COPPER METABOLISM[1]

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I. INTRODUCTION

In preparing this review of copper metabolism we have attempted to select material critically, and have not tried to include all of the very large number of empirical observations which have been made in relation to copper metabolism. We have, in general, referred to animal studies only when their results fill an obvious gap in our knowledge of human copper metabolism such as, for example, in the case of copper deficiency.

II. ROUTES FOLLOWED BY INGESTED COPPER

The daily American diet contains about 2 to 5 mg of copper (33, 79). Practically all of this is excreted in the feces (204). The path followed by copper which has been absorbed from the gastrointestinal tract has been observed by several investigators using radioactive copper^{64} (10, 14, 16, 29, 57, 103, 126, 139, 192, 221).

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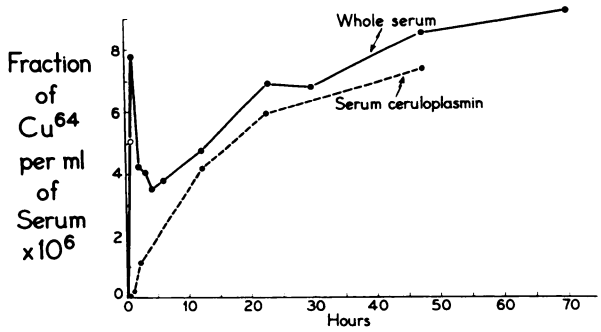

FIG. 1. The amount of copper^{64} in 1 ml of serum (——) and the amount of copper^{64} in the ceruloplasmin contained in 1 ml of serum (-----) following the ingestion of 2.0 mg of copper^{64} as cupric^{64} acetate in a normal adult male.

These studies show that copper^{64} first appears in the plasma as cupric ion which is probably loosely bound to albumin. Within about two hours the initial rise in total plasma copper^{64} concentration is followed by a sharp fall. During both this rise and fall there is a continued uptake of copper^{64} by the liver (29, 139), and there appears in the blood a gradually increasing concentration of copper^{64} which is tightly bound to the plasma copper-protein, ceruloplasmin (Fig. 1). Liver uptake may be a prerequisite for copper^{64} to appear in ceruloplasmin since the available evidence suggests that ceruloplasmin is synthesized in the liver (114).

Several different forms of hepatic copper can be distinguished. First, of approximately 24 μg of copper in a gram of dry liver in an adult (183) much is assumed to be "storage" copper, but whether this is combined with proteins or other constituents of the liver is unknown. It is likely that some is in transit between the intestine and the blood. Second, cytochrome C oxidase contains copper (215) and, since the liver is relatively rich in this enzyme (70), it accounts for some of the organ's copper. Third, a fraction of the metal is almost certainly part of other hepatic copper-proteins which have been isolated from various species, for example, hepatocuprein (122), and an unnamed copper-protein (131).

The amount of copper in liver—and quite possibly its form—are functions of 1) age, 2) diet and 3) various clinical conditions. For example, 1) the copper concentration in the liver of the newborn infant is considerably greater than that of the older child or adult (77, 85, 138, 204, 220a). 2) In rats (53), cattle (84) and sheep (98) the hepatic concentration of copper varies in the same direction as the dietary intake of the metal.[2] 3) Wilson's disease is associated with a great increase in the concentration of copper in the liver (20, 22, 31, 37, 43, 45, 183). We may note the somewhat paradoxical finding that in both the neonatal state and Wilson's disease, hepatic copper concentration is *elevated*, and there is a marked *decrease* in ceruloplasmin-copper (12, 17, 19, 20, 37, 169, 176, 179, 185, 189, 217).

[2] In rats (53) and cattle (84) the concentration of copper in the serum also varies in the same direction as the dietary intake.

The copper in blood is present in several different forms in plasma and red cells (41, 79, 112, 140). There are no data concerning copper in white blood cells or platelets. In the plasma of Americans and Europeans there is approximately 100 μg of copper per 100 ml (79, 85, 112a, 145, 199, 201, 204). Most of this appears to be bound tightly to ceruloplasmin. Indeed, almost all investigators believe that 95% (79, 124, 208), or even all (90), of plasma copper is part of the ceruloplasmin molecule. A contrary opinion (46), stating that a considerable portion of plasma copper may be bound to other globulins, has been changed recently by its authors (45a).

Most investigators agree that there is a small proportion of plasma copper which is not part of ceruloplasmin. This is present as free cupric ion which is in equilibrium with copper loosely bound to albumin (14, 63, 79, 203, 204, 206). When copper first enters the plasma from the gastrointestinal tract it is probably in this form. Thereafter, it is transferred progressively, presumably in the liver, (114) to ceruloplasmin (14) in which it is tightly bound. The loosely bound copper is probably that portion in transport (79), for, unlike copper bound to ceruloplasmin, it can diffuse freely across semipermeable membranes like the blood-brain barrier (79a), the red cell membrane, the glomerular membrane and the placenta (178).

Copper in red cells, the total concentration of which is also about 100 μg per 100 ml (103, 112a, 140, 142), is found in at least two forms. A minute portion is in diffusion-equilibrium with plasma (28, 103). The major portion is not diffusible and is tightly bound to a copper-protein of red cells, erythrocuprein (107, 123) or hemocuprein (122) (Table 2).

Normal urine contains extremely small amounts of copper. The mean 24-hour urinary excretion of 18 μg obtained in a careful investigation by Butler and Newman (30) is confirmed by the results of other studies, in dogs as well as in human beings, in indicating that less than 1% of the probable daily intake of copper is excreted in the urine (40, 120). Thus, an insignificant amount of copper, relative to intake or, as we shall see, body content, is lost in the urine of normal individuals. This is probably due to the binding of almost all blood copper to ceruloplasmin (120, 124), and to the absence of a specific tubular secretory mechanism for copper.

III. THE PRINCIPAL MAMMALIAN COPPER-PROTEINS

From plasma copper goes to a great many other sites in the body (Table 1). There are undoubtedly several forms in which copper may exist *in vivo* and these include free cupric, or cuprous ions, and combinations of copper with amino acids, purines, pyrimidines, nucleotides, DNA, RNA (67, 174, 214) and proteins. In general, the biological significance of these combinations is not well known although there has been considerable study of specific copper-proteins in the hope of elucidating their physiologic roles. Nine such proteins[3] which have been studied in mammals seem to represent important end-points of the probably divergent

[3] In some instances it is possible that one protein may have been given two or more different names.

TABLE 1

Copper content of normal adult human tissues
(μg/g dry tissue)

	Cartwright, G. E.* *et al.* (37)	Bickel, H.* *et al.* (20)	Others
Liver	12.2	44.0	23.6 (183)†
Spleen	3.3	15.0	
Kidney	10.0	14.0	
Adrenal	5.0	7.0	
Heart	12.2	22.0	
Lung	6.7	17.0	
Muscle	4.6	—	
Stomach	—	12.0	
Jejunum	—	18.0	
Colon	—	13.0	
Cerebral cortex (white)	13.9	20.0	33.0 (43)‡
Cerebral cortex (grey)	15.9	—	62.0 (43)‡
Cerebellum (white)	23.3	24.0	
Cerebellum (grey)	33.0		
Basal ganglia	24.6	27.0	
Thalamus	—	30.0	59.0 (43)‡
Brain stem	8.0	—	
Spinal cord	12.0	9.0	
Placenta	—	—	13.5 (130)§

* These results were obtained from one subject in each instance. They represent the only analyses of copper of many tissues from a single individual reported on a dry-weight basis. A few data, for normal brain and liver, reported on the same basis, and gathered prior to 1948, are given in Chapter 5 of ref. 45. The same chapter summarizes data, as μg per 100 ml, concerning copper in bile (8.3 to 19.8) and cerebrospinal fluid (10.0 to 2.40). The copper content of plasma, red cells and urine is discussed in the text of the present review.

† Mean of 5 subjects; range: 16.1 to 31. Ref. 183 gives the results of the determinations of the copper content of liver by 7 other groups of investigators.

‡ Mean of 9 subjects; range, cerebral cortex (white): 11.0 to 82.0; range, cerebral cortex (grey): 24.0 to 99.0; range, thalamus: 31.0 to 124.0.

§ Mean of 8 mature placentas; range: 11.8 to 16.6.

paths followed by ingested copper from the intestine. It is also likely that specific physiologic functions will ultimately be shown to depend on the chemical uniqueness of these copper-proteins, as has already been shown for tyrosinase (26, 117). We have summarized in Table 2 some of the chemical data available concerning these proteins which, together with physiologic information, deserve further discussion.

A. Ceruloplasmin

Ceruloplasmin is an *alpha*-globulin of plasma with a molecular weight of 151,000 (91, 105). The best preparations of Holmberg and Laurell, who first isolated, crystallized and characterized this protein, contained about 0.3% copper, which corresponded to eight atoms of copper per molecule. Ceruloplasmin

TABLE 2

Characteristics of mammalian copper-proteins

Protein	Source	Molecular Weight	Sedimentation Constant, $S_{20,w} \times 10^{-13}$	Diffusion Constant, $D_{20,w} \times 10^{-7}$ cm²/sec	Isoelectric Point	Electrophoretic Mobility			Copper Content	Color	Absorption Spectrum		Enzymatic Activity*	Copper Removed from Protein by			Reversibility of Copper-Protein Bond*	Immunochemical Specificity*	Physiologic Role	References
						μ cm²/volt·sec. $\times 10^{-5}$	pH	$\Gamma/2$			$E^{1\%}_{1\,cm}$	λ		pH‡	Cyanide‡	DTC†				
									%			mμ								
Cerebrocuprein I	Human brain	30,000–40,000	3.1	—	—	α_2-β globulin			0.29	Bluish-green	0.14 9.7	665 270	ND	<2.0	—	<5%	ND	ND	Unknown	152
Ceruloplasmin	Human plasma	151,000	7.29	4.5	4.4	−3.46 −5.32	7.0 8.6	0.2 0.1	0.34	Blue	0.68 5.5	605 280	D	<3.0	Yes	No	D	D	Unknown	91, 171, 123, 115
Cytochrome C oxidase	Beef heart	93,000	—	—	—	—	—	—	0.07	—	—	—	D	—	Yes	—	ND	ND	Aerobic oxidation	6, 171a
Erythrocuprein	Human red blood cells	28,000 ±2,000	3.02	9.3 ± 0.7	5.3	—	—	—	0.32–0.36	Light green-blue	0.10 6.6	655 265	ND	<1.0	Yes	No	ND	D	Unknown	107, 123
Hemocuprein	Ox and human red blood cells and serum	35,000	—	—	—	—	—	—	0.34	Blue	—	—	ND	<1.0	—	No	ND	ND	Unknown	122
Hepatocuprein	Ox liver	—	—	—	—	—	—	—	0.34	Almost colorless	—	—	ND	<1.0	—	No	ND	ND	Unknown	122
Horse liver copper-protein	Horse liver	30,000–40,000	2.9	—	—	2.35¶	6.8	0.1	0.3–0.4	Blue-green	—	—	ND	<5.0	Yes	—	ND	ND	Unknown	131
Tyrosinase	Mouse melanoma	—	—	—	—	—	—	—	0.22–0.25	—	—	—	D	—	Yes	—	D	ND	Production of melanin	25, 26, 117
Uricase	Hog liver	100,000 (approx.)	5.5	—	—	3.8¶	10.5	0.1§	0.05	Colorless	11.5	276	D	No	No	No	ND	ND	Oxidation of uric acid	119b

* D = Demonstrated; ND = Not Demonstrated.

† DTC = Diethyldithiocarbamate. The reaction of protein-bound copper with this compound is so complex that the various studies are not strictly comparable. For example, the time over which the reaction is observed, the pH of the solution and the presence of other proteins can affect the reaction sufficiently so that its strict characterization can be given only if the conditions of the reaction are precisely defined.

‡ To a lesser extent the same considerations apply to the reaction of protein-bound copper with H^+ and CN^- as with DTC.

§ Uncertain from text if this is ionic strength or molarity.

¶ Data insufficient to determine sign of mobility.

is, as one might expect from the name, deep blue, and it contains hexosamine, hexose (86) and neuraminic acid (115). It can be purified and crystallized by methods based on 1) its solubility at different temperatures in aqueous solutions of varying concentrations of small ions, and organic compounds, and on 2) its chromatographic behavior on columns of diethylaminoethylcellulose and calcium phosphate (20a, 24, 50, 91, 115, 134, 171, 190).

Quantitative analysis of ceruloplasmin in plasma or in serum may be made in four ways: by measurement of the absorption of light of 605 mμ by ceruloplasmin before and after destruction of its blue color (92, 181); by measurement of the total copper after removal of free or loosely bound copper (94, 181); by measurement of the rate of oxidation of paraphenylenediamine, or similar compounds, in a Warburg apparatus (94) or a spectrophotometer (87, 179, 181); and by immunochemical precipitation of ceruloplasmin and quantitative estimation of the specific precipitate (68, 124, 179). Various simpler qualitative and semiquantitative modifications of these methods have been devised (3, 4, 75, 97, 100, 156, 203), often by combining the method with electrophoretic analysis (78, 207, 208).

Four interesting aspects of the protein are 1) its oxidase activity toward certain polyphenols and polyamines, particularly paraphenylenediamine (94); 2) the reversibility of its copper-protein bond (133, 181); 3) its heterogeneity (24, 134, 206); and 4) inherited deficiency of normal ceruloplasmin.

1. The physiologic significance of its oxidase activity, although the most intensely studied characteristic of ceruloplasmin, remains completely unknown. A decade ago Holmberg and Laurell (94) showed ceruloplasmin to be the only oxidase in human plasma. This has been confirmed since, particularly by the finding that plasma which contains essentially no ceruloplasmin, as determined by measurement of blue color or by immunochemical analysis, also shows complete lack of oxidase activity toward paraphenylenediamine. Although ceruloplasmin's oxidase activity is most conveniently studied with paraphenylenediamine, or one of its derivatives as substrate, numerous other substrates have been found including benzidine (94), dihydroxyphenylalanine (DOPA) (92), serotonin (148) and epinephrine (92, 116) and their metabolic relatives (49, 87). It has been stated that ceruloplasmin is an ascorbic acid oxidase (91, 92), but solutions of purified human ceruloplasmin which have been carefully freed of non-ceruloplasmin ionic copper, by means of ion-exchange resins, show no oxidase activity toward ascorbic acid (132). Such activity may have been ascribed to ceruloplasmin because of the incidental presence of free cupric ions in its solutions (175).

It is possible that ceruloplasmin's oxidase activity has no physiologic significance (106) unless, as will be considered below, it be connected, by a presently unknown mechanism, with control of copper absorption (181) or excretion (49).

2. The copper of ceruloplasmin, although normally very tightly bound to the protein, can be reversibly removed from the latter *in vitro* if the copper is first reduced by ascorbic acid (181). A white, copper-free protein—apoceruloplasmin—can be obtained which is capable of recombining with cuprous ions, in the presence of ascorbate, to form ceruloplasmin again (133). We have speculated (181),

on the basis of this reversible copper-protein bond, that ceruloplasmin may transport copper much as transferrin transports iron (115). However, such transport of copper has never been demonstrated (221) and no one has yet shown that the copper-protein bond of ceruloplasmin is reversible *in vivo*.

The nature of the copper-protein bond in ceruloplasmin has been investigated independently in two laboratories. The experiments which first illustrated the reversibility of this bond (181) indicated that four of the eight copper atoms of each ceruloplasmin molecule seemed more readily exchangeable than the remaining four. In apparent confirmation of this, Curzon (48) demonstrated that digestion of ceruloplasmin by chymotrypsin made only half of the protein-bound copper dialyzable. Nevertheless, all eight of the copper atoms in ceruloplasmin could be reversibly removed in preparing apoceruloplasmin (133), suggesting that any difference in the nature of the binding of the eight copper atoms of the protein may be only relative. Results of preliminary acid-base titrations and electrophoretic analyses of ceruloplasmin and apoceruloplasmin suggested that each copper atom may be bound to two negatively charged carboxyl groups in the intact protein (177). Curzon (49) has suggested that the firm copper-protein link can be envisaged as the result of the co-ordination of copper with four groups on parallel polypeptide chains. The copper-protein bonds in ceruloplasmin which have been regenerated from apoceruloplasmin and copper may be different from those in the native protein since the regenerated protein possesses roughly 30% more oxidase activity, per atom of ceruloplasmin-copper, than the native protein (133).

3. Ceruloplasmin, like hemoglobin, albumin, haptoglobin and transferrin, is heterogeneous (24, 134). It has not yet been proven that this heterogeneity is genetically determined as is the heterogeneity of the other proteins listed. Four human ceruloplasmins have already been described (134) and two more have since been noted (135). These ceruloplasmins have been distinguished chromatographically and electrophoretically. All normal individuals so far studied possess at least two ceruloplasmins. The significance of this chemical heterogeneity of ceruloplasmin remains obscure. The differences so far detected are in electrophoretic mobilities, just as is true of those proteins where heterogeneity has already been demonstrated to be genetically determined (101). Since all the demonstrated variants of transferrin are alike in their ability to bind iron (202), it is interesting that two of the different ceruloplasmins are indistinguishable with respect to such characteristic properties as color, copper content, copper exchangeability and oxidase activity (134).

4. Inherited abnormalities of ceruloplasmin will be considered briefly together with one of tyrosinase, and more extensively subsequently.

B. Tyrosinase

Tyrosinase is a mammalian copper-protein with considerable chemical resemblance to ceruloplasmin. Its molecular weight and copper content are not accurately known although the latter seems to be of the order of 0.1 to 0.2% (25, 26). Four of its principal characteristics correspond closely to properties of cerulo-

plasmin: 1) oxidase activity toward poly- and monophenols, 2) the reversibility of its copper-protein bond, 3) possible heterogeneity, and 4) inherited deficiency.

1. The oxidase activity of tyrosinase is most marked toward the polyphenol, DOPA, although, unlike ceruloplasmin, it also seems able to oxidize the monophenol tyrosine and some of its derivatives which possess a free amino group. Tyrosinase oxidizes tyrosine at a slower rate than it oxidizes DOPA and may require the presence of the latter to "spark" the oxidation of the former (26).

2. Tyrosinase is also similar to ceruloplasmin in the reversible dissociability of its copper-protein bond (117). Lerner's studies of apotyrosinase indicate that gold, silver, mercury and copper can compete with each other for its copper-binding sites although the apoprotein has greater affinity for copper than for any of the other three metals. When copper is restored to apotyrosinase enzymatic activity is also restored, but the induction period, and the requirement for *l*-DOPA to "spark" the oxidation of tyrosine, are both increased (26).

3. There is evidence, obtained by chromatographic studies, that the tyrosinase of mouse-melanoma may be heterogeneous. Three fractions, with indistinguishable enzymatic activity, have been eluted from columns of diethylaminoethylcellulose (26).

4. Rare individuals exhibit an autosomally and recessively inherited deficiency of either normal ceruloplasmin or normal tyrosinase. Studies of subjects with either of these deficiencies have shed considerable light on the physiologic role of these proteins. Deficiency, or absence, of ceruloplasmin occurs in almost all patients with hepatolenticular degeneration (Wilson's disease). Considerable data suggest, as we shall discuss further below, that ceruloplasmin is connected with the regulation of copper metabolism. The physiologic role of tyrosinase has been elucidated by comparing normally pigmented subjects with complete albinos. The latter possess no detectable tyrosinase activity (88), which is thus confirmed as being responsible for the production of melanin in the skin and uveal tract of normal individuals.

Deficiencies of both proteins have never been observed in one individual, nor have albinism and Wilson's disease been reported in the same patient. An albino boy had a quantitatively normal concentration of serum ceruloplasmin (135) and there is no evidence of diminution of melanin-formation in patients who lack ceruloplasmin. It is clear, therefore, that despite their similarities, ceruloplasmin and tyrosinase are different proteins the synthesis of each of which is governed by different genes.

C. Cerebrocuprein

Cerebrocuprein is the name given by Porter and his co-workers (149–154) to a copper-protein isolated from bovine and human brain. Its copper content of 0.29%, representing 2 atoms of copper per molecule, its bluish-green color, and the relative stability of its copper-protein bond to acid and the diethyldithiocarbamate ion are similar to properties of ceruloplasmin. There is suggestive evidence, too, that copper in cerebrocuprein is bound to carboxyl groups (152,

153). But cerebrocuprein differs from ceruloplasmin in having a molecular weight of only 30,000 to 40,000 and in lacking any enzymatic activity toward paraphenylenediamine.

D. Erythrocuprein

Erythrocuprein is a copper-protein which has been isolated from human red blood cells by Markowitz, Cartwright and Wintrobe (123). This protein binds almost all the copper in erythrocytes. Detailed and valuable chemical information about it has been obtained by these investigators and their collaborators (107). Confusion about the relationship between ceruloplasmin and erythrocuprein, which resulted from the earlier conclusion of Mann and Keilin (122) that there was only *one* copper-protein in blood, has been dispelled by these studies on erythrocuprein. This protein differs from ceruloplasmin 1) in being only faintly greenish-blue, 2) in having less than one-fourth the molecular weight, 3) in possessing a different isoelectric point (107), 4) in lacking oxidase activity toward paraphenylenediamine, 5) in having different absorption spectra in both visible and ultraviolet light, 6) in immunochemical specificity (123), and 7) in being present in its usual concentration in a patient with Wilson's disease (34a). On the other hand, both proteins 1) bind copper tightly enough to prevent, almost completely, its direct reaction with diethyldithiocarbamate (79, 123), 2) are glycoproteins, 3) contain the same amount of copper by weight, and 4) lose their copper in the presence of sufficient cyanide or hydrogen ion, although the copper-protein bond of erythrocuprein seems somewhat more resistant to acid than that of ceruloplasmin.

Porter and Ainsworth (152) have also pointed out several similarities between erythrocuprein and cerebrocuprein. No individuals who are deficient in erythrocuprein or cerebrocuprein have yet been discovered, and insight into the function of these proteins is essentially nil.

E. Cytochrome C oxidase

Cytochrome oxidase—more specifically cytochrome C oxidase—has well-known importance in aerobic oxidation. It has only recently been demonstrated definitely that this protein contains copper. This was shown by Wainio (215) and his collaborators in experiments in which copper content, heme content and enzymatic activity were related, and in which the removal of copper by 0.1 M NaCN destroyed activity and reduced the characteristic light absorption at 445 mμ. One molecule of heme and one atom each of iron and copper are present in each water-soluble monomer of cytochrome oxidase (6). Paramagnetic resonance studies indicate that the copper of cytochrome oxidase is reduced specifically by cytochrome C, and probably by ascorbate as well, and that this reduction may be connected with the function of the enzyme (171a).

These chemical data are consonant with results, to be discussed below, that cytochrome oxidase activity is markedly diminished in experimental and natural copper deficiency.

F. Copper-proteins in liver

The functions of hepatocuprein (122) and a copper-protein isolated from horse liver by Mohamed and Greenberg (131) are unknown. It is possible that these are the same protein. Porcine hepatic uricase, which catalyzes the oxidation of uric acid to allantoin, possesses "enzymatic activity [which] appears to be related to the presence of copper in the enzyme . . ." (119b). Some doubt persists that uricase is a copper-protein since neither dialysis against a solution of cyanide ion nor exposure to a solution of pH 1.0 at 0°C for 10 minutes removes copper from the protein (119b), whereas one or both of these procedures removes copper from all other copper-proteins listed in Table 2.

Butyryl coenzyme A dehydrogenase, previously reported as containing copper (119a), has recently been convincingly shown to be free of the metal (195). Early preparations of δ-aminolevulinic acid dehydrase contained 0.1% copper but cyanide did not inhibit enzymatic activity (102), and subsequent work (220b) showed that the copper could be removed from the enzyme without a parallel loss in enzymatic activity. These are two instances where copper, at first thought to be an integral part of a protein, was subsequently shown to be a contaminant. It seems reasonable to assume that some other organic compounds which have been found to contain trace amounts of copper will also prove ultimately merely to have been contaminated by the metal.

IV. PHYSIOLOGIC, PATHOLOGIC AND PHARMACOLOGIC ALTERATIONS IN THE CONCENTRATION OF CERULOPLASMIN AND COPPER IN BLOOD

Copper metabolism seems peculiarly susceptible to alteration in a wide variety of clinical conditions. There are a considerable number of empirical observations in this area, most of which, in human beings, have been concerned with changes in the concentration of blood copper.[4] Plasma, or serum, has been most intensively investigated usually by measurements of total copper or of ceruloplasmin, the normal concentrations of which are about 100 μg and 30 mg, respectively, per 100 ml. Copper bound to ceruloplasmin, however, accounts for almost all plasma copper—except in patients with Wilson's disease, discussed below—so that both copper and ceruloplasmin almost always vary in the same direction (2, 94, 124, 137) and either is a good index of the other. A great many clinical conditions are accompanied by a marked increase in plasma copper and ceruloplasmin. We understand virtually nothing of the significance of these increases. Perhaps their chief importance to date lies in the conclusion that serum copper, like the sedimentation rate or the white blood-cell count, is an "acute phase reactant."

Most striking, perhaps, is the rise in ceruloplasmin and plasma copper which is seen in the latter part of pregnancy (2, 90, 112, 124, 169, 178, 197, 201). By term, maternal serum may contain two to three times the normal adult con-

[4] Quantitative and semiquantitative analyses of copper in blood and other biological material can be carried out accurately and precisely by a variety of methods. These involve colorimetry (2, 59, 80a, 113, 145a, 181, 224), amperometry (62), emission spectroscopy (31, 41), activation analysis (23) and histochemical techniques (76, 99, 138, 209).

centration of ceruloplasmin (124, 176). This is likely to be related to the fact that the administration of estrogens can regularly induce a sharp increase in plasma ceruloplasmin (60, 128, 166, 197, 203), and these hormones have even been used in attempts to correct the hereditary deficiency of ceruloplasmin associated with Wilson's disease (12, 167). Maternal ceruloplasmin either does not cross the placental barrier or does so to a small degree only, since the newborn infant has a plasma concentration of this protein which is not only much lower than his mother's at delivery but is only about a third of the average concentration seen several weeks after birth or in adults (94, 169, 176, 178, 185).

The concentration of ceruloplasmin, and copper, in serum has also been found to rise significantly in infections (90, 112, 124), myocardial infarction (2), neurologic disorders other than Wilson's disease (124, 180), iron deficiency anemia (112, 141, 142), hyperthyroidism (145, 160), portal cirrhosis (15, 79a, 141), biliary tract infection (141), hepatitis and post-hepatitic cirrhosis (158), pellagra (65), chronic alcoholism (164), Hodgkin's disease (112, 141, 199), acute (141) and chronic leukemia (79, 112, 157, 199), carcinoma (140, 141), and following the prolonged administration of testosterone (104).

Following elevation of the plasma ceruloplasmin concentration a return to a normal concentration may require several weeks. Thus, in experimentally induced increase in the concentration of ceruloplasmin by administration of estrogen or androgen, the concentration of the protein returned to normal by 8 weeks after the drug was discontinued (104). Infusion of ceruloplasmin, either in the form of fresh normal blood (180) or as purified material (20a, 192) prepared from human plasma, into subjects with an hereditary absence of this protein permitted the rate of disappearance of the protein to be determined by measurement of the oxidase activity of the subject's serum at daily intervals. In three individuals, six experiments yielded half-lives of ceruloplasmin of 4.69, 4.40 and 3.21 days when whole blood was infused (180), and 5.6, 7.0 and 7.2 days when purified ceruloplasmin was administered (192, 194).

In 1957 Akerfeldt (4) reported findings which were interpreted as indicating that the concentration of ceruloplasmin was elevated in schizophrenic patients and in patients with other mental diseases. In the succeeding years, however, it has become clear that this is not the case, and Akerfeldt's conclusion that measurements of ceruloplasmin's properties can be used as a diagnostic aid in mental disease is incorrect (144, 173, 182). That error was the result, chiefly, of neglect of the effects of nutrition or intercurrent disease in chronically ill psychiatric patients (1, 68, 73, 89, 96, 106, 127, 222).

A plasma concentration of ceruloplasmin of less than 15 mg per 100 ml, which is about the lowest value reported in normal children and adults, is found more or less regularly in infants who are newly born (94, 169, 185, 212) or who suffer from severe hypoproteinemia, hypocupremia and hypoferremia (198, 226) and in patients with Wilson's disease (12, 19, 124, 179, 203, 208), tropical (32) and non-tropical sprue (34, 79, 191), the nephrotic syndrome (36, 112, 124), kwashiorkor (110, 161) and intestinal malabsorption due to scleroderma (191). In the nephrotic syndrome, ceruloplasmin is apparently lost in the urine in an amount

sufficient to result in hypoceruloplasminemia. In myxedema, elevated concentrations of ceruloplasmin (191), as well as low concentrations (160), have been found.

V. COPPER DEFICIENCY

Deficiency or toxic excess of iron can occur not infrequently. In contrast, unequivocal and significant deficiency of copper has never been reported in human beings and a toxic excess of copper is rare. There seem to be two reasons which account for this fortunate pair of facts: 1) normal diets, in the United States and probably in most parts of the world, contain a large amount of copper relative to needs and, 2) a specific regulatory mechanism seems to keep this dietary surfeit of copper from becoming toxic.

That copper is essential metabolically in mammals has been demonstrated by observations made in cattle, chickens, sheep, dogs, pigs and rats (47, 69, 125, 204, 205). Some of these data were gathered as a result of experimentally induced copper deficiency while others have been observed in naturally occurring deficiency. The metabolic disturbances which inadequate amounts of copper induce are seen in A) various aspects of iron metabolism, B) phospholipid synthesis, C) osteoblastic activity, and D) keratin and pigment formation. It is worth noting that the pathologic effects seen in tissues as a result of copper deficiency such as, for example, the demyelination in copper-deficient lambs, may result from a combination of these metabolic disturbances. Furthermore, several of the specific copper-containing proteins seem to be reduced in amount in copper deficiency, but their relation to corresponding pathologic changes is obscure.

A. Iron metabolism

Evidence that absorption and utilization of iron are abnormally low in copper deficiency (168) is found in the work of Gubler (81) and Bush (27) and their collaborators. The iron deficiency seen in copper-deficient swine (111) and rats (38) cannot be corrected by feeding iron alone. Furthermore, if iron is supplied parenterally to such pigs the effects of its deficiency are not corrected unless copper is also supplied (35). An earlier conclusion (81) that mobilization of iron stores is also impaired in copper deficiency is not borne out by recent work (27) of Cartwright's group, which shows that the turnover of iron is, in fact, increased from the rate of 0.10 mg/kg per day in control pigs to a rate of 0.52 mg/kg per day in copper-deficient pigs.

Three groups of investigators have shown independently that the enzymatic activity of the heme-protein, cytochrome oxidase, is markedly diminished in copper deficiency. Gallagher, Judah and Rees (70) demonstrated in copper-deficient rats a decrease in cytochrome oxidase activity which was most marked in the liver but was noted also in kidney, heart and brain. These authors were able to show also that extracts of liver of these copper-deficient rats contained less heme α than those of control rats, suggesting that the deficiency of cytochrome oxidase might be a consequence, at least in part, of insufficiency of the enzyme's porphyrin. Gubler, Cartwright and Wintrobe (80) demonstrated an

eight-fold decrease in the activity of this enzyme in heart and a three-fold decrease in its activity in the liver of copper-deficient swine compared to control pigs. Howell and Davison (98) showed a significant reduction of the activity of cytochrome oxidase in the brains of naturally copper-deficient, demyelinated lambs.

Such deficiency in cytochrome oxidase may be the result of several factors. First, we have already pointed out that each monomeric molecule of cytochrome oxidase contains one atom of copper. Second, there is evidence suggesting that synthesis of heme can be diminished in copper deficiency, as demonstrated in red cells *in vitro* (8). Third, the interference with iron absorption found in copper deficiency (81) may diminish the iron available for synthesis of this protein (8).

Anemia has been observed in experimental copper deficiency (27). In pigs this anemia is hypochromic and microcytic and, thus, morphologically indistinguishable from that induced by iron deficiency (35). In addition, the mean half-life of erythrocytes in copper-deficient pigs as measured by chromium^{51} is 9 days as compared to 17 days in control pigs (27). That this is in part a defect in the corpuscle itself is shown by the fact that the shortened red cell life-span is not completely corrected when these cells are transfused into a normal pig. The origin of this corpuscular defect is unknown but it may be related to the abnormality of phospholipid metabolism about to be described.

An anemia has also been reported in dogs made experimentally copper-deficient. In contrast to the anemia in pigs, that in dogs is normochromic and normocytic. (211a).

B. Phospholipid synthesis

In careful studies designed to measure defects in synthesis occurring in experimental copper deficiency, Gallagher, Judah and Rees (71) demonstrated that a deficiency in phosphatidic acids in copper-deficient rats was probably due to a diminished capacity for coupling coenzyme A-activated fatty acids to glycerophosphate. These authors suggested that this finding may be important in understanding the pathogenesis of the demyelinating disease, "swayback," which is seen in newborn lambs dropped by ewes which are copper-deficient. If this defect in phospholipid synthesis had occurred *in utero* at a time when myelin, which is rich in phospholipid, was being formed, demyelination might have resulted. In addition, deficiency in cytochrome oxidase activity may play a role in producing demyelination since the latter can be caused by agents, like potassium cyanide, which are known to inhibit cytochrome oxidase (69, 70, 71).

C. Osteoblastic activity

In copper-deficient dogs (11), swine (66) and chickens (69) a defect in osteoblastic activity is demonstrable, although chondroblastic activity and calcification of cartilage are unimpaired. The defect closely resembles that seen in deficiency of ascorbic acid (66). In the natural copper deficiency of cattle and

lambs, and in the experimental deficiency of dogs, skeletal changes similar to those seen in scurvy often develop (204).

D. Keratin and pigment formation

In sheep suffering from naturally occurring copper deficiency a defect in wool-keratin has been noted (204). This has been thought to be related to an abnormality in the cross-linkages of keratin which normally occur through disulfide bridges. The wool of such sheep (204), and the hair of rats (69) made copper-deficient may also show achromotrichia and it is likely that this is due to deficiency of tyrosinase.

Although naturally occurring copper deficiency in animals is usually the result of a simple lack of copper in the pasture (less than 6 parts of copper per million is considered to be the critical level) (204), deficiency may also occur in the presence of adequate concentrations of copper in the forage. Thus, sheep feeding on certain seaweeds of Iceland develop copper deficiency despite a copper content of these plants of more than 9 parts per million (143). Mills (129) has shown recently that some copper in herbage is effective in the treatment of copper deficiency, while other forms of herbage-copper are relatively inefficient in this respect. The fraction of copper most effective in relieving copper deficiency can be extracted with saline but is *not* dialyzable. Furthermore it has been known for some time that molybdenum and copper are interrelated nutritionally (47). Copper deficiency seems aggravated by feeding molybdenum (56, 125, 204) and chronic copper toxicity in sheep seems to be ameliorated if the animals are given ammonium molybdate (146, 200). In contrast, there is evidence that manganese may increase the retention of copper in the body (83).

E. Human beings

The foregoing pathologic effects of copper deficiency have not been seen in human beings (79). There are, it is true, conditions where the concentrations of serum copper are below normal but if there are simultaneous significant decreases of total body copper, or clinical consequences, they have yet to be defined. Thus, for example, in infants who suffer from hypocupremia, hypoferremia, anemia and hypoproteinemia (198, 226), no evidence has been adduced to show that the hypocupremia produces any ill effect. No one has yet treated these infants with copper alone and the available evidence indicates that iron and protein can produce clinical remission just as quickly without as with added copper. Nor could any of the effects of copper deficiency found in animals be observed in small, premature infants fed only 15 μg of copper per kg a day for a two-month period (220a).

Nevertheless, in addition to the evidence from the observations made in animals, the existence of the unique copper-proteins in human beings which we have discussed is presumptive evidence for the essential nature of copper in man. To varying degrees the functions of ceruloplasmin, tyrosinase and cytochrome oxidase are known and it hardly seems likely that the other copper-proteins would exist if they had no function.

TABLE 3

Dietary supply, body stores and loss with blood of iron and copper in a 70-kg man

	Daily Dietary Supply	Total Amount in Body	Per Cent of Body Content Lost with 100 ml of Blood	References
	mg	*mg*		
Iron	12–15	4000–5000	1.0–1.25	204
Copper	2–5	75–150	0.067–0.13	79

Copper deficiency does not occur in human beings apparently because of the facts that dietary copper is abundant relative to body requirements and that loss of copper is insignificant. A comparison of copper with iron is interesting in these respects. Table 3 shows that the ratio of dietary supply to body content is almost ten times greater for copper than for iron. On the other hand, bleeding removes only one-tenth as much body copper as body iron, relative to body content. Since, for all practical purposes, even iron deficiency occurs only in the presence of blood loss, growth or pregnancy, it is not surprising that copper, with greater supply and smaller losses, hardly ever, if ever, falls to levels of deficiency. With respect to the needs of the body, consequently, there is a surfeit of copper in almost any diet.

VI. COPPER TOXICITY

If copper deficiency fails to develop in human beings because there is, relatively, so much dietary copper one may ask whether copper toxicity results. It is rather remarkable that despite the dietary copper, the widespread use of copper for plumbing, kitchen utensils, beer-brewing kettles and whiskey-stills (42), and the exposure of many kinds of workers to high concentrations of copper (21, 40, 121), poisoning by this metal is almost, if not quite, as unusual as copper deficiency. The toxicity that does occur is either acute or chronic, and despite the clinical rarity of either form, discussion of them may aid in understanding copper metabolism.

A. Acute copper toxicity

The incidence of acute copper toxicity has been discussed at length by Davenport (51) in a survey of health hazards related to copper. The ingestion of more than 10 to 15 mg of copper at one time will cause nausea and vomiting and perhaps diarrhea and cramps (95, 223). As a result, very little copper is left for absorption. One subject, who apparently ingested 20 grams (*sic*!) of copper sulfate, and had been doing so several times weekly for months, was admitted to a hospital with hemolytic anemia (163). Nevertheless, in general, copper is benign in human beings.

B. Chronic copper toxicity

1. Excessive intake of copper. There is, however, evidence that copper can be more toxic in animals than in man, and such toxicity in animals is usually chronic

(146,200). Thus, sheep have developed, experimentally and naturally, anemia, hemolytic crises and liver disease (5, 125, 146, 200), and chickens have been made to develop hemolytic anemia as a result of copper poisoning (74). Fish, too, have been poisoned with copper experimentally (213), as have rats (221a). Copper toxicity, therefore, probably does not develop in man because the metal is excreted, or is incompletely absorbed, rather than because of an inherent lack of toxicity of copper. The evidence for this statement again lies in a consideration of dietary and body copper. If 3.0 mg is a reasonable figure for the average daily ingestion of copper by an American (79) from birth on, then, in 20 years, about 22 g of copper will have been consumed. Yet the body of this individual will, at that age, contain less than 150 mg of copper (79, 85, 145, 204), or considerably less than 1% of the amount eaten. What has happened to the other 99%?

We have already indicated that in normal individuals urinary excretion of copper is almost nil (30, 33, 79, 136). Consequently, more than 99% of dietary copper must ultimately be excreted *via* the feces. But, has fecal copper simply passed through the gastrointestinal tract unabsorbed, or has it been absorbed and excreted, or have both occurred?

An answer to this question can be obtained only through studies with isotopically labelled copper since conventional balance studies, which are, incidentally, inaccurate and extremely difficult to carry out (36), can supply only information as to net absorption. Unfortunately, the only isotope of copper which has so far been available for use in such studies is radioactive copper^{64}. This has a half-life of 12.8 hours (220), so that orally administered copper^{64} can be traced for only three or four days. Studies made by Matthews (126), Bush (29), Bearn (16), Jensen (103) and their collaborators indicate that the amount of copper absorbed is quite variable in human beings. All of these experiments involved feeding cupric salts but they are not comparable in that the amount of copper fed varied from 1.0 to 12.5 mg. The amounts of copper^{64} recovered from the feces, in normal individuals, ranged from 26% of a 12.5 mg dose (16) to 95% of a 1 mg dose (29). Since all of these values are incompatible with the fact that only 1% of dietary copper is retained in the body, the conclusion seems warranted that an appreciable amount of copper is absorbed and is excreted through the feces over a period of more than four days.

Fecal excretion of copper has been demonstrated experimentally in dogs and in human beings. In dogs 7% of intravenously administered copper was excreted, and of this, 82% was excreted in the bile, 13% *via* the intestinal wall, and only 5% in the urine (120). In humans 12.0 to 33.4% of 1.25 mg of intravenously administered copper^{64} was recovered in the feces (16), and, as in dogs, a considerable fraction of this reached the intestinal lumen *via* the bile. In two experiments where 2.0 mg of copper^{64} as cupric^{64} acetate were administered intravenously to patients with external drainage of bile *via* T-tube, 0.3 and 4.2% of the copper^{64} was recovered in the bile within 73 hours (194). Other investigators have detected considerable concentrations of copper (5.6 to 205 μg/100 ml) in human bile (55, 155).

2. Normal intake of copper and an hereditary abnormality. There is, however, one very small group of individuals in whom there regularly develops progressive and fatal copper toxicity although they eat no more dietary copper than other individuals. They suffer from hereditary hepatolenticular degeneration (Wilson's disease). We shall consider, in these subjects, a) the evidence that control of copper balance is defective, b) the nature of their inherited defect in regulation of net copper absorption, and c) the effects of this disturbance in regulation.

a. Evidence that control of copper balance is defective in patients with Wilson's disease.

1. Balance studies, though not very accurate, generally indicate that copper absorption is greater in patients with Wilson's disease than in control subjects. Thus, Cartwright and his collaborators (36) found that four patients with Wilson's disease were in positive balance while three normal subjects were in almost perfect balance. Similarly, Zimdahl and his co-workers (225) found a marked positive balance of copper in three patients with Wilson's disease. Bickel, Neale and Hall (20) have pointed out most pertinently that balance studies are, at best, a poor way to investigate Wilson's disease since "it does not . . . seem necessary to suppose more than a very slightly positive balance to produce the . . . amounts of deposited copper which are found" after 10 to 20 years.

2. Studies with radioactive copper^{64} have usually indicated that diminished excretion of copper exists in patients with Wilson's disease (16, 29, 103, 126). Following both oral feeding and intravenous administration of copper^{64} these patients excrete less copper^{64} in the feces than normal individuals. These experiments do not conclusively demonstrate less excretion of copper^{64} in patients with Wilson's disease since it is possible that the absorbed or injected copper^{64} is diluted by their larger copper-pool to a greater extent than by the smaller pool of normal subjects (29).

3. The liver (183), brain (22, 45, 118, 149, 150) and, in fact, almost all tissues (20, 37) of patients with Wilson's disease contain amounts of copper which are much greater than normal.

b. The nature of the inherited defect in regulation of net copper absorption. The nature of the inherited defect has not been definitely established, but certain facts indicate that it is closely related to deficiency of normal ceruloplasmin.

1. Practically all of these patients exhibit a deficiency of the plasma copper-protein, ceruloplasmin (12, 15, 19, 20, 22, 37, 72, 78, 88, 124, 179, 180, 189, 203, 212, 217). Suggestive evidence exists that at least in those few patients in whom there is a normal concentration of ceruloplasmin (61, 165, 172) the ceruloplasmin may be qualitatively abnormal (135a).

2. In several young individuals deficiency of ceruloplasmin was found to antedate all of the other clinical and laboratory manifestations of Wilson's disease (72, 119, 183).

3. Since the disease is inherited in an autosomal recessive manner (13, 88), one pair of abnormal genes is presumably present in patients. In view of recent evidence that each gene's action is to direct the quantitative and qualitative syn-

thesis of the structure of a single protein, or even of one polypeptide chain of several in a protein (101), it seems unlikely that this one pair of abnormal genes is responsible also for an abnormality in another protein, in addition to ceruloplasmin, unless the former is a precursor or metabolic product of ceruloplasmin.

c. The effects of this disturbance in regulation of copper metabolism. Several investigators (12, 17, 19, 20, 22, 37, 45, 180, 225) believe that the pathology of Wilson's disease is consequent to the deposition in tissues of excessive amounts of copper. Striking evidence for this conclusion has come from continued clinical and chemical examination of certain young children, which indicates that deposition of excess copper precedes pathologic effects. One such boy, whose older sister has aceruloplasminemia and severe Wilson's disease, has never been found to have any ceruloplasmin from ten months of age, when he was first seen, to his present age of six. Clinically he has been a completely normal child. Biopsy of his liver at three, and again at four years of age, showed it to be grossly and microscopically normal, except for the presence of moderate numbers of glycogen nuclei, but to contain an enormous concentration of copper (183).

Studies in other patients have produced data which strongly suggest that, as time goes on, the excessive copper-deposits gradually induce pathologic changes in several organs. Liver parenchyma degenerates and collapses and is replaced by fibrous tissue to produce a pathologic picture indistinguishable from posthepatic cirrhosis (7). Progressive brain damage is seen with prominent symptoms and signs of involvement of the basal ganglia, and often with findings indicative of widespread affection of the brain (22, 45, 109, 162). Kidney function, although normal early in the disease (184), eventually shows tubular abnormalities (12, 18, 20) and, later, glomerular malfunction (12, 18, 188). Deposits of copper in the cornea manifest themselves as the highly characteristic Kayser-Fleischer rings. Progressive pathologic involvement of the liver, brain, or both, is the eventual cause of death in the untreated patient.

A relevant and interesting experimental study has shown that severe neurotoxic and nephrotoxic, though not hepatotoxic, effects can be induced regularly in goldfish by keeping them in water containing 1 μg of copper per ml (213).

Further evidence that chronic copper toxicity is the basis of these pathologic changes is found in the results of treatment of patients with Wilson's disease. It is a fair statement that no therapy was of more than palliative value in this illness until measures were introduced which prevented the further accumulation of copper in the body and removed some of the copper already deposited. Such specific therapy has included: i) diets low in copper which exclude, in particular, such copper-rich foods as shellfish, liver, mushrooms, nuts and chocolate (184), ii) agents, such as cation-exchange resins and potassium sulfide (29, 37, 147, 225) which serve to render dietary ionic copper insoluble and unabsorbable, and iii) agents, such as 2,3-dimercaptopropanol (BAL) (44, 55, 225) or β,β-dimethylcysteine (penicillamine), which chelate copper and thereby increase its urinary excretion (12, 139, 216). With a regimen based on these measures there is little question that marked and sustained improvement in the neurologic aspects of Wilson's disease can be produced (147, 184, 186, 196). The causal relation of copper-deposits to these manifestations is, consequently, made more likely.

It is true that very little change in liver function has regularly accompanied this neurologic improvement but treatment has usually not been started until advanced structural and functional liver disease is present.

For several years we have been continually treating with "anti-copper" regimens (184) three young boys, including the one just described, all of whom are asymptomatic and hypoceruloplasminemic siblings of patients with Wilson's disease. If manifest disease can be averted, or long delayed in onset (in comparison with the ages of onset in their siblings) in these boys, this will provide even firmer evidence that Wilson's disease is chronic copper toxicity in human beings.

A different concept of the pathogenesis of Wilson's disease has been advanced by Uzman (210). He has postulated the presence of "genetically determined . . . proteins with high copper avidity" as the cause of the increased copper in the tissues of patients with Wilson's disease. The deficiency of ceruloplasmin exhibited by almost all of these patients is assumed to be due to the fact that these abnormal proteins have so much greater affinity for copper than apoceruloplasmin that little or no copper is available to complete the synthesis of ceruloplasmin. It seems unlikely, however, that ceruloplasmin deficiency can be due merely to a lack of copper (17, 217) since there is evidence that deficiency of another copper-protein, cerebrocuprein (149), does not occur in these patients. Furthermore, there is, in fact, a much greater amount of dialyzable copper in patients with Wilson's disease than in normal subjects as shown by the high concentration of direct-reacting plasma copper (12, 37), urinary copper (17–20, 37, 57, 103, 136, 139, 184, 216, 225) and spinal fluid copper (37) in the former.

The conclusion that human chronic copper intoxication results only when there is prolonged deficiency of ceruloplasmin really rests on the finding that patients with Wilson's disease lack normal ceruloplasmin, more or less completely. The consequences of this deficiency seem to be that copper i) is not incorporated into this protein (16, 57, 103, 172, 192), ii) is relatively free to diffuse from the vascular compartment into tissues, and iii) is not so readily excreted *via* the feces as ceruloplasmin copper. Several facts have been reported which are, however, difficult to reconcile with such a central role for ceruloplasmin in regulating copper metabolism. First, the age at onset and the severity of Wilson's disease in a patient are not well correlated with his serum ceruloplasmin concentration. Second, a small number of patients with indubitable Wilson's disease have concentrations of ceruloplasmin which are only slightly lower than the concentrations in some normal individuals (15, 124), and at least three patients with Wilson's disease have had perfectly normal concentrations of ceruloplasmin (61, 165, 172). Third, parents of patients never develop the disease, and although only heterozygous for the abnormal gene (193), may, rarely, have concentrations of ceruloplasmin low enough to be indistinguishable from those in patients (12, 20, 136, 194). Lack of relation of age at onset and severity of the disease to ceruloplasmin deficiency may be due to variations in copper intake and absorption. But what about patients with normal concentrations, and healthy heterozygotes (parents) with low concentrations, of ceruloplasmin? Two explanations may be thought of in patients, the second of which could apply to the heterozygotes.

First, a few patients with Wilson's disease, intially found to have characteristically low concentrations of ceruloplasmin, have, on subsequent examination, been shown to have perfectly normal concentrations of this protein. In some instances this change has almost certainly been the consequence of an increase in estrogens (page 365, above), brought about either exogenously (12, 167), or endogenously during pregnancy (194). The suggestion has been made that the concentration of endogenous estrogens may also rise in these patients as a result of hepatic decompensation (61) and we have seen a marked increase in the concentration of ceruloplasmin in one patient entirely consistent with this idea (191).

Second, we have already summarized the evidence that ceruloplasmin is heterogeneous and there is suggestive electrophoretic evidence that one of the patients with Wilson's disease and 27 mg of ceruloplasmin per 100 ml of serum (172) may have had an abnormal ceruloplasmin (135a). Thalassemia, which has generally been assumed to represent a deficiency of hemoglobin A, may, in fact, be the result of inheritance of a gene directing the synthesis of a very small amount of an abnormal hemoglobin (101), although one where the amino acid substitutions do not affect electrophoretic behavior. It is possible that many, if not all, inherited, specific protein deficiencies are due to the synthesis of an abnormal form of the particular protein. It is consistent with such a thesis that all the abnormal adult hemoglobins, except hemoglobin J, appear to be synthesized in lesser amounts than hemoglobin A (101). Accordingly, deficiency of ceruloplasmin in patients with Wilson's disease may represent merely the observable result of the synthesis of a small amount of an abnormal ceruloplasmin, of which there may be several varieties. Development of the disease in some patients could conceivably be the consequence of the presence of an abnormal ceruloplasmin rather than simply of quantitative deficiency of the normal protein. Such an hypothesis might explain, too, why heterozygotes for the, or a, Wilson's disease gene, with as little as half the normal amount of ceruloplasmin would escape the disease, since most of the ceruloplasmin which they have is of a normal variety.

Finally, there is no evidence that the aceruloplasminemic brother of a patient with Wilson's disease already referred to has any detectable physiologic, psychologic or chemical abnormality other than disturbance in copper metabolism. There is, therefore, no evidence for a function of ceruloplasmin other than its possible one in regulating the metabolism of copper.

VII. CONCLUSION

There are two reasons which justify the appearance of this paper in *Pharmacological Reviews*, even though copper has no systemic pharmacologic use. First, we have presented evidence that copper is essential to metabolism although it so happens that copper is so relatively abundant in our foods that human copper deficiency occurs very rarely, if at all. Therefore, copper need never be added to a human diet therapeutically, or prophylactically. Second, pharmacology subsumes toxicology. The fact that in man copper poisoning occurs virtually only in subjects with deficiency of ceruloplasmin suggests that the prevention of

copper toxicity is as subtle and complex an aspect of copper metabolism as is the essentiality of copper in metabolic processes.

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