Role of Copper in Mitochondrial Iron Metabolism

By D. M. Williams, D. Loukopoulos, G. R. Lee, and G. E. Cartwright

Heme synthesis by copper-deficient cells was investigated to elucidate the nature of the defect in intracellular iron metabolism. Iron uptake from transferrin by copper-deficient reticulocytes was 52% of normal, and the rate of heme synthesis was 33% of normal. Hepatic mitochondria isolated from copper-deficient animals were deficient in cytochrome oxidase activity and failed to synthesize heme from ferric iron (Fe III) and protoporphyrin at the normal rate. The rate of heme synthesis correlated with the cytochrome oxidase activity. Heme synthesis from Fe(III) and protoporphyrin by normal mitochondria was enhanced by succinate and inhibited by malonate, antimycin A, azide, and cyanide. It is proposed that an intact electron transport system is required for the reduction of Fe(III), thereby providing a pool of ferrous iron (Fe II) for protoheme and heme a synthesis.

SWINE, late in the course of copper deficiency, develop severe microcytic–hypochromic anemia, reticulocytopenia, hyperferremia, and an increase in bone marrow sideroblasts.[1] Although this constellation of abnormalities suggests impaired hemoglobin synthesis, no abnormality has been detected in the porphyrin biosynthetic pathway or in the biosynthesis of heme from protoporphyrin and ferrous iron.[2] However, because of the presence of multiple defects in iron metabolism in other tissues,[3] we have suggested that the defect within normoblasts could affect a biochemical reaction involving the movement of iron from transferrin to hemoglobin.[1] Such a defect might not be detected when heme synthesis from ferrous iron (Fe II) and protoporphyrin is measured in hemolysates of copper-deficient reticulocytes.

The studies reported herein were designed to study the intracellular movement of iron from transferrin to heme. Heme synthesis from ferric iron (Fe III) and protoporphyrin by isolated normal and copper-deficient mitochondria was studied to elucidate the nature of the intracellular defect in iron metabolism.

MATERIALS AND METHODS

Copper-deficient, iron-replete swine were raised as described previously.[3] Plasma iron and copper were measured by atomic absorption spectroscopy[4] as were mitochondrial iron and copper after wet digestion.[1] Protein was measured by the method of Lowry et al.[5] Cytochrome oxidase activity was assayed by the method of Wharton and Tzagoloff.[6] Heme A was extracted in acidified acetone and quantitated by difference spectroscopy.[7] Heme was crystallized twice by a modification of the method of Shemin et al.[2,8]

Iron Uptake by Reticulocytes

Sufficient ferrous sulfate was added to a large pool of normal porcine plasma to produce a transferrin saturation of 50%. The pool was stored in frozen aliquots. Radioactive iron ($^{59}FeCl_3$,

From the Department of Medicine, University of Utah College of Medicine, Salt Lake City, Utah.

Submitted June 17, 1975; accepted February 18, 1976.

Supported by Research Grant AM-04495 and Graduate Training Grant 2A-5098 from the National Institutes of Health.

Address for reprint requests: D. M. Williams, M.D., University Hospital, 50 North Medical Drive, Salt Lake City, Utah 81432.

Table 1. Heme Synthesis by Isolated, Intact Mitochondria as Measured by the Radioiron Method and by the Heme Chromogen Method

Conditions	Radioiron Method (nmoles/mg protein/hr)	Heme Chromogen Method (nmoles/mg protein/hr)
Copper deficient	0.0	0.0
	0.0	0.0
	0.3	0.4
	0.7	1.1
	0.8	0.5
	0.8	0.6
Copper deficient + succinate (4 mM)	1.0	0.7
	1.8	1.7
	1.8	1.7
Control + succinate (4 mM)	1.5	1.6
Mean	0.87	0.83

10 μCi/ml; New England Nuclear, Boston, Mass.) was added to an aliquot of this pool and incubated at 37°C for exactly 30 min.

Five milliliters of packed red cells were separated from heparinized whole blood by centrifugation, washed once with 0.15 *M* NaCl at 4°C, and resuspended in 5.0 ml of the unlabeled pooled plasma. Suspensions of cells prepared from one to three copper-deficient animals and a littermate control were incubated simultaneously at 37°C for 15 min, whereupon 2.0 ml of labeled plasma were added. The incubation was continued for 2 hr in an aerobic atmosphere. The reaction was stopped by transfer of 5.0 ml of the suspension to 150 ml of 3:1 acetone:glacial acetic acid mixture containing 0.5% $SnCl_2$, from which hemin was subsequently recovered. An additional 5.0 ml of suspension was transferred to 0.15 *M* NaCl at 4°C, and the erythrocytes were washed four times with 0.15 *M* NaCl, hemolyzed with distilled water, and transferred quantitatively to counting vials. The radioactivity contained therein was assayed in a Nuclear Chicago C120 automatic gamma counter. The quantity of iron taken up by the cells and the quantity of heme synthesized were measured and expressed in terms of the numbers of reticulocytes in the suspension, as calculated from the reticulocyte count and mean corpuscular hemoglobin of the original blood sample and of the hemoglobin concentration of the washed suspension.

Antimycin and Cyanide Effects on Iron Uptake by Reticulocyte Hemolysates

Heparinized blood was obtained from normal animals with reticulocytosis induced by phlebotomy. A reticulocyte-rich fraction of cells was obtained by centrifugation, washed twice with 0.15 *M* NaCl, mixed with an equal volume of deionized water and hemolyzed by sonication. A solution of sodium cyanide, antimycin A, or an equal volume of water was added to 4.0 ml of hemolysate. Radioactive $FeCl_3$ (100 nmoles) and protoporphyrin (1 μmole) were added and the volume adjusted to 5.0 ml. The reaction flask was flushed with nitrogen for 30 sec, capped and incubated for 2 hr at 37°C. Heme synthesis was measured as described above.

Isolation of Mitochondria

Mitochondria were isolated from the livers of exsanguinated animals by the method of Schneider.[9] The mitochondrial pellet was resuspended in 0.25 *M* sucrose and centrifuged at 300 *g* for 10 min; the sediment was discarded. Mitochondria were stored at 4°C and used within 2 hr.

Mitochondrial Heme Synthesis

Mitochondria (3–5 mg protein/ml) were incubated in a total volume of 3.0 ml containing $^{59}FeCl_3$ (0.033 m*M*), protoporphyrin (0.167 m*M*), KH_2PO_4 (8 m*M*), and Tris-HCl (8 m*M*), pH 8.0. Other reagents were added as noted elsewhere. Reaction vessels were flushed with nitrogen, capped, and incubated for 2 hr in the dark at 37°C. The reaction was stopped by adding the contents to 150 ml of acetone:acetic acid containing 0.5% $SnCl_2$.

Table 2. Iron Uptake and Heme Synthesis by Normal and Copper-deficient Reticulocytes

Determination	Normal	Copper Deficient	
		Early	Late
Number of animals	19	19	9
Volume of packed red cells (ml/100 ml)	42 ± 0.7	35 ± 0.7	25 ± 2.6
Reticulocytes (× 10^6/ml)	245 ± 28.8	200 ± 20.1	90 ± 14.3
Serum iron (μg/100 ml)	161 ± 6.4	82 ± 4.5	194 ± 20.0
Serum copper (μg/100 ml)	175 ± 6.0	9 ± 1.1	17 ± 2.0
Iron uptake (nmoles/10^9 retics/2 hr)	6.7 ± 0.55	6.5 ± 0.52	3.5 ± 0.43
Heme synthesis (nmoles/10^9 retics/2 hr)	4.2 ± 0.44	2.3 ± 0.26	1.4 ± 0.20

Values refer to mean ± 1 SE.

To verify the validity of the radioiron method for measuring heme synthesis, the amount of heme synthesized was measured directly by the pyridine hemochromogen method of Porra and Jones.[10] In ten paired determinations (Table 1), the correlation coefficient was +0.9.

Mitochondrial Respiration

Standard manometric techniques[11] were used to measure mitochondrial oxygen consumption at 30°C. The reaction mixture contained mitochondria (3–5 mg protein/ml), KH_2PO_4 (0.0133 *M*), cytochrome C (0.01 m*M*, horse heart, type III; Sigma, St. Louis, Mo.), K succinate (10 m*M*), K-ATP (2 m*M*), $MgSO_4$ (0.5 m*M*), glucose (16.6 m*M*), and hexokinase (0.008 U/ml, yeast, type C300; Sigma) in a total volume of 3.0 ml at pH 7.4. Glucose and hexokinase were added from a side arm after 10 min of temperature equilibration.

RESULTS

Iron Uptake and Heme Synthesis by Intact Normal and Copper-deficient Reticulocytes

Early in the course of the deficiency, the cellular uptake of iron was normal, although heme synthesis was impaired (Table 2). Late in the course of the deficiency, the cellular uptake of iron by copper-deficient reticulocytes was 52% of that of normal cells, and the amount of heme synthesized was 33% of normal. Thus, both heme synthesis and cellular iron uptake were decreased in copper-deficient reticulocytes as compared with normal, and heme synthesis was impaired to a greater extent than was the cellular uptake of iron.

Inhibition of Heme Synthesis in Hemolysates of Normal Reticulocytes

Heme synthesis in hemolysates of phlebotomy-induced reticulocytes was inhibited completely by concentrations of sodium cyanide greater than 8 μmoles/10^9 reticulocytes. Antimycin A in concentrations greater than 1 mmole/10^9 reticulocytes inhibited heme synthesis by 90%.

Heme Synthesis by Isolated Normal Hepatic Mitochondria

Heme synthesis by isolated, normal hepatic mitochondria was studied to define the optimal conditions for heme synthesis from Fe(III) and protoporphyrin and to determine the role of the electron transport system in this pathway (Table 3).

Table 3. Influence of Isocitrate, Succinate, Malonate, and Rotenone on Heme Synthesis From Fe(III) and Protoporphyrin by Isolated Intact Normal Hepatic Mitochondria

Additive	Determinations	Heme Synthesis (nmoles/mg protein/hr)
None	18	0.4 ± 0.08
Isocitrate (4 mM)	4	1.6 ± 0.64
Isocitrate (4 mM) + rotenone (0.17 mM)	3	0.7
Succinate (4 mM)	18	2.0 ± 0.14
Succinate (4 mM) + malonate (12 mM)	7	0.7 ± 0.16

Values refer to mean ± 1 SE.

The rate of heme synthesis from Fe(III) and protoporphyrin was negligible in the absence of substrates for the electron transport system. The addition of either succinate or isocitrate enhanced heme synthesis. Heme synthesis in the presence of these substrates was as great from Fe(III) (2.0 ± 0.6 nmoles/mg protein/hr) as from Fe(II) (2.1 ± 0.6 nmoles). Malonate (12 m*M*) inhibited succinate stimulation of heme synthesis from Fe(III) by 65% and rotenone (0.17 m*M*) inhibited isocitrate stimulation by 58%. Antimycin A inhibited heme synthesis from Fe(III) and protoporphyrin (Fig. 1). Sodium azide in low concentrations partially inhibited oxygen utilization without decreasing heme synthesis (Table 4). At higher concentrations both respiration and heme synthesis were inhibited by azide. Sodium cyanide inhibited both respiration and heme synthesis.

Heme Synthesis by Isolated Copper-deficient Hepatic Mitochondria

The rate of heme synthesis from Fe(III) and protoporphyrin, in the presence of succinate, was decreased in isolated copper-deficient hepatic mitochondria ($N = 20$, $p < 0.01$) (Table 5). Mitochondria from copper-deficient animals were severely depleted of copper and consumed less oxygen than normal mitochondria. The cytochrome oxidase activity and heme A content were decreased, and the rate of heme synthesis correlated with the cytochrome oxidase activity ($N = 12$; $r = +0.78$; $p < 0.001$).

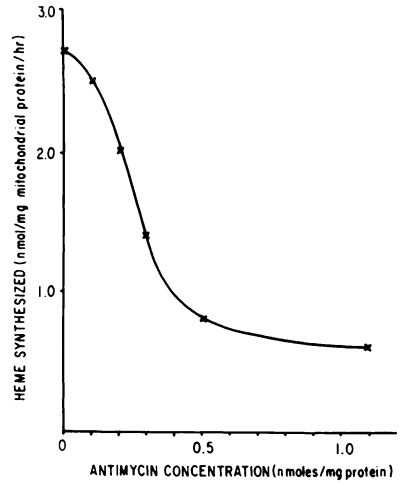

Fig. 1. Effect of antimycin A concentration on heme synthesis from Fe(III) and protoporphyrin by isolated intact normal hepatic mitochondria. Succinate (4 mM) was added as substrate.

Table 4. Azide and Cyanide Inhibition of Oxygen Consumption and Heme Synthesis in Isolated Normal Hepatic Mitochondria

Inhibitor (μmoles/mg)	Per Cent Inhibition	
	Oxygen Consumption	Heme Synthesis*
Sodium azide		
1	60	0
2	67	20
3	70	25
10	75	52
Sodium cyanide		
1	98	60
2	99	72
3	100	80
10	100	100

*From Fe(III) and protoporphyrin in the presence of 4 mM succinate.

DISCUSSION

In studies previously reported, we observed that the synthesis of heme from ^{14}C-glycine, ^{14}C-aminolevulinic acid, and ^{59}Fe and protoporphyrin by hemolysates of copper-deficient reticulocytes is either normal or increased.[2] In these studies Fe(II) was used as a substrate in the reactions. Our present studies indicate that the cellular uptake of iron from Fe(III)-transferrin and the synthesis of heme by intact copper-deficient reticulocytes is impaired. Thus, there is an abnormality in the pathway from Fe(III)-transferrin to heme in such cells, and this abnormality would appear to account for the defect in the heme biosynthetic pathway that we have observed in vivo.[1] This observation is in keeping with the studies of Goodman and Dallman.[12] These workers observed that the uptake of iron by mitochondria of erythroid cells is impaired in copper-deficient rats.

The validity of traditional methods for labeling transferrin in vitro with iron salts has been questioned recently.[13] However, in our experiments, normal and copper-deficient reticulocytes were incubated simultaneously with the same pool of labeled plasma, and the method of labeling the transferrin could not explain the differences in cellular iron uptake and heme synthesis observed between normal and copper-deficient reticulocytes.

Table 5. Characteristics of Normal and Copper-deficient Mitochondria

Determination	Normal	Copper Deficient
Heme synthesis* (nmoles/mg protein/hr)	2.6 ± 0.19	1.3 ± 0.10
Cytochrome oxidase (nmoles cyt C oxidized/mg protein/min)	244 ± 14.5	72 ± 13.8
Heme A (nmoles/mg protein)	0.28 ± 0.026	0.07 ± 0.010
O_2 consumption (μl O_2/mg protein/hr)	2.4 ± 0.18	1.8 ± 0.20
Copper (ng/mg protein)	61 ± 30.1	4 ± 0.9
Iron (μg/mg protein)	1.2 ± 0.28	4.9 ± 0.92

Values represent mean ± 1 SE.

*From Fe(III) and protoporphyrin in the presence of 4 mM succinate.

Heme synthesis from Fe(III) and protoporphyrin by isolated normal hepatic mitochondria was enhanced by succinate and isocitrate and inhibited by malonate, rotenone, antimycin A, azide, and cyanide. These studies may be interpreted as evidence that the electron transport system is required for heme synthesis because these substrates and inhibitors acted as would be expected in the modification of respiration. Unfortunately, this interpretation is complicated by the iron-chelating potential of succinate, isocitrate, antimycin A, cyanide, and azide. However, it seems unlikely that the action of these agents can be explained solely on the basis of iron chelation. This effect would not explain the stimulation of heme synthesis by succinate and its inhibition by malonate. Both are weak iron chelators. Nor would it explain the stimulation of heme synthesis by isocitrate and its inhibition by rotenone. The latter is not an iron chelator. Furthermore, antimycin A, cyanide, and azide inhibited respiration to a greater extent than heme synthesis. However, the mechanisms of action of these substrates and inhibitors of mitochondrial respiration have not been defined by our experiments.

Isolated hepatic mitochondria from copper-deficient animals were unable to synthesize heme at the normal rate when Fe(III) was supplied as substrate. The concentration of copper in these mitochondria was reduced by 93%. The mitochondria also consumed less oxygen than normal, and they were severely depleted of cytochrome oxidase as indicated by a 70% reduction in enzymatic activity and a 75% reduction in heme A concentration. This finding is not surprising since cytochrome oxidase contains two atoms of copper per molecule, and the copper is essential for the enzymatic activity of the protein.[14] A decrease in cytochrome oxidase activity and content in experimental dietary copper deficiency has been demonstrated many times.[15-17]

The function of cytochrome oxidase in iron metabolism may be best understood by first reviewing recent information on the role of the electron transfer system in intracellular iron metabolism.

The final step in the heme biosynthetic pathway, the incorporation of iron into protoporphyrin, is catalyzed by the mitochondrial enzyme, heme synthetase (ferrochelatase, protoheme ferro-lyase EC 4.99.1.1).[10,18] The substrate for this enzyme is Fe(II), not Fe(III), and an anaerobic environment is required.[10,18] Since transferrin-bound iron is in an oxidized state,[19] the requirement for an iron-reducing system is apparent.

It has been recognized for some time that as the reticulocyte matures and loses its mitochondria, the cell is unable to take up iron from transferrin.[20] Only recently has the role of mitochondria in the uptake, accumulation, and metabolism of iron come to be appreciated.[21] Morgan et al.[22,23] have shown that the rate of release of iron from transferrin and its uptake by reticulocytes is dependent upon an active electron transport system. Barnes et al.[24,25] have studied the reduction of Fe(III) and the incorporation of the resulting Fe(II) into heme by mitochondria and have concluded that the reduction depends upon the electron transport system. The accumulation of Fe(III) by isolated rat liver and reticulocyte mitochondria has been studied in detail by Romslo and Flatmark.[26-32] These workers have found that Fe(III) accumulates by both an energy-dependent (cyanide-sensitive) and an energy-independent mechanism. In

the energized accumulation of iron by mitochondria, Fe(III) is bound to the cytosolic side of the inner mitochondrial membrane. Reducing equivalents are supplied by the electron transfer system and Fe(II) then crosses the inner membrane. The Fe(II) that reaches the matrix is not reoxidized and is available for the biosynthesis of heme.

The step at which the electron transport system reduces iron is uncertain. Barnes et al.[24] have suggested that the reduction of Fe(III) occurs on the matrix side of the inner mitochondrial membrane, possibly at the flavoprotein, succinic dehydrogenase, or NADH dehydrogenase site. Flatmark and Romslo[32] have proposed that the Fe(III) ligands on the cytosolic side of the inner mitochondrial membrane give the metal a half-reduction potential that is sufficiently high to establish an oxidation–reduction equilibrium with the respiratory chain at the level of cytochrome C. In our studies, heme synthesis from Fe(III) is impaired in mitochondria deficient in cytochrome oxidase, and the rate of heme synthesis correlates with the cytochrome oxidase activity. Since copper-deficient mitochondria are not deficient in cytochrome C,[17] it is tempting to suggest that electrons are donated to Fe(III) directly from cytochrome oxidase. The cytochrome A component of cytochrome oxidase is located on the cytosolic side of the inner mitochondrial membrane near cytochrome C. However, the effect of cytochrome oxidase deficiency on the rate of heme synthesis may be due to the lowering of the specific respiratory rate of these mitochondria. In any event, it is apparent that an active and intact electron transport system is required for the cellular uptake of iron, the reduction of Fe(III) on the inner mitochondrial membrane, and for a supply of Fe(II) as substrate for the heme synthetase reaction.

Heme A, the prosthetic group of cytochrome oxidase, is an iron–porphyrin compound.[33] The decrease in heme A content observed by ourselves and others[16,17,34] in copper-deficient tissues is not readily explained by failure to synthesize the holoenzyme. This finding has led Lemberg et al.[34] to postulate that copper is also essential for the synthesis of heme A. The reduced concentration of heme A can now be explained by the role of the electron transport system in the reduction of Fe(III). It would be anticipated that the supply of Fe(II) for the synthesis of heme A would be impaired as it is for the synthesis of protoheme (Fig. 2).

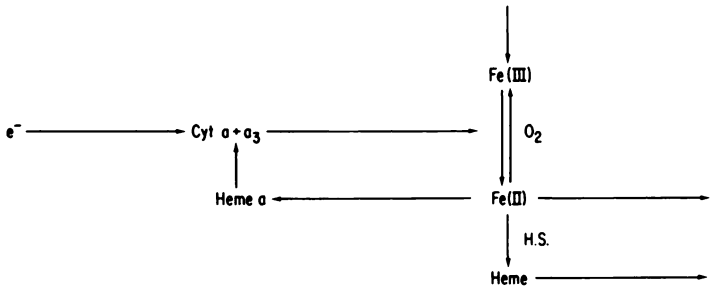

Fig. 2. Diagrammatic representation of the proposed scheme for iron reduction. Cyt a + a_3 refers to cytochrome oxidase. H.S. refers to heme synthetase.

Four defects in iron metabolism have now been delineated in copper-deficient swine: (1) impaired synthesis of heme from Fe(III) and protoporphyrin; (2) impaired mobilization of iron from reticuloendothelial cells to transferrin; (3) impaired mobilization of iron from hepatic parenchymal cells to transferrin; and (4) impaired absorption of iron from the gastrointestinal tract.[1] We propose that the first of these defects is due to a deficiency of cytochrome oxidase. The second and third defects are due to a deficiency of ceruloplasmin.[3] The nature of the defect in the mucosal cell has not yet been defined, but it could be due to a deficiency of both cytochrome oxidase and ceruloplasmin. Thus, it is apparent that at least two copper proteins, cytochrome oxidase and ceruloplasmin, are involved in the movement of iron.

ACKNOWLEDGMENT

We are indebted to Miss Jacqueline Thomas, Mrs. Alice Tustison, Mr. George Trappett, and Mr. Dale Chlarson for technical assistance. Heparin was the gift of the Upjohn Co., Kalamazoo, Mich. Iron dextrin (Pigdex) was kindly supplied by the American Cyanamid Co., Princeton, N.J.

REFERENCES

1. Lee GR, Nacht S, Lukens J, Cartwright GE: Iron metabolism in copper-deficient swine. J Clin Invest 47:2058–2069, 1968

2. Lee GR, Cartwright GE, Wintrobe MM: Heme biosynthesis in copper-deficient swine. Proc Soc Exp Biol Med 127:977–981, 1968

3. Roeser HP, Lee GR, Nacht S, Cartwright GE: The role of ceruloplasmin in iron metabolism. J Clin Invest 49:2408–2417, 1970

4. Trinder P: The improved determination of iron in serum. J Clin Pathol 9:170–172, 1956

5. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951

6. Wharton DC, Tzagoloff A: Cytochrome oxidase in beef heart mitochondria, in Estabrook RW, Pullman ME (eds): Methods in Enzymology (vol 10). New York, Academic, 1967, p 245

7. Rieske JS: The quantitative determination of mitochondrial hemoproteins, in Estabrook RW, Pullman ME (eds): Methods in Enzymology (vol 10). New York, Academic, 1967, p 488

8. Shemin D, London IM, Rittenberg D: The synthesis of protoporphyrin in vitro by red blood cells of the duck. J Biol Chem 183:757–765, 1950

9. Schneider WC: Methods for the isolation of particulate components of the cell, in Umbreit WW, Burris RH, Stauffer JF (eds): Manometric Techniques (ed 4). Minneapolis, Burgess, 1964, p 177

10. Porra RJ, Jones OTG: Studies on ferrochelatase. I. Assay and properties of ferrochelatase from pig liver mitochondria extract. Biochem J 87:181–185, 1963

11. Potter VR: The homogenate technique, in Umbreit WW, Burris RH, Stauffer JF (eds): Manometric Techniques (ed 4). Minneapolis, Burgess, 1964, p 159

12. Goodman JR, Dallman PR: Role of copper in iron localization in developing erythrocytes. Blood 34:747–753, 1969

13. Bates GW, Schlabach MR: The reaction of ferric salts with transferrin. J Biol Chem 248:3228–3232, 1973

14. Beinert H: Cytochrome C oxidase: Present knowledge of the state and function of its copper components, in Peisach J, Aisen P, Blumberg WE (eds): The Biochemistry of Copper, Proceedings of the Symposium on Copper in Biological Systems. New York, Academic, 1965, p 213

15. Schultze MO: The effect of deficiencies in copper and iron on the cytochrome oxidase of rat tissues. J Biol Chem 129:729–737, 1939

16. Gallagher CH, Judah JD, Rees KR: The biochemistry of copper deficiency. I. Enzymological disturbances, blood chemistry and excretion of amino acids. Proc R Soc B 145: 134–150, 1956

17. Gubler CJ, Cartwright GE, Wintrobe MM: Studies on copper metabolism. XX. Enzyme activities and iron metabolism in copper and iron deficiencies. J Biol Chem 224:533–546, 1957

18. Jones MS, Jones OTG: The structural or-

ganization of haem synthesis in rat liver mitochondria. Biochem J 113:507–514, 1969

19. Gaber BP, Aisen P: Is divalent iron bound to transferrin? Biochim Biophys Acta 221:228–233, 1970

20. Jandl JH, Inman JK, Simmons RL, Allen DW: Transfer of iron from serum iron-binding protein to human reticulocytes. J Clin Invest 38:161–185, 1959

21. Borová J, Poňka P, Neuwirt J: Study of intracellular iron distribution in rabbit reticulocytes with normal and inhibited heme synthesis. Biochim Biophys Acta 320:143–156, 1973

22. Morgan EH, Baker E: The effect of metabolic inhibitors on transferrin and iron uptake and transferrin release from reticulocytes. Biochim Biophys Acta 184:442–454, 1969

23. Kailis SG, Morgan EH: Transferrin and iron uptake by rabbit bone marrow cells in vitro. Br J Haematol 28:37–52, 1974

24. Barnes R, Connelly JL, Jones OTG: The utilization of iron and its complexes by mammalian mitochondria. Biochem J 128:1043–1055, 1972

25. Barnes R, Jones OTG: The availability of iron for haem synthesis in red blood cells. Biochim Biophys Acta 304:304–308, 1973

26. Romslo I, Flatmark T: Energy-dependent accumulation of iron by isolated rat liver mitochondria. I. General features. Biochim Biophys Acta 305:29–40, 1973

27. Romslo I, Flatmark T: Energy-dependent accumulation of iron by isolated rat liver mitochondria. II. Relationship to the active transport of Ca^{2+}. Biochim Biophys Acta 325: 38–46, 1973

28. Romslo I, Flatmark T: Energy-dependent accumulation of iron by isolated rat liver mitochondria. III. Submitochondrial localization of the iron accumulated. Biochim Biophys Acta 347:160–167, 1974

29. Romslo I: Energy-dependent accumulation of iron by isolated rabbit reticulocyte mitochondria. Biochim Biophys Acta 357:34–72, 1974

30. Romslo I: Energy-dependent accumulation of iron by isolated rat liver mitochondria. IV. Relationship to the energy state of the mitochondria. Biochim Biophys Acta 387:69–79, 1975

31. Romslo I, Flatmark T: Energy-dependent accumulation of iron by isolated rat liver mitochondria. V. Effect on factors controlling respiration and oxidative phosphorylation. Biochim Biophys Acta 387:80–94, 1975

32. Flatmark T, Romslo I: Energy-dependent accumulation of iron by isolated rat liver mitochondria. J Biol Chem 250:6433–6438, 1975

33. Caughey WS, Smythe GA, O'Keeffe DH, Maskasky JE, Smith ML: Heme A of cytochrome *c* oxidase. Structure and properties: Comparison with hemes B, C, and S and derivatives. J Biol Chem 250:7602–7622, 1975

34. Lemberg R, Newton N, Clarke L: Decrease in haem A content in the heart muscle of copper-deficient swine. Aust J Exp Biol Med Sci 40:367–372, 1962

1976 48: 77-85

Role of copper in mitochondrial iron metabolism

DM Williams, D Loukopoulos, GR Lee and GE Cartwright

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.