese in the diet that interfered with hemoglobin formation was estimated to be between 50 and 125 ppm. A supplement of 400 ppm of iron in the diet overcame the depressing effect of 2000 ppm of manganese on the hemoglobin formation of baby pigs. A supplement of either 1250 or 2000 ppm of manganese depressed growth which was probably the result of the anemia rather than the direct effect of manganese on rate of gain.

When hay containing insufficient amounts of zinc (< 20-25 mg per kg) was supplemented in a sheep diet with ZnSO₄ (2.5 mg Zn per kg body weight) growth was stimulated. Molybdenum administered as sulfate replaced zinc in some tissues and organs, which was accompanied by increased excretion of zinc in urine (Kholod, Shpak and Gutkovich, 1968).

In vitamin B₁₂ deficiency the average amount of sodium, potassium, magnesium, zinc, calcium and copper in red blood cells was increased (Table 9), but only zinc was raised in all human objects observed (Valberg, Holt and Brown, 1965).

In iron deficiency the average amount of sodium, potassium, magnesium, copper, and zinc in the red blood cell was decreased (Table 10). The diminution in sodium, magnesium, copper, and zinc was proportional to the decrease in cell volume.

In investigations of Ganther and Baumann (1962b), rats given labeled selenate excreted substantially more selenium into the urine when sulfate was injected or was present in the diet. Although the increased urinary excretion of selenium was accompanied by a decreased excretion into the gastrointestinal tract, there were small but consistent decreases in the retention of selenium in the blood, liver, kidney and
Table 9. Chemical composition of erythrocytes in iron deficiency (mean value ± SD)

<table>
<thead>
<tr>
<th>Substance</th>
<th>mcg/g</th>
<th>mcmoles/g</th>
<th>mcmoles x 10^{-9} /cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>193.7 ± 28</td>
<td>8.42 ± 1.2</td>
<td>0.733 ± 0.099†</td>
</tr>
<tr>
<td>Potassium</td>
<td>3,903 ± 240*</td>
<td>99.81 ± 6.1*</td>
<td>8.71 ± 0.73†</td>
</tr>
<tr>
<td>Magnesium</td>
<td>55.3 ± 6.7</td>
<td>2.27 ± 0.28</td>
<td>0.199 ± 0.036†</td>
</tr>
<tr>
<td>Zinc</td>
<td>10.9 ± 1.9</td>
<td>0.167 ± 0.03</td>
<td>0.0145 ± 0.0019†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>mcg/g</th>
<th>mcmoles x 10^{-12} /cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>3.60 ± 1.23*</td>
<td>89.8 ± 31*</td>
</tr>
<tr>
<td>Copper</td>
<td>0.96 ± 0.22</td>
<td>15.2 ± 3.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>mcg x 10^3/m</th>
<th>mcmoles/ml</th>
<th>mcmoles x 10^{-9} /cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>310.0 ± 35</td>
<td>4.65 ± 0.49</td>
<td>0.339 ± 0.047†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>mcg x 10^3/g</th>
<th>mcmoles</th>
<th>mcmoles x 10^{-9} /cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>49.2 ± 2.8†</td>
<td>1.76 ± 0.10†</td>
<td>154.0 ± 21†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substance</th>
<th>mcg x 10^3/g</th>
<th>mcl/g</th>
<th>mcl x 10^{-9} /cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>707.8 ± 21.4*</td>
<td>707.8 ± 21.4*</td>
<td>61.8 ± 5.1†</td>
</tr>
<tr>
<td>Mean cell volume</td>
<td>72 ± 7.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean level increased significantly.
†Mean level decreased significantly.
(From Valberg, Holt and Brown, 1965, p. 1228)
Table 10. Chemical composition of erythrocytes in vitamin B<sub>12</sub> deficiency (mean value ± SD)

<table>
<thead>
<tr>
<th></th>
<th>mcg/g</th>
<th>mcmoles/g</th>
<th>mcmoles x 10^{-9}/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>255.7 ± 28*</td>
<td>11.1 ± 1.2*</td>
<td>1.70 ± 0.19*</td>
</tr>
<tr>
<td>Potassium</td>
<td>3,636 ± 113</td>
<td>92.99 ± 2.9</td>
<td>14.3 ± 1.4*</td>
</tr>
<tr>
<td>Magnesium</td>
<td>69.3 ± 16*</td>
<td>2.85 ± 0.65*</td>
<td>0.440 ± 0.12*</td>
</tr>
<tr>
<td>Zinc</td>
<td>15.5 ± 2.9*</td>
<td>0.237 ± 0.04*</td>
<td>0.0363 ± 0.0076*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mcg/g</th>
<th>mcmoles</th>
<th>mcmoles x 10^{-12}/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>2.48 ± 0.43</td>
<td>61.8 ± 11</td>
<td>9.54 ± 2.1*</td>
</tr>
<tr>
<td>Copper</td>
<td>0.83 ± 0.13†</td>
<td>13.2 ± 2.1†</td>
<td>2.01 ± 0.30*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mcg x 10^3/ml</th>
<th>mcmoles/ml</th>
<th>mcmoles x 10^{-9}/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>337.5 ± 41</td>
<td>5.04 ± 0.62</td>
<td>0.611 ± 0.061*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mcg x 10^3/ml</th>
<th>mcmoles</th>
<th>mcmoles x 10^{-9}/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>54.5 ± 2.7</td>
<td>1.95 ± 0.10</td>
<td>298 ± 20*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mcg x 10^3/g</th>
<th>mcl/g</th>
<th>mcl x 10^{-9}/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>676.3 ± 16</td>
<td>676.3 ± 16</td>
<td>103 ± 10*</td>
</tr>
</tbody>
</table>

Mean cell volume 123 ± 9.3*

*Mean level increased significantly.
†Mean level decreased significantly.
(From Valberg, Holt and Brown, 1965, p. 1220)
carcass. Sulfate was much less effective in altering the distribution or excretion of labeled selenite. Measured by the growth of young male rats, dietary sulfate partially alleviated a chronic selenium toxicity induced with selenate.

When weanling pigs were fed a soybean diet in which the zinc level was 0.2 or 0.4 percent, evidence was obtained that using the 0.4 percent of zinc resulted in a significant reduction in liver iron without a concomitant loss of liver copper. On the other hand, although 0.2 percent of zinc in the diet caused a significant increase of liver zinc, the iron content in the liver decreased.

Davis, Norris and Kratzer (1962) raised chicks on diets containing three levels of zinc, manganese, copper and iron and without ethylenedia-minetetraacetic acid in order to measure the effect of isolated soybean protein on the availability of these elements. Isolated soybean protein contained a component which combined with the zinc, manganese, and copper. Chicks developed the respective deficiency symptoms because of the unavailability of these minerals. The addition of EDTA to this diet reduced the chick's requirement for these trace elements, and produced the lowest tibia length-to-body weight ratio. This finding was interpreted to indicate that the zinc from the isolated protein-bound zinc made available to the chick met its requirement for normal bone development. The iron in isolated soybean protein appeared to be available for growth, hemoglobin and packed cell volumes. EDTA had no effect on the requirement for iron. Hemoglobin and packed cell volume values were decreased to a greater extent with a deficiency of iron than with a deficiency of copper.
Van Campen (1969) suggested that there was a mutual antagonism between zinc and copper during the adsorption process and this antagonism apparently took place either in or on the intestinal epithelium. Using Zinc-65 and copper administered intraduodenally into isolated duodenal segments depressed subsequent absorption of zinc-65. When zinc-65 was given intraduodenally and copper was given intraperitoneally, there was no depression during absorption.

Starcher (1969) thought that zinc and cadmium were inhibitors of copper absorption, which acted as copper antagonists by binding to, and displacing copper from the duodenal protein.

Bunn and Matrone (1966) observed the interactions of cadmium, copper, iron and zinc in mice and rats. Cadmium decreased weight gains and hemoglobin level significantly in copper-depleted or normal rats and mice. Feeding a combination of copper and zinc largely overcame the adverse effects of cadmium for copper-deficient rats and mice, but did not depress the effect of cadmium in rats and mice on normal diets. Dietary cadmium increased zinc concentrations in liver and testis more markedly in normal-fed mice than in copper-depleted animals. Dietary zinc increased liver iron concentration in all cases.

Dowdy and Matrone (1968b) fed a purified, low copper diet which contained different levels of molybdenum and inorganic sulfate. Anemia developed only in those animals receiving molybdenum. All the sheep showed a decrease in plasma copper. High levels of dietary molybdenum did not significantly depress the induction of ceruloplasmin activity in chicks stressed with fowl typhoid. These workers (1968a) also fed baby pigs with Cu-Mo complex as the only copper supplement, serum copper levels and ceruloplasma activity were similar to those in pigs fed copper
sulfate. In sheep given intravenous injection of the Cu-Mo complex made from Copper-64 and Molybdenum-99 the following results were shown: The rates of removal from the blood of the copper and molybdenum were equal; and this rate was more rapid than the removal of molybdenum when molybdenum-99 was injected alone. Conversely, the rate of urinary excretion of molybdenum from the Cu-64-Mo-99 injected sheep was slower than from the molybdenum-99 injected animals. They suggested that these results supported the hypothesis that copper bound in a Cu-Mo complex was biologically unavailable and indicated that such a complex might exist in vivo.

Copper-deficient swine failed to absorb dietary iron at the normal rate (Lee et al., 1968). Increased amounts of stainable iron were observed in fixed sections of duodenum from such animals. When iron-59 was given orally, the mucosa of copper-deficient pigs extracted iron from the duodenal lumen at the normal rate, but the subsequent transfer to plasma was impaired. Intramuscular iron supplements given to copper-deficient pigs, increased iron in the reticuloendothelial system, the hepatic parenchymal cells, and in normalblasts. When red cells that were damaged by prolonged storage were administered, the reticulaendothelial system failed to extract and transfer the erythrocyte iron to the plasma at the normal rate. Administration of copper to copper-deficient animals with normal iron stores increased the plasma iron immediately.

David Arthur (1965) observed the interrelationship of molybdenum and copper in the diet of the gunea pig. He found that molybdenum ingestion increased the molybdenum content of blood, liver, kidney and hair and decreased the copper content of hair. The addition of
copper to molybdenum supplemented diets increased the copper content of kidney and hair.

The utilization of one dietary nutrient might be profoundly influenced by the presence of another. In particular, dietary interrelationships existed between copper, molybdenum and zinc. As reported by Kinnamon (1963), dietary copper and zinc induced a mechanism of competition within the fetus and placental structures in rats resulting in a lower uptake of radiozinc in these tissues. A high level of dietary molybdenum appeared to counteract completely the influence of an increased level of dietary copper in the absence of increased dietary zinc, and partially overcame the influence of excess copper when it was fed in combination with a high level of zinc. Molybdenum did not elicit a similar action on dietary zinc. A highly significant ($P < 0.01$) increase in zinc-65 urine excretion was observed in rats fed any ration high in zinc and a significant ($P < 0.05$) increase was noted in animals fed an excess copper and molybdenum diet.

In vivo interactions of cadmium with copper, zinc and iron in chicks were studied by Hill et al. (1963). Their results showed that cadmium was toxic to chicks at dietary levels of 25 to 400 ppm in a copper and iron deficient diet. The toxicity resulted in a reduced growth rate, mortality, microcytic hypochromic anemia, atony and elongation of gizzard. The growth depression and gizzard abnormality were corrected by increased dietary zinc. The mortality was reversed by added copper indicating that the speculation which prompted the study was valid. Increased dietary iron partially corrected both the mortality and the growth depression, indicating a previously unsuspected iron component of cadmium toxicity.
The uptake of oxygen was observed to decrease with the increase of chromium dosage, but had an opposite effect on ascorbic acid concentration (Verzhikovskaya, 1966). He also added to a diet amounts of chromium that were above or below the physiological level. Results showed an increased serum level of sodium and potassium, with no effect on the serum protein level. The amount of albumin decreased. The content of alpha-, beta and gama-globulins were enhanced, whether the diet was above or below the physiological level.

The results from a Latin square designed metabolism experiment with added Fe, Cu, Mn, and Co showed a marked interaction in digestibility between manganese and cobalt when manganese was added with cobalt (Pfander, Beck and Preston, 1966). In vitro studies with radioactive elements showed that the manganese interfered with cobalt uptake by rumen micro-organisms. Four factorially designed experiments have been conducted with lambs fed basal rations containing approximately 2 percent and 100 Kcal net energy per Kg ration. These trials indicated that manganese source, cobalt level and source, protein source and fat level all contribute to the observed changes in ration digestibility, liver weight gain, fat deposition, endocrine development and zinc availability. The large biological response obtained from manganese salts added at a level below that present in many natural feedstuffs appeared to be due to action on both the microorganisms and the host.

Several researchers had suggested the possibility of a dietary interrelationship between cobalt and selenium. Bunyan, Edwin and Green (1958) demonstrated that cobalt was partially protective against dietary liver necrosis produced by feeding rats a diet of 30 percent bakers yeast, sucrose, stripped lard, vitamins and minerals. However, Schwarz,
Roginski and Folz (1959) found no protective effect against dietary liver necrosis in rats fed a similar diet with torula yeast as the protein source.

Cobalt-deficient sheep were shown to be more susceptible to selenium toxicity than cobalt dosed sheep (Gardiner, 1966). In New Zealand, which has both cobalt and selenium deficient soils, Andrews, Grant and Stephenson (1964) found that sheep raised on cobalt-deficient diets averaged significantly more selenium in the kidney on a per gram or per organ basis.

The main functions of the trace elements have in many instances been determined, however the body can use more than one pathway or more than one trace mineral to get the same results. Also some of the trace elements act as antagonists against each other competing for use at the same site which may cause a deficiency in the biological active element and abnormalities to occur.

The biological activities of trace elements depend on the content and chemical compounds that exist in the foods consumed. Utilization of the elements also may influence their biological activity such as the pH of the stomach and the intestine, other physiological conditions and the presence of diseases.

Additional relationships will be identified as intensive research on these elements continues.
SUMMARY AND CONCLUSION

Anemia is a condition in which the number of red blood cells, the amount of hemoglobin, and the volume of packed red blood cells are less than normal. Iron is the most important element that is deficient in anemia. However, a deficiency, toxicity, or both of certain trace elements may cause anemia by the failure in absorption or utilization of the iron.

Arsenic, lead and selenium cause hemolytic anemia as excessive amounts of the metals attach to hemoglobins and destroy the red blood cells, thus decrease the number of red cells.

High levels of molybdenum produce an anemia of low hemoglobin concentration and reduced red blood cell counts. The physiologic action of molybdenum in causing anemia is unknown. However, excessive amounts of cadmium, manganese and zinc all cause an anemia similar to the anemia of iron-deficiency as they interfere directly with absorption or utilization of iron. Among the metals that have been discussed, the action of zinc was more indirect in the cause of anemia than that of the other metals. The mode of action of zinc is believed to be through a copper-deficient state due to zinc-copper antagonism which is followed by the iron-deficiency.

Insufficient copper has two pathways by which it causes an iron-deficient anemia: (1) by interfering with the absorption or mobilization of iron and (2) by decreasing the enzymes for the biosynthesis of hemoglobin.
Chromium activity was found to be associated with globin, thus increased plasma iron turnover occurred due to the fact that utilization of iron by red cells had been decreased.

Cobalt—vitamin B\(_{12}\), when deficient causes a different anemia called pernicious anemia. This anemia is due to a lack of intrinsic factor. The absorption of vitamin B\(_{12}\) requires the presence of the factor as a carrier.

It is apparent that anemia is caused either by an excessive amount of one or more of the trace elements mentioned above or by their deficiencies or both.

The clinical incidences of anemia have not yet been found that caused by a deficiency or excessive amounts of arsenic, manganese, molybdenum and selenium. The anemia caused either by deficiency or toxicity of cadmium, copper and zinc are rare. The anemia caused by chromium deficiency and lead toxicity is more prevalent in children, whereas that caused by cobalt—vitamin B\(_{12}\), is predominantly a disease of adult life though a few cases have been reported in children.

Iron deficient or nutritionally caused anemia is the most prevalent disease in the world with a relatively high incidence among children and women, especially during pregnancy.


Popov, V. V. 1966. Effect of varying amounts of manganese and chromium in a ration on the tissue respiration of parenchymatous organs and on their ascorbic acid level. Gig. Pitan 76-78. (Original not seen; abstracted in Chemical Abstracts, 1969, 70:17963j).


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