

CHROMIUM - METABOLISM AND BIOCHEMICAL
INTERACTIONS IN ANIMALS AND HUMANS

by

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B.S., Michigan State University, 1965

A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

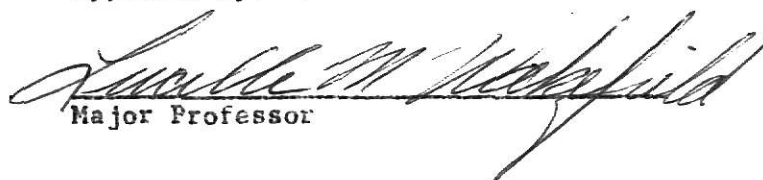
MASTER OF SCIENCE

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1975

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INTRODUCTION

Research during the past twenty years has demonstrated the essential role of chromium for animals and humans. Progress has been most rapid since chromium was identified as the active component in the "glucose tolerance factor" in 1959. Chromium in this biologically active form is necessary for normal glucose, protein and lipid metabolism through its relationship to insulin. Nanogram quantities of chromium are required for the optimal effect of insulin in every insulin-dependent system that has been investigated. It appears that chromium initiates the formation of disulfide linkages between the intra-chain disulfide of insulin and sulfhydryl groups of the cell or mitochondrial membrane by participating in a ternary complex.

Chromium deficiency in laboratory animals is characterized by impaired glucose tolerance. Chromium deficient rats incorporate less of certain amino acids into body tissue, have higher circulating cholesterol levels and an increased incidence of aortic plaques.

Tissue levels of chromium are lower in persons in the United States than in other areas of the world; levels also decline markedly with age. Chromium levels are lower in the food supply of the United States and are further lowered by the refining of sugar and milling of flour. A nutritional deficiency of chromium is therefore likely in the United States.

Therapeutic trials of chromium supplementation have indicated that a deficiency may exist in impaired glucose tolerance of the middle-aged and elderly, diabetes mellitus and protein-calorie malnutrition, all of which are characterized, in part, by impaired glucose tolerance. There is also indirect evidence of chromium malnutrition in gestational diabetes and atherosclerosis.

Chromium research is hampered by difficulties in analysis of the extremely low concentrations in which it occurs in biological materials. Various methods of analysis have been used with widely different results. Reports of chromium levels in foods or tissues can therefore be compared only within a study and cannot be considered as absolute.

REVIEW OF LITERATURE

Inorganic Chemistry

Chromium, with an atomic number of 24 and a mass of 52.0 daltons, belongs to the first series of transition elements. It is surrounded on the periodic table by three elements with known biological function: vanadium, manganese and molybdenum. Chromium can occur in every one of the oxidation states from -2 to +6, but only 0, +2, +3 and +6 are common. Cr^0 is inert and not thought to have a biological role. The divalent forms are unstable unless protected from oxidation and are unlikely to occur in biological systems.

The hexavalent form is almost always linked with oxygen and is a strong oxidizing agent. The important ions of Cr^{+6} are chromates, CrO_4^{-2} , and dichromates, $\text{Cr}_2\text{O}_7^{-2}$, both of which are easily reduced to Cr^{+3} in acidic solution according to the following formula:



The trivalent is the most stable oxidation state and has a strong tendency to form coordination compounds, complexes and chelates. The rate of ligand exchange is very slow. Trivalent chromium has a coordination number of six, with the direction of the ligands pointing to the corners of an octahedron. Some of the more common ligands are water, ammonia, urea, ethylenediamide, anions like halides, sulfate and anions of many organic acids. Free chromic ion does not exist in aqueous solution, it is always coordinated, either with water or other ligands in the solution (1).

At acidic pH ($\ll 4$) these compounds are quite stable. If the concentration of OH^- groups is increased (by raising the pH) hydrolysis of the coordinated water begins to occur, resulting in coordination of hydroxo groups in place of the water. This results in bridge formation through the hydroxo groups. This process, known as olation, leads to the formation of polynucleate complexes. Once a chromium aquo complex has begun olation, the process continues, forming macromolecular complexes that precipitate out of solution as biologically inert chromium hydroxides. Because of the strong tendency of Cr^{+3} for olation, simple trivalent chromium compounds are insoluble in the nearly neutral pH of the blood, and must be complexed or chelated to stay in solution (2).

Olation is believed to be the process by which chromium tanning of leather occurs, but tanning is a total saturation of protein and does not normally occur in the living organism. Olation may however play an important role in the biological action of chromium in the living organism (2).

There are several chemical properties of chromium which must be considered before discussing its possible biological functions:

a) The difference in oxidation potential between Cr^{+2} and Cr^{+3} or between Cr^{+3} and Cr^{+6} are so great that reversible transition between oxidation states is unlikely in biological systems. This rules out any role similar to that of iron in the cytochrome system which depends on the transition of iron from Fe^{+2} to Fe^{+3} and back. Chromium probably functions only as Cr^{+3} and therefore does not participate in oxidation-reduction reactions.

- b) Trivalent chromium occurs in only one configuration, the octahedral.
- c) Trivalent chromium is not subject to high-spin, low-spin transitions such as those of Fe^{+2} as the carrier of oxygen in hemoglobin.
- d) Trivalent chromium shows a very slow rate of ligand exchange which makes it unsuitable as an active site of enzymes. This property is more compatible with structural function such as binding of hormones to receptor sites or the stabilization of tertiary structure of proteins or nucleic acids.
- e) Trivalent chromium aquo complexes have such a great tendency to hydrolyze and olate that there can be no small, soluble aquo complexes at physiological pH (2).

Occurrence

Chromium is ubiquitous and is the fourth most abundant of the twenty-nine metals in the universe. It ranks sixth in abundance in the earth's crust, fifteenth in sea water and fifteenth in the human body.

Methods of Analysis

There are many methods of analysis that can be used for the determination of chromium in biological materials. These have been reviewed by Mertz (2) and the short-comings of each are stated. The two methods most commonly used at the present time are colormetric methods, which are very sensitive, but subject to interference from other ions, and atomic absorption spectrophotometry. Both of these methods require digestion or ashing, the disadvantages of which will be discussed in a later section.

Neutron activation and laser excitation spectrography do not require ashing, but still are not widely used.

Water and Air

Federal drinking water standards reject water with a concentration in excess of 0.05 ppm hexavalent chromium (3) although symptoms of excess dietary intake of chromium have not been demonstrated. Schroeder et al. (4) reported the consumption of water with a wide range of chromium levels over long periods with no toxic effects. Low chromium content of drinking water has been linked to deficiency symptoms in infants suffering from protein-calorie malnutrition (5) but, in general, chromium in drinking water does not contribute significantly to chromium intake.

Chromium concentration in the air varies considerably being much higher in cities with adjacent industries (2). The chromium in the air is quite insoluble and inhaled chromium remains in the lung, accounting for the observed increase in lung chromium level with age (4). Particles may, however, settle on food and be ingested. This probably accounts for very little chromium absorption, but more severe chromium deficiency can be observed in laboratory animals only if airborne contamination is excluded from the environment in which they are kept (6).

Soil and Plants

The concentration of chromium (as Cr_2O_3) in soil varies over a wide range, with no apparent geographical distribution pattern. Because chromium availability is complex and influenced by many factors such

as chemical binding, solubility, competition by other elements and the organic composition of the soil, chromium deficiency is defined in relative terms; i.e. chromium deficiency exists if the addition of chromium increases crop yield. Chromium deficiency has been demonstrated in the soil in Germany, France, Poland and Russia (2).

Schroeder et al. (4) analyzed samples of leaves and needles from a forest and grasses from a pasture both thought to be free from industrial pollution. They found chromium levels to be much higher than in vegetables purchased at local supermarkets, indicating that human food sources may be limited in chromium.

Animals and Humans

In the past, analytical methods were not always sensitive enough to detect chromium in animal tissue and when it was found, it was regarded as a contaminant (2). It has since been demonstrated in all animals and in all tissues in man, although the concentration varies markedly from species to species, from individual to individual and from tissue to tissue.

Schroeder et al. (4) found the concentration of chromium in kidney and liver of nearly all domestic and wild animals to be higher than that in man, often many times higher. They also reported a difference in chromium levels in man according to geographical location. Tissue samples from the United States were generally lower in chromium than those from the Far East, Near East and Africa. Tissue levels in Americans varied from city to city. Chromium levels were generally higher in lung, heart and aorta than in other tissues.

Chromium concentration in tissue samples examined after death indicate that chromium levels are exceptionally high at birth and decline with advancing age, unlike any other element tested (4,7). This is true of all organs except the lungs, where chromium accumulates as the result of the inhalation of environmental pollutants. This chromium is thought to be relatively insoluble and makes little or no contribution to the body pool of chromium (4).

Fasting serum levels of chromium are not a good indication of chromium nutrition, but only a measure of recent dietary intake (2,7). Ingestion of glucose is followed by a two- to five-fold increase in plasma chromium level in 30-90 min, followed by a subsequent decline to fasting levels; this rise is a much more sensitive indication of chromium nutritional state than the fasting level (8).

Hair is readily available and is relatively high in chromium (from 0.2 - 20 ppm) which makes it an ideal tissue for measurement of chromium nutrition (9,10). Recent studies employing emission spectrochemical techniques have shown chromium content in the same range as found in earlier studies using atomic absorption (7). Levels of chromium in hair are very high at birth and throughout the first year of life. There is a rapid decline during early childhood, then levels tend to remain constant from 3-40 years of age as shown in figure 1 (7,11).

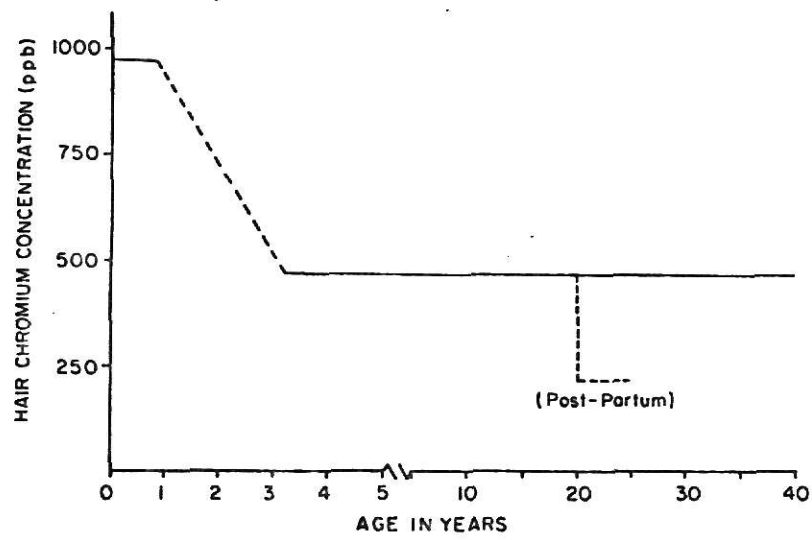


Fig. 1. Variation in hair chromium concentration with age (7).

Chromium Content of Food

Analysis of chromium in food presents many problems. First, chromium exists in different forms whose chemical and biological characteristics are not well defined. Second, chromium exists in such small quantities in food that analysis requires extremely sensitive instruments and techniques. At present, atomic absorption is the most widely used technique, but this requires ashing of the sample. Wolf¹ recently reported that chromium levels can vary by a factor of 15 in brewer's yeast and by a factor of 12 in bovine liver, depending on the type of destructive sample preparation used. This casts doubt on the validity of any values for chromium content reported in the literature, except for comparison of levels within any one study, if all samples were treated in the same way.

Toepfer et al. (12) attempted to determine the biologically available chromium in various foods by in vitro effect of food extracts on glucose oxidation activity of fat from chromium deficient rats. They were able to show that there is no significant relationship between total chromium determined colorimetrically and the biological activity. On the basis of the bioassay, they proposed a comparative ranking of foods (table 1) which is probably the most valuable indication of chromium now available to the nutritionist although it is far from complete. Hambidge (13) pointed out that modern methods of food processing such as the milling of wheat and the refining of sugar remove a high percentage of chromium.

¹Wolf, W.R. (1975) Uncertainties in the analysis of chromium in biological materials. Fed. Proc. 34, 927. (Abstr.)

TABLE 1

Calculated chromium biological values of selected foods,
edible portion as purchased (12)

Sample	Relative biological value	Sample	Relative biological value
Yeast, Brewer's	44.88	Peppers, chili (fresh)	2.27
Pepper, black	10.21	Wheat bran	2.21
Liver, calf's	4.52	Vegetarian chicken	2.16
Cheese, American	4.39	Cornmeal, white	2.09
Wheat germ	4.05	Shrimp	2.03
Bread, whole wheat	3.59	Grits	1.97
Cornflakes cereal	3.01	Lobster	1.95
Bread, white	2.99	Mushrooms	1.92
Spaghetti	2.89	Chicken leg	1.89
Beef round	2.89	Haddock	1.86
Wheat grain	2.86	Patent flour	1.86
Butter	2.81	Beer	1.77
Bread, rye	2.67	Egg white	1.77
Margarine	2.48	Chicken breast	1.75
Oysters	2.43	Vegetarian choplets	1.72
Cornmeal, yellow	2.35	Skimmed milk	1.59

Due to difficulties in assessment of chromium in foods, biological tissue, urine and feces it is not possible to perform accurate balance studies (14). Thus, no dietary recommendations have been made by the National Research Council (15) or the World Health Organization (16). Both agencies, however, recognize chromium as an essential nutrient and are supporting further research to determine the adequacy of this mineral in the diet.

Biochemical Interactions

Low Molecular Weight Substances vs. the Glucose Tolerance Factor (GTF):

As stated earlier, free Cr^{+3} hexaquo complex does not exist at physiological pH. Chromium must be protected from elation by ligands that successfully compete with OH^- at neutral pH. The following ligands have been shown to maintain chromium in diffusable form and in the following order: pyrophosphate methionine serine glycine leucine lysine proline. Glucose had no effect (2).

Mertz et al. (17) were the first to recognize the importance of chromium as a cofactor for the blood-glucose lowering action of insulin. The glucose tolerance factor was discovered in 1955 as a result of feeding a *Torula* yeast based diet to rats. There was a significant impairment of glucose tolerance within a few weeks (17). This diet also produced necrotic liver degeneration which could be prevented by the addition of vitamin E, factor 3, sulfur-containing amino acids or selenium to the diet, but none of these prevented the decline of glucose tolerance. However, glucose tolerance remained normal if a small percent

of brewer's yeast was added to the diet (18).

Purified fractions were obtained from brewer's yeast which not only prevented the development of impairment of glucose tolerance, but cured it overnight in very small oral doses (19). Trivalent chromium was identified as the active component in the glucose tolerance factor (20) and it was proposed that the chromic ion did not act as a simple complex, but was incorporated into an organic complex before becoming biologically active. This was demonstrated by an immediate response to brewer's yeast extract to a low chromium in vitro system. There was little or no response to the addition of chromium salts in such small concentrations².

These and other observations led Mertz to the conclusion that there are two categories of chromium compounds. The first are quite simple such as chloro, aquo or acetato coordinate compounds and a large number of complexes having organic acids, sugars and certain vitamins as ligands. These have limited insulin potentiating ability. The second group, which is still not completely characterized, appears to be one or more closely related compounds with the following characteristics: low molecular weight, water soluble, heat stable in solution and an absorption maximum at 262 nm. GTF chromium differs from simple chromium complexes by its much greater stimulation of the effect of insulin on glucose metabolism on isolated epididymal fat tissue. Much smaller amounts of chromium in the form of GTF are needed than if it is administered as a simple salt. Chromium GTF

²Burkeholder, J.N. & Mertz, W. (1966) Incorporation and effect of chromium (III) in brewer's yeast. Fed. Proc. 25, 759. (Abstr.)

crosses the placenta of rats easily (14), while chromium in the form of simple salts has not been demonstrated to do so. Purification of the glucose tolerance factor from brewer's yeast has revealed the presence in addition to chromium, of nicotinic acid, glutamic acid, glycine and a sulfur-containing amino acid (21).

Mertz and coworkers have synthesized a tetra-aquo-dinicotinato chromium complex and proposed the structure shown in figure 2. This complex improved defective glucose tolerance in chromium deficient rats, potentiated the effect of insulin on isolated epididymal fat tissue of chromium deficient rats and was better absorbed than chromium chloride when administered by stomach tube³. This compound was not stable over time, suggesting weak coordination and a tendency toward olation. The naturally occurring compound is probably protected against olation by coordination with amino acids. Another compound was synthesized with the addition of glutamic acid, glycine and cysteine. It had properties very similar to naturally occurring GTF, although glutamic acid was not present and placental transport has not been demonstrated (21). The proposed structure for this compound is shown in figure 3.

Chromium deficient animals exhibit an impaired response to exogenous insulin when compared with chromium supplemented controls when serum glucose response, incorporation of amino acids into protein or cell transport of an amino acid analog are measured (22). These and

³Mertz, W. (1974) Biological function of nicotinic acid-chromium complexes. Fed. Proc. 33, 659. (Abstr.)

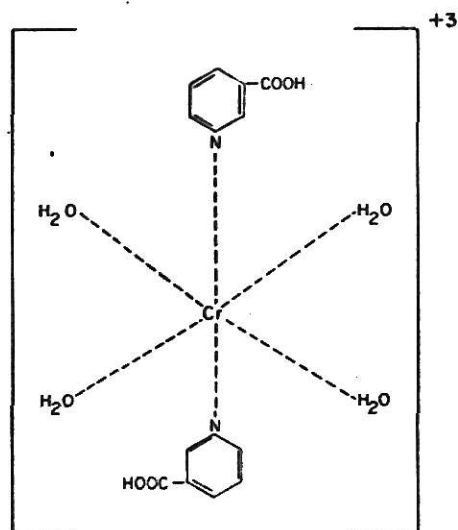
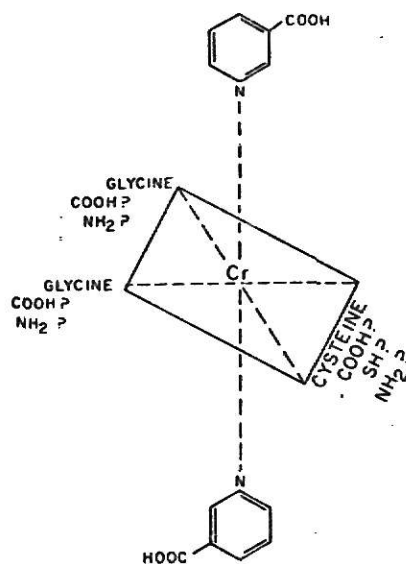


Fig. 2 Suggested structure for tetra-aquo-dinicotinato Cr-complex (21).



STEREO CHEMISTRY: CIS-TRANS ?
CO-ORDINATION TO FUNCTIONAL GROUPS OF AA ?
DISTRIBUTION OF CHARGES ?

Fig. 3 Possible structure of dinicotinato-amino acid Cr-complex (21).

the results of polarographic studies (23) led to the hypothesis that chromium can form a ternary complex between insulin and the receptor sites of the cell membrane which facilitates the initial insulin-tissue interaction (14). A model, proposed by Mertz⁴, suggests a coordination of chromium to two sulfhydryl groups of the cell membrane and the two sulfur atoms of the A chain disulfide bridge of insulin. This model (fig. 4) does not account for the two remaining coordination sites of chromium. Mertz suggested that ligands coordinated to the two remaining sites may account for the difference between the relatively small effect of many synthetic chromium compounds and the much stronger glucose tolerance factor from natural sources (21). These ligands might also account for the ability of natural GTF to cross the placenta while synthetic GTF apparently does not (21).

Animals, and probably man, have a limited ability to incorporate chromium into GTF, and appear to be dependent on an exogenous supply; in this respect the glucose tolerance factor could be regarded as a vitamin (13).

Protein Metabolism

Chromium was observed experimentally to form stable cross-linkages between protein strands. It is not known whether chromium performs a similar function in vivo (24). Chromium is bound to serum proteins for transport. The binding is firm enough to prevent non-specific tanning

⁴Mertz, W. (1967) Biological role of chromium. Fed. Proc. 20, 186. (Abstr.)

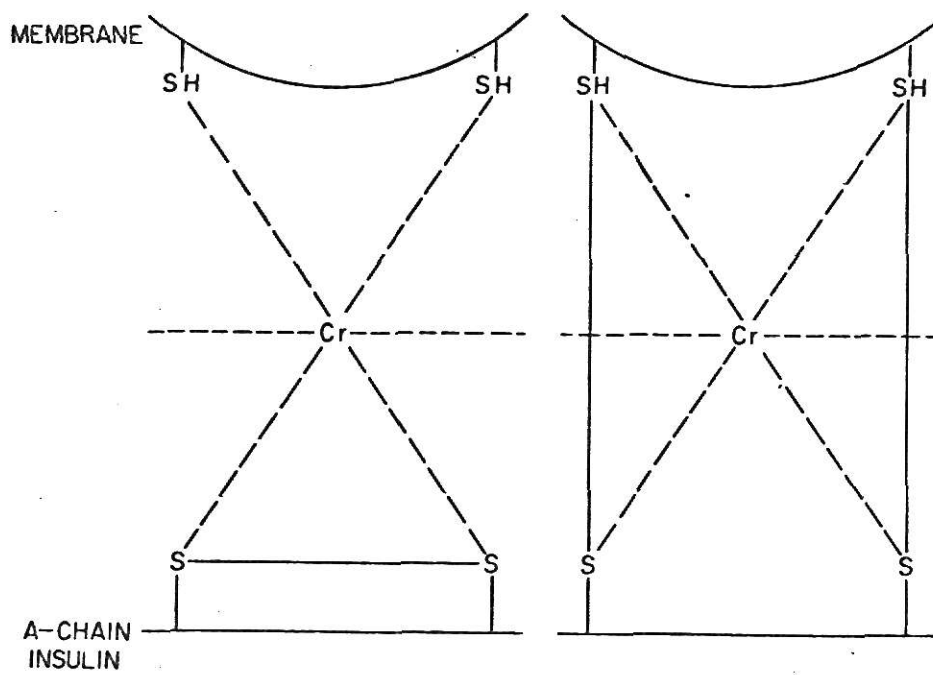


Fig. 4 Hypothetical ternary complex of chromium at site of action (21).

reactions with other proteins, but labile enough to allow chromium to be taken up by the tissues (2). Siderophilin bound almost all of the chromium administered to rats in physiological doses (25), and this was assumed to be the mechanism by which ingested chromium was transported in man. Doisy et al. (26) have since reported that in humans only 30-40% of ^{51}Cr was found in the globulin fraction, which includes siderophilin.

Insulin is known to enhance the uptake of amino acids by body tissue, particularly muscle (27). Roginski and Mertz (22) demonstrated increased incorporation of three amino acids into heart protein in chromium supplemented rats in comparison to non-supplemented rats fed diets low in protein and chromium. Gürson and Saner (28) found that chromium supplementation increased growth in some infants suffering from protein-calorie malnutrition, and suggested that chromium be considered a growth factor.

Enzymes

Chromium has been reported to enhance the action of succinic-cytochrome c dehydrogenase (29) and phosphoglucomutase (30) but follow-up research has apparently not been done and nothing is known about possible mechanisms (2). Chromium also stimulates the conversion of acetate to CO_2 , cholesterol and fatty acids by rat liver in vitro. These reactions require very high doses of chromium and may be a pharmacological rather than naturally occurring effect (2).

The digestive enzyme trypsin appears to contain one atom of chromium

per molecule in a dializable form; the residual activity of trypsin after dialysis is only 5% of the original which can be restored to normal by the addition of chromium (2).

Various enzyme systems are inhibited by excessive amounts of chromium. These include bacterial urease (31), thromboplastic activity (32) and beta glucuronidase (33). These reactions are not specific for chromium, however, and probably do not occur in animals under normal conditions since there is precise control of absorption, binding and excretion of trace metals in the intact organism (4).

Ribonucleic Acid (RNA)

Chromium levels have been found to be very high in RNA varying from 400 ppm in horse kidney RNA to 18 ppm in calf pancreas s-RNA. There was no apparent stoichiometric ratio between chromium and RNA, but the ratio of total metals to phosphorus was nearly constant, suggesting that many of the transition metals may stabilize the tertiary structure of RNA (34).

Red Blood Cells (RBCs)

The great affinity of RBCs for hexavalent chromium makes possible their permanent tagging and is a valuable diagnostic and research tool. Hexavalent chromium penetrates the erythrocyte readily without reacting with any component of the plasma; the trivalent form, on the other hand, attaches to plasma proteins and does not penetrate the erythrocyte membrane. Once inside the erythrocyte, Cr^{+6} is converted to Cr^{+3} in which form it cannot pass through the cell membrane. It is bound to

hemoglobin, resulting in the stable tagging of the erythrocyte.

Chromium concentration in mature RBCs in normal individuals is about the same as in plasma. This may be due to incorporation during maturation, since Cr^{+3} cannot penetrate the membrane of the mature cell. The function of chromium in the erythrocyte is not known (2).

Adipose Tissue

Mertz (35) reported that glucose uptake of isolated epididymal fat from chromium deficient rats was enhanced by the addition of physiological concentrations of chromium. This will raise the impaired activity to normal, but not beyond. In other words, it acts only to correct a deficiency. Nanogram quantities of chromium are required for optimal effect of every insulin dependent system that has been investigated and an uncorrected chromium deficiency requires the addition of high, non-physiological amounts of insulin to give a normal response (2). In the presence of insulin, addition of chromium to epididymal fat tissue from deficient animals increases the rate of glucose uptake (35), the incorporation of glucose carbon into fat (35) and the oxidation of glucose to carbon dioxide (36). Chromium also facilitates the insulin-mediated entry of D-galactose into the cell without the lag phase that is observed when no chromium is present (37).

The technique for measuring in vitro production of CO_2 by epididymal fat has been adapted for the measurement of biologically active chromium in foods. Relative biological activity of sample extracts were determined by measuring CO_2 production from ^{14}C labeled glucose when the extract and insulin were added to the system (12).

Mitochondria

Neubert and Lehninger (38) observed that water intake of isolated liver mitochondria was enhanced by the addition of insulin. Using liver mitochondria from chromium deficient rats, Campbell and Mertz (39) were able to induce mitochondrial swelling with one-tenth as much insulin if chromium was added to the system than if it was not present. Polarographic studies by Christian et al. (23) led to the conclusion that chromium facilitates the formation of disulfide links between insulin and the mitochondrial membrane in the same way as it does between insulin and the cell membrane.

Metabolism

Absorption

Trivalent chromium is poorly absorbed from the gastrointestinal tract. Various studies have reported absorption of only about 0.5-2% of the orally administered dose of chromic chloride (2,40). This is probably due to its poor solubility, the tendency to oxidation at physiological pH and the presence of phytate in foods (41). It appears that chromic chloride is absorbed by simple diffusion and no active transport is involved. Poisoning of energy generating systems within the cell does not depress chromium absorption (42). The rate of transport diminished with increasing dose, which suggested a mechanism of transport similar to facilitated diffusion. This hypothesis is supported by the observation that other metals, in less than toxic amounts, but in ratios similar to those found in vivo, depress chromium transport. These

include iron, manganese, calcium and titanium; but not copper and zinc. The former are therefore thought to share the same transport system with chromium, while the last two do not (42).

Chen et al. (41) reported that the midsection of the rat intestine is the most permeable to chelated chromium, followed by the ileum and the duodenum. They also found that fasted rats absorbed chromium more rapidly than non-fasted controls.

Chromium excretion in human urine is in the range of 4-15 ug/day (42). Estimates of dietary chromium intake average 50 ug/day (4,42); thus total chromium absorption of only 0.5-2% reported in previous studies (2,40) is impossible. Some dietary chromium must be more readily available than simple chromium salts. Administration of ⁵¹chromic chloride to rats resulted in a retention of only 0.5%, whereas administration of extract from brewer's yeast grown in ⁵¹chromic chloride, resulted in retention of up to 25% of administered chromium (42). Since extracts from brewer's yeast are known to contain large amounts of glucose tolerance factor (6), chromium in the form of GTF must be better absorbed and retained than chromium in the form of simple salts.

Synthesis of chromium-nicotinic acid complexes which possess glucose tolerance factor activity in vitro, have made it possible to study long-range effects of dietary supplementation in animals (21). Administration of these compounds improved both growth and glucose tolerance in deficient animals. These chromium complexes were better absorbed than chromium chloride and were found to label the pool from which the acute plasma chromium increment is derived on challenge

with glucose. Tissue distribution of chromium was different when the labeled Cr-nicotinic acid complexes were administered than if chromium was given as ⁵¹chromium chloride. No toxic effects were noted after 21 weeks of feeding very high levels of the chromium-nicotinic acid compounds⁵.

Transport

Hopkins and Swartz (25) reported that nearly all of the chromium absorbed by rats was transported via siderophilin. Doisy et al. (26) found that only 30-40% of chromium absorbed in humans is found in the globulin fraction, of which siderophilin is a component. The rest is bound to proteins in the albumin fraction.

Injected chromium chloride disappears rapidly from the blood. Mertz (2) and Hambidge (7) agree that blood or plasma chromium is not a meaningful indicator of nutritional state of the individual, but only a measure of recent intake. Fasting levels in normal individuals are extremely low (7).

Tissue Distribution

Tissue levels of chromium are very high at birth and rapidly decline to adult levels by age three (7). Schroeder, Balassa and Tipton (4) analyzed human tissues from the United States and other parts of the world, as well as tissues from wild and domestic animals. Chromium is

⁵Mertz, W. & Roginski, E.E. (1975) Some biological properties of chromium-nicotinic acid complexes. Fed. Proc. 34, 922. (Abstr.)

particularly low in some elderly persons in the United States, but low levels are not confined to the elderly. Kidney and liver appear to maintain neonatal concentrations until the second decade of life; lung, aorta, heart and spleen, in contrast, lose chromium early in life. Chromium levels were considerably lower in kidney and liver of adults in the United States than in adults from the Far East, Near East and Africa. Tissue levels were also much lower in American adults than in the same tissues of wild and domestic animals. Chromium levels are generally lower in the food supply in the United States than in pasture or forest plants or the food supply of other countries (4). These results suggest that lower tissue chromium levels are related to intake (4,7).

The injection of physiological doses of ^{51}Cr chromium chloride into rats was followed by a considerable accumulation of chromium in the spleen, probably indicative of the removal of colloidal (precipitated) chromium from the blood (43). Chromium administered in the form of glucose tolerance factor from yeast extract did not accumulate in the spleen (42). The greatest amount of GTF chromium accumulated in the liver and uterus, followed by kidney and bone. Lung, heart, intestines, spleen, pancreas and brain accumulated about half as much as liver. Muscle, ovaries and aorta accumulated about one quarter as much as liver (42).

The values above contrast with chromium levels reported by other authors from human autopsies (15). There is general agreement that the highest tissue concentration is found in the lung and is increased with age, suggesting that the lung accumulates chromium from exposure to

airborne contamination and that this chromium does not readily exchange with body pools (2,3,15). Values for other tissues vary so much from one study to the next that no generalizations can be drawn.

Excretion

The major excretory route for chromium is via the kidney and most urinary chromium, in contrast to plasma chromium, is in a dialysable form of low molecular weight (44). Characteristics of this chromium compound are similar to those of GTF chromium and is believed to reflect the chromium status of the individual (45). Chromium that is present in the blood, coordinated to small molecular ligands, is filtered at the glomerulus and up to 63% is reabsorbed in the tubules; protein-bound chromium is excreted only to a small degree (44). A small amount of injected chromium is excreted via the feces (26).

Due to difficulties of analysis at the low concentrations at which chromium is present in the urine, a wide range of values have been reported. More recent studies have reported mean 24 hr urinary chromium excretion at 8.4 μg (7) and 7.2 μg (45). Very high levels of chromium excretion have been detected in some insulin-dependent diabetics (7).

Response to Glucose

Glinsman et al. (46) reported that an oral dose of glucose administered to young healthy individuals resulted in an acute increase in plasma chromium concentration. This increase was not observed in elderly persons with impaired glucose tolerance except after they were

given daily chromium supplements for 7-13 weeks. Chromium response was also improved when chromium supplements were given to diabetics, but not all individuals responded with improved glucose tolerance, and of those who did, none returned to normal. The lack of a plasma chromium response and its reappearance after chromium supplementation suggests that this plasma chromium increment is derived from a specific body pool that may be deficient in some persons and may be replenished by supplementation with chromium salts (2,21).

The plasma chromium increment is thought to be GTF chromium which is released from a physiologically important body pool probably in the liver. The release of GTF appears to be mediated through the release of insulin in response to a rise in blood glucose. It is, in turn, utilized to facilitate the peripheral action of insulin (13). When $^{51}\text{chromium}$ GTF extracted from brewer's yeast was administered intravenously to rats, there was a substantial increase in plasma radioactivity following subsequent administration of insulin. When inorganic $^{51}\text{chromium}$ was substituted for the labeled GTF, the plasma response was smaller and occurred only if insulin was administered at least 3 days after the $^{51}\text{chromium}$. This time lag is thought to be required for the incorporation of inorganic chromium into the glucose tolerance factor (42).

An increased excretion rate of chromium has been reported to follow an oral glucose load (6). This was thought to be part of the GTF chromium released into the blood in response to the glucose. From this assumption it was reasoned that the consumption of a high carbohydrate diet could lead to a depletion of body stores of chromium. However, results of a

study measuring chromium excretion of individuals consuming diets of different sucrose contents do not suggest that increased carbohydrate consumption increases chromium requirements (21).

Placental Transport

Chromium accumulates in the fetus during gestation and is present in the newborn and very young child at higher levels than at any other time in life (4). The fetal chromium pool cannot be labeled by the administration of ^{51}Cr chromium chloride in drinking water, by stomach tube, or intravenous injection of the mother rat (47). The chromium concentration in the newborn is influenced by the naturally occurring chromium in food, and the young of rats fed a Torula yeast diet accumulated only half as much chromium as the young of mothers fed a natural diet high in chromium. This indicates that placental transport requires chromium in natural form, probably in the form of glucose tolerance factor (7,42,47). In this respect, if it is shown that synthesis of GTF from chromium salts does not occur rapidly enough to fill the demands of the fetus, it may be elevated to the role of a vitamin (13).

The premature infant has low tissue stores of chromium, while infants at term have very high tissue levels (7,11). Low hair chromium levels in post-partum and multiparous women indicate that pregnancy depletes maternal stores of chromium in humans as well as laboratory animals (7,10). It is not clear that this represents a health hazard to the mother, but it may be related in some way to gestational diabetes (7).

Toxicity

Each of the essential metals has a low toxicity and even if the total estimated quantity found in the body of man is ingested, only a local irritation of the gastrointestinal mucosa results. Even with large daily doses, there is little tendency for excess accumulation in body tissues. Chromium is one of the least toxic of the trace elements. One or two hundred times the total body content has been tolerated by animals in oral doses (4). Symptoms of excess dietary chromium in man are unknown (2,4,15).

Hexavalent chromium is thought to be in the range of 100 times as toxic as trivalent chromium, but large doses were administered to laboratory animals without ill effect, although chronic toxicity can be observed in some species at concentrations over 5 mg/liter in drinking water. Symptoms of chromium toxicity include acute hypertension, hypocholesterolemia and hypoglycemia (48). The chromate ion penetrates cell membranes quite readily and is a strong oxidizing agent. These properties account for its irritating effect and higher toxicity than that of the trivalent form. Increased incidence of respiratory and gastrointestinal diseases have been reported among chromate workers and is probably due to inhaled and ingested chromate particles. Cancers have been induced by the implantation of poorly soluble chromates, but only at the site of the implant (2).

Toxicity of trivalent chromium appears to be restricted to parenteral administration and the therapeutic : toxic ratio for intravenously injected trivalent chromium is estimated to be approximately 1 : 10,000 (2).

Chromium Nutrition in Humans

Methods of Assessment

The determination of chromium levels in various tissues obtained at autopsy has provided valuable data for comparing differences in tissue levels in various geographical and age groups. Chromium levels decline in most tissues with age in the United States population, with the exception of the lungs, and are generally low when compared to other parts of the world (4). These findings indicate that there may be marginal chromium deficiency within the United States (15).

Measurement of plasma chromium levels are not very meaningful, as ingested or injected chromium is rapidly cleared from the blood and fasting levels of plasma chromium are very low (2). However, after an oral glucose challenge there is a temporary increase in the plasma chromium level and this increment is apparently dependent on adequate body stores of metabolically active chromium. This pool can be replenished from dietary sources of chromium (8). Glinsman et al. (8) noted that maturity-onset diabetics who did not show this chromium response began to respond after a period of chromium supplementation. In a study involving pregnant women, the percentage of "responders" during the ninth month of gestation was significantly less than in non-pregnant women and no response was detected in 58% of the women who had evidence of impaired glucose tolerance (7).

This test requires the performance of a glucose tolerance test and at least five blood samples (2). The increment of plasma chromium is

extremely small; Hambidge (13) estimated that an increment of less than 1 ppb could be more than adequate to combine with plasma insulin in the circulation or at the site of peripheral action. Analysis of such small quantities is difficult and elimination of any contamination is extremely important. This test is therefore of little clinical value at this time.

The chromium content of hair seems to provide a useful index of chromium nutritional status (9-11,49). There is a relatively high concentration of chromium in hair and there is no turnover once chromium has been deposited in the shaft (7). Investigation by this technique has demonstrated that parous women had much lower hair chromium levels than women of the same age who had never borne children (10). This low level was probably due to depletion of maternal stores of chromium by the fetus and agrees with the findings of depletion in maternal liver chromium in laboratory animals (4).

Hair chromium levels are higher in newborns than in any other age group (7) in agreement with the findings of Schroeder et al. (4) of very high chromium tissue levels in infants. The decline in hair chromium levels during infancy has been studied with serial sample collection from the same subjects, analyzing the portion of hair nearest the scalp (13). Changes with age have also been studied by analyzing portions of the hair shaft at increasing distances from the scalp. Mean hair chromium content varied directly with distance from the scalp, demonstrating that most recently grown hair was lower in chromium than that formed at a younger age (11). Hair chromium concentrations of premature infants

delivered before 36 weeks of gestation are generally lower than those of full-term infants and may indicate an increased risk of chromium deficiency in later infancy or childhood (7).

Comparison of hair chromium levels of normal children and children with juvenile diabetes showed a much lower concentration in those with diabetes (9). Hambidge advanced two possible explanations: a) a reduction of total body chromium, possibly from nutritional deficit, could have preceded the onset of clinical diabetes in these genetically predetermined individuals, or b) depleted levels of chromium in the tissues may be iatrogenic in the insulin-treated diabetic. There has apparently been no follow-up research to resolve these possibilities (7).

Hambidge et al.⁶ found that the decreased chemotaxic index of polymorphonuclear leukocytes in some insulin-dependent diabetics and gestational diabetics could be increased to normal by incubating the leukocytes in a solution containing chromium chloride. Further research is necessary to determine if this finding will provide a reliable assay of chromium deficiency.

Although urinary excretion of chromium has received little study, it may prove a valuable tool in the investigation of chromium nutrition (6). Chromium in the urine is derived from the dialyzable fraction of serum chromium (44) which includes GTF chromium (7). Thus, measurement of variations in urine chromium should provide an indirect measurement of

⁶Hambidge, K.M., Martinez, B., Jones, J.A., Boyle, C.E., Hathaway, W.E. & O'Brien, D. (1972) Correction of impaired chemotaxis of polymorphonuclear leukocytes from patients with diabetes mellitus by incubation with trivalent chromium in vitro. *Pediatric Res.* 6,393. (Abstr.)

serum chromium. Abnormally high levels of chromium excretion were found in two of seven insulin-treated juvenile diabetics (7). This loss of chromium may be due to the large amount of insulin which enters the circulation in an unphysiological manner, i.e., upon injection. Urine chromium excretion was normal in two children at the clinical onset of diabetes before initiation of insulin treatment.(7).

It has been demonstrated that a challenge with glucose or insulin results in an acute rise in urinary chromium (6). It would seem that any condition which increased serum insulin levels would increase the dietary chromium requirements, although increased carbohydrate intake did not appear to increase chromium excretion in a recent study (21).

Final proof of chromium deficiency can only come from therapeutic trial with chromium supplements. Because of poor absorption, tests with chromium salts are of limited use, but will be discussed in a later section.

Decline of Tissue Levels of Chromium with Age

The decline of tissue chromium with age has been recognized since the early 1960's. Although a decrease of several elements have been observed in infants, chromium is unique in that it continues to decline throughout life (2). The decline in infancy may be a normal physiological process, since the need for chromium is greatest in a rapidly growing organism. But tissue chromium levels are high in mature animals and in mature humans from other countries, so that continued decline may not be normal.

The other possible explanation is nutritional. This assumes a suboptimal intake of chromium as the cause of declining tissue levels in the population of the United States (7).

Dietary Intake

Although there are chromium values for food in the literature (4,50), Toepfer et al. (12) have demonstrated that there is no significant relationship between total chromium in foods and the biological activity of extracts from these foods. For this reason, research aimed at determining total chromium intake from various diets is for the most part meaningless. Several factors may contribute to suboptimal intake of chromium in the United States. The initial decline in infancy may be physiological, but the low chromium content of cow's milk and some infant formulas could contribute to this decline and could be particularly serious for infants born prematurely with low chromium stores. By comparison, human milk is somewhat higher in chromium, but there is no information on the availability of chromium from these sources (table 2) (7). Not only is chromium low in the United States food supply (4), but modern methods of food processing remove a large percentage of chromium from such foods as flour (51) and refined sugar (52).

Effective prevention of chromium deficiency cannot be achieved by supplementing food with inorganic chromium which does not substitute for the biologically active GTF chromium found in some foods (13). Ten to 25% of GTF chromium is absorbed compared to about 1% of inorganic chromium (42). Since GTF is not available at the present time for

TABLE 2
Chromium Content of Milk (7)

	ng Cr/mg Ash	ng Cr/ml Milk
Human		
Subjects: a	2.1	18.5
b (mean of 4)	1.6	6.4
c (mean of 8) 2.3	2.3	7.9
d	2.0	11.2
e (mean of 2)	<u>1.8</u>	<u>14.1</u>
Mean =	2.0	11.6
Cow		
Undiluted	0.5	8.0
Infant Formula (20 cal/oz)		
Similac	0.8	5.6
Enfamil	2.2	10.5
Modilac	0.5	5.0

supplementation, foods high in GTF activity (12) are the only sources of chromium that could be recommended.

Low Chromium States in Animals

Mertz has used the term "low-chromium state" or "chromium deficiency" to describe the results of suboptimal intake of biologically available chromium. A low-chromium state is characterized as an impairment of some function that can be prevented or cured by physiological amounts of the element (2).

Anatomical Lesion

When weanling rats are maintained on a 10% soy protein diet containing less than 100 ppb chromium a grossly visible eye lesion develops in 10-15% of the animals (53). The first symptom, a dull appearance of one eye, appears in 2-3 weeks. This may disappear spontaneously or worsen to complete opacity of the cornea, dilation of blood vessels in the iris, and neovascularization of the cornea. The lens and conjunctivas are not affected. This eye lesion is completely prevented by chromium supplementation, but once developed, the defect cannot be corrected by feeding chromium, high protein, or both. Low protein may be a conditioning factor, since the eye lesion is rarely observed in rats fed a protein-sufficient *Torula* yeast diet as low in chromium as the soy protein diet. Researchers have been unable to explain the eye lesion in terms of a biochemical defect in the cornea. Glucose metabolism in this tissue in vitro did not appear to respond to insulin, regardless of the chromium state of the animal (2).

Glucose Tolerance

Impaired glucose tolerance in rats fed a 30% Torula yeast diet was the first laboratory observation of chromium deficiency, although it was not immediately recognized as such (2). Necrotic liver degeneration and death occurred in these animals in a few weeks (54). This diet was known to be deficient in vitamin E and factor 3 and low in sulfur-containing amino acids. The addition of vitamin E (17), factor 3 (19) or cystine (18) prevented the liver necrosis, but did not improve glucose tolerance. Glucose tolerance remained normal if brewer's yeast or table scraps were added to the Torula yeast diet (18). Purified glucose tolerance factor obtained from brewer's yeast or pork kidney powder not only prevented the development of impaired glucose tolerance, but cured the impairment overnight with a single dose of 100 μ g/100 g of body weight (19).

Forty-seven elements were administered to GTF-deficient rats and only trivalent chromium compounds improved the impaired glucose tolerance (20). The difference in dose and absorption of chromium salts and naturally occurring GTF chromium has been discussed.

Insulin-induced Hypoglycemia and Glycogen Formation

To further demonstrate the interaction of chromium and insulin, rats raised on a 10% soy protein diet also very low in chromium were injected with insulin after an 18 hr fast (22). The hypoglycemic response was significantly smaller (about half) in the deficient animals when compared to chromium supplemented controls. The incorporation of glucose 1- 14 C into tissue glycogen by the deficient animals was only half that in

supplemented animals for the organs studied (heart, liver and diaphragm). Thus impaired glucose tolerance in chromium deficiency may be due to a decreased response of the deficient animal to its endogenous insulin (22).

Syndrome Simulating Diabetes Mellitus

A more severe impairment of glucose tolerance develops when rats are kept in a laboratory from which all traces of chromium are eliminated (55) or in isolators (56). Fasting hyperglycemia and glucosuria were found in chromium deficient rats and was particularly marked in their offspring (57). The air in industrial areas is known to contain appreciable amounts of chromium (2). Even though there is evidence that inhaled chromium is not absorbed (4) some particles may be deposited on cage surfaces or food and be ingested, so air entering the laboratory must be filtered. Metal cages also seem to contribute to chromium intake, and plastic cages with plastic and glass equipment must be used to prevent contamination.

Circulating Vascular Lipids

Curran (58) demonstrated that chromium stimulates hepatic synthesis of cholesterol in vivo and vitro. A very high dose of chromium was necessary, suggesting that the mechanism by which this is accomplished is different than that involved in glucose metabolism, i.e., it is not dependent on insulin (2). The large dose necessary to increase cholesterol synthesis is nearly toxic (36) suggesting that this might be a pharmacological rather than physiological effect.

Using more physiological levels of chromium, Schroeder (59) demonstrated a decrease in serum cholesterol with a supplement of 5 ppm in the drinking water of rats. This treatment also reduced the incidence of aortic plaques (60). Preston⁷, however, reported that F-1 offspring of Guinea pigs fed a low chromium diet had lower serum cholesterol levels than those of control animals, exactly opposite the findings with rats. The possible relationship between human atherosclerosis and chromium nutrition will be discussed later.

Amino Acid Metabolism

When rats are fed a 10% soy protein ration low in chromium, chromium supplementation of 2 ppm in drinking water results in stimulation of growth (22). This has been shown to be due to actual increase in tissue protein. When a radioactively labeled non-utilizable amino acid analogue was injected into chromium deficient and chromium supplemented rats, there was increased uptake in the tissues of the chromium supplemented rats. Cell transport of amino acids is mediated by insulin and is independent of the presence of glucose (2).

A second site of action of insulin in amino acid metabolism is the ribosome where it acts to increase protein synthesis (22). Rats treated as described above were injected with insulin and labeled amino acids. The supplemented rats incorporated more glycine, methionine and serine

⁷Preston, A.M. & Dowdy, R.P. (1975) Effect of dietary chromium on glucose tolerance and serum cholesterol in Guinea pigs. Fed. Proc. 34,927. (Abstr.)

into heart tissue and more methionine into the liver. However, these results could not be demonstrated in the absence of exogenous insulin (22).

Life Span, Growth and Survival Under Stress

The first indications of the benefits of dietary chromium were observed in the early 1960's under conditions designed to detect toxicity symptoms of chromium. Rats were given 2.2 ppm chromate in drinking water. The chromium content of the diet was not determined, but was probably not chromium deficient (2). In later experiments performed by Schroeder in a controlled environment similar results were observed in mice (61) and rats (62). Mortality was decreased in the supplemented mice. Survival (in % of total) was 68.8 vs. 92.6 at 12 months and 47.3 vs. 61.1 at 18 months for deficient and supplemented mice respectively. Rats were not affected in terms of early mortality, but the life span of the last surviving 10% of the male rats was considerably prolonged.

The growth stimulating effects of chromium are not observed if animals are kept under normal laboratory conditions on a low chromium Torula yeast diet. However, when the rats were maintained in plastic cages on a 10% soy protein ration also low in chromium (>100 ppb), those receiving chromium supplementation consistently grew at a faster rate than the deficient animals. The average increase in body weight was 8% (63).

Because it is difficult to lower the chromium content of diets below 100 ppb, Mertz and Roginski (63) attempted to increase the severity of the symptoms of deficiency by applying stress. Rats were fed a diet

containing only 10% protein, thereby imposing nutritional stress. Subjecting the animals to controlled exercise or blood loss further aggravated the low chromium state. Rats in both groups ran an average of 11.5 km/week. The chromium supplemented rats gained weight from the beginning of the experiment; non-supplemented rats lost weight for the first twelve days. After 140 days, mean weight gain of non-supplemented rats was less than 40 g and that of the supplemented rats was nearly 80 g. Acute stress was applied by the withdrawal of blood equal to 3% of the body weight from the jugular vein of anesthetized rats. Mortality was higher in the non-supplemented group. Initial weight loss of the survivors was the same, but recovery weight gain in the next five days was considerably greater in the chromium supplemented group (63).

Low Chromium States in Humans

In the past 10 years, evidence of a nutritional requirement for chromium and of human chromium deficiency has been accumulated. Therapeutic trials of chromium supplementation have been limited in number, but there is good evidence that chromium plays a role in at least three conditions: "maturity-onset" diabetes, impaired glucose tolerance in the middle-aged and elderly, and protein-calorie malnutrition in infants, each of which is characterized in part by impairment of glucose tolerance (2). Chromium undernutrition has also been implicated in atherosclerosis as a result of animal studies and the finding of low tissue levels of chromium in some atherosclerotic persons (50).

Diabetes

Results of research on chromium undernutrition as a causative factor in diabetes mellitus has been somewhat contradictory. As mentioned before, the most prominent and readily detectable feature of chromium deficiency in laboratory animals is impaired glucose tolerance (35,57). It was logical, therefore, to test the effect of chromium supplementation on human diabetics. Short term supplementation of 1-7 days had no effect (2). Four male diabetics were treated in a metabolic ward for an extended period of time. Three showed marked improvement of oral glucose tolerance. Schroeder reported improvement of glucose metabolism in only 4 of 12 diabetic patients given doses of up to 1 mg daily for six months (2). In none of these cases did supplementation reduce the need for hypoglycemic medication. Chromium is therefore not considered to be a clinically useful therapeutic agent in diabetes (46).

Acute plasma increment of chromium has been shown to follow a glucose or insulin challenge in young healthy persons (8). Part of this chromium is subsequently lost in the urine (6,7). This strongly suggests that insulin therapy can lead to considerable loss of chromium, particularly with the administration of fast-acting insulin (42). Morgan (64) reported significantly lower chromium levels in the livers of persons with diabetes. Hambidge et al. (9) reported much lower chromium levels in the hair of children with juvenile diabetes who had been receiving insulin therapy for several years. They advanced two possible explanations: a) a reduction of total body chromium, possibly due to nutritional deficiency, may precede clinical symptoms in the genetically predetermined juvenile

diabetic; or b) low tissue levels may be due to increased excretion in response to injection of unphysiological therapeutic doses of insulin. This later explanation was confirmed by the finding of abnormally high levels of chromium in the urine of two insulin-treated juvenile diabetics; urinary excretion of chromium was near normal in two others at the time of the clinical onset of diabetes, but before insulin treatment was begun (7).

Recent studies with labeled chromium confirm increased urinary excretion of orally administered chromium chloride by insulin-requiring diabetics (26). It could not be shown if this was due to increased absorption or under-utilization of chromium, although both are likely. It is also not known whether this is a cause or result of the disease. Diabetics are known to excrete 2-3 times as much zinc as normal individuals, and there may be a kidney defect associated with diabetes that is responsible for abnormal loss of trace metals (9).

Impaired Glucose Tolerance in the Middle-aged and Elderly

Within 7 years after chromium was shown to be an essential micro-nutrient for rats, it was implicated in impaired glucose tolerance in humans (46). Levine et al. (65) treated 10 elderly men with impaired glucose tolerance with daily chromium supplements for up to four months. No improvement was noted in six, but glucose tolerance improved to within normal ranges in the other four. Tests remained normal for about one month after supplementation stopped.

Mertz reported that Hopkins performed similar tests on middle-aged persons and found half of the subjects responded to supplementation with chromium chloride within six months. He noted that the persons who responded to chromium treatment were of normal weight while non-responders were overweight (2). As noted earlier, tissue chromium levels decline with increasing age in the United States (4) while the incidence of impaired glucose tolerance increases (2).

Pregnancy

It has been shown that the fetus accumulates high levels of chromium during the later part of pregnancy (4,7). This may cause a severe depletion of maternal stores of chromium if dietary intake is limited (7). Chromium levels have been shown to be much lower in the hair of multiparous women than in nuliparous women of the same age (10). No effort was made to correlate chromium levels with the number of pregnancies or the length of time since the last pregnancy.

Gestational diabetes is a temporary derangement of glucose tolerance which occurs during pregnancy and returns to normal after delivery (66). The gestational diabetic is characterized by increased peripheral resistance to the action of insulin (7). There was no serum chromium response in seven of twelve gestational diabetics when challenged with an oral glucose load (7). There have apparently been no attempts to alleviate gestational diabetes with chromium supplementation, presumably because with the limited absorption of chromium chloride and conversion to GTF, the pregnancy would in most cases be completed before the effect of supple-

mentation was shown.

Breast milk has been shown to contain an appreciable amount of chromium (7), so lactation may impose further demands on maternal chromium stores.

Protein-calorie Malnutrition

Chromium supplementation has been found effective in some but not all cases of children suffering from protein-calorie malnutrition, which is frequently accompanied by disorders of carbohydrate metabolism. Clinical symptoms may include fasting hypoglycemia or impairment of glucose tolerance (2).

The first trial of chromium supplementation was undertaken by Hopkins et al. (5) in Jordan with infants from the Jordanian Hills and the Jordan River Valley admitted to the hospital suffering from severe malnutrition. The children from the hill village showed severe impairment of glucose tolerance which responded overnight to a single dose of chromium chloride. The children from the valley had normal fasting blood glucose levels and glucose removal rate, although they showed the same degree of malnutrition. The main source of protein for both groups was milk powder provided by a United Nations relief agency. Analysis of 10 sources of drinking water detected three times as much chromium in the water from the valley as in the hills. Six malnourished infants from Nigeria responded favorably to the same oral dose of chromium chloride (5). Initial impairment of glucose tolerance was not as severe as in the Jordanian Hills infants. A common food staple for Nigerian children is

maize pap which contains only about 10 ppb chromium (13).

In contrast to these results, Carter et al. (67) found that the impaired glucose tolerance of malnourished infants in Cairo, Egypt, improved spontaneously with routine hospital care and did not occur more rapidly with chromium administration. They analyzed local food and drugs used in the hospital treatment of these infants and found some to be very high in chromium.

Gürson and Saner (68) administered a single dose of chromium chloride to 14 malnourished infants in Istanbul, Turkey. Nine of the children responded with an improvement in glucose tolerance, and this was maintained throughout the period of observation, which varied from 8 to 40 days, without further supplementation. In a later publication (28) they reported that the infants who responded to chromium with improved glucose tolerance also gained weight more rapidly. On this basis they proposed that chromium be considered a growth factor for human infants.

Regional differences in quantities of dietary chromium, including chromium in the water supply, probably account for the different responses to chromium supplementation by infants suffering from protein-calorie malnutrition. Thus chromium supplementation is effective in the treatment of this condition only if there is also a deficiency of dietary chromium or low body stores. The overnight response to chromium administered in the form of chromium chloride is in striking contrast to the very slow improvement of glucose tolerance in middle-aged and elderly persons, and indicates a more efficient absorption and conversion of inorganic chromium to GTF chromium in the very young (28).

Atherosclerosis

Severe atherosclerosis in man is usually associated with abnormalities of glucose metabolism, often with impaired glucose tolerance (50). The association of diabetes and atherosclerosis is well-known; atherosclerosis is a leading cause of death among diabetics (69). Insulin is vital to both lipid and glucose metabolism (27). A syndrome similar to human atherosclerosis has been produced in rats by chronic chromium deficiency (60).

Tissue samples from areas of the world where atherosclerosis is mild or rare show higher levels of chromium than do those from the United States or other countries where the disease is common (6,50). In analysis of post-mortem tissues of 18 persons with coronary arterial disease, chromium was detected in the aorta of only two, whereas it was detected in all but two of 13 persons with no evidence of the disease. There was no significant differences in chromium in nine other tissues of these subjects. This relative deficiency of aortic chromium appeared to be confined to cases of atherosclerotic heart disease; there was no difference in other trace metals (50).

Administration of chromium acetate for extended periods of time reduced serum cholesterol levels in 7 of 10 institutionalized patients by an average of 12.2%. Serum cholesterol also declined in 3 of 5 diabetics taking larger doses (6).

Normal circulating blood levels of cholesterol are considerably lower in other parts of the world than those considered "normal" in the United States, which have been increasing over the past 30 years.

Food and tissue levels of chromium are generally high in areas of the world where atherosclerosis is mild or absent. It is therefore possible that chromium deficiency is a cause of human atherosclerosis, although many other factors, both dietary and genetic, are probably involved (50).

SUMMARY

Chromium has been shown to be the active component in the "glucose tolerance factor" which appears to be a dietary essential for animals and humans. Chromium, in very small quantities is necessary for the optimal effect in every insulin-mediated system that has been tested in vitro. This, and the results of polarographic studies indicate that chromium in a biologically active form initiates the linkage between insulin and the cell membrane at its receptor sites. The glucose tolerance factor appears to be a complex containing an atom of chromium surrounded by two molecules of nicotinic acid and four amino acid molecules.

Administration of either synthetic glucose tolerance factor or that purified from biological material cures the impairment of glucose tolerance in chromium deficient laboratory animals in extremely small doses.

Evidence of chromium deficiency in at least part of the population of the United States is accumulating. This may account, in part, for the high incidence of diabetes and impaired glucose tolerance in the middle-aged and elderly. It may also be involved in gestational diabetes and atherosclerosis.

Due to difficulties of analysis, the chromium content of food is not known with certainty. In addition, the amount of inorganic chromium in a food gives no indication of availability or biological

activity, which can be determined only with great difficulty. A dietary recommendation for chromium must therefore await the refinement of analytic techniques for food and other biological materials so that nutritional balance studies can be performed.

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CHROMIUM - METABOLISM AND BIOCHEMICAL
INTERACTIONS IN ANIMALS AND HUMANS

by

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B.S., Michigan State University, 1965

AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1975

ABSTRACT

Chromium has been identified as the active component in the "glucose tolerance factor", a dietary component necessary for the optimal effect of insulin in carbohydrate, protein and lipid metabolism.

Chromium deficiency in laboratory animals is characterized by impaired glucose tolerance, decreased incorporation of certain amino acids into body tissue, elevated serum cholesterol and an increased incidence of aortic plaques.

There is evidence of possible dietary inadequacy of chromium in the United States. This may contribute to the high incidence of diabetes mellitus, impaired glucose tolerance in the middle-aged and elderly, atherosclerosis and gestational diabetes.

Analyses of the low levels at which chromium occurs in biological materials are difficult and yield inconsistent results. Thus there is no dietary recommendation for chromium at the present time, although it is recognized as an essential trace mineral.