

Role of Ceruloplasmin (Plasma Ferroxidase) in Hypoferremia Associated with Chronic Inflammation and Endotoxin¹ (37197)

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Hypoferremia develops in the anemia of chronic disorders (1) and following the administration of bacterial endotoxin (2), as a consequence of impaired flow of iron from reticuloendothelial cells to plasma. A similar type of kinetic abnormality in iron metabolism, which is corrected by the administration of ceruloplasmin, has been observed in copper-deficient swine (3, 4). The *in vitro* iron-oxidizing activity (ferroxidase activity) of ceruloplasmin has been studied extensively (5-7), and it has been proposed that *in vivo* ceruloplasmin enhances the movement of iron from reticuloendothelial cells to transferrin by oxidation of iron on cell surfaces, thereby releasing the iron to transferrin (4).

Although it is recognized that the ceruloplasmin concentration is increased in the plasma of patients with the anemia of chronic disorders (1), studies have not been reported on the ferroxidase activity of the protein under such circumstances. Therefore, it seemed pertinent to investigate the ferroxidase activity of ceruloplasmin in this disorder and particularly to inquire if inhibition of the ferroxidase function of ceruloplasmin could account for the hypoferremia. Studies on plasma ferroxidase activity in patients with hypoferremia associated with the anemia of chronic disorders (rheumatoid arthritis) in man and in the plasma of swine following endotoxin-induced hypoferremia are reported in this paper.

Materials and Methods. Plasma ferroxidase activity was measured by a modification of the method of Osaki *et al.* (5, 8). Three milliliters of incubation mixture contained 1 ml of appropriately diluted (1:10 to 1:100)

plasma, apotransferrin (30 μM), ferrous ammonium sulfate (30 μM), and ascorbic acid (300 μM) in 0.0133 *M* sodium phosphate buffer, pH 6.7. After the addition of the iron-ascorbate solution, the rate of increase in absorbance at a wavelength of 460 *m μ* was measured at room temperature in a model 15 Cary recording spectrophotometer. The determined values were corrected for the nonenzymatic rate of transferrin formation, which was measured in the presence of all of the above reagents except plasma. The nonceruloplasmin ferroxidase was measured in the presence of 100 μM sodium azide (8). The ceruloplasmin ferroxidase activity was calculated by subtracting the nonceruloplasmin ferroxidase activity from the total plasma ferroxidase activity. Plasma copper was determined, after deproteinization with 2 *N* HCl and 20% trichloroacetic acid, with oxalyldihydrazide as the colorimetric reagent (9). Plasma iron was measured by a method described elsewhere (10). The method of Ravin (11) was used for the measurement of para-phenylenediamine (*p*PD) oxidase activity; the pH of the final reaction mixture was 5.5. Porcine ceruloplasmin was prepared by chromatography on DEAE Sephadex columns (12). The material was greater than 70% pure as estimated by absorbance characteristics at 610 and 280 *m μ* .

The method for inducing copper deficiency in swine (3) has been published. Acute hypoferremia was induced in swine by the intravenous administration of bacterial endotoxin (lipopolysaccharide of *E. coli*, Difco, 0.55:B5), 2 $\mu g/kg$ of body wt.

Results. *Plasma ferroxidase activity in rheumatoid arthritis.* Plasma iron, copper, para-phenylenediamine (*p*PD) oxidase activity, total plasma ferroxidase activity, cerulo-

¹ Supported by a Research Grant (AM-04489) and a Training Grant (AM-5098) from the National Institutes of Health, Bethesda, Maryland.

TABLE I. Plasma Ferroxidase in Normal Subjects and in Patients with Rheumatoid Arthritis.

Determination	Normal subjects	Rheumatoid arthritis
Number	30	11
Plasma iron ($\mu\text{g}/100\text{ ml}$)	108 \pm 25*	42 \pm 33
Plasma copper ($\mu\text{g}/100\text{ ml}$)	117 \pm 20	153 \pm 25
pPD oxidase ($A, 530\text{ m}\mu$)	0.31 \pm 0.05	0.41 \pm 0.05
Total ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	53 \pm 7	76 \pm 9
Ceruloplasmin ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	36 \pm 8	60 \pm 9
Nonceruloplasmin ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	17 \pm 5	16 \pm 5

* Values refer to mean \pm 1 SD.

plasmin ferroxidase activity, and nonceruloplasmin ferroxidase activity were measured in 30 normal subjects and in 11 patients with rheumatoid arthritis (Table I). There was a decrease in plasma iron and an increase in copper, pPD oxidase, total ferroxidase, and ceruloplasmin ferroxidase activity in the plasma of the patients as compared with normal subjects. The ceruloplasmin ferroxidase activity increased to a greater degree than did the plasma copper or the pPD oxidase activity.

Plasma ferroxidase activity during endotoxin-induced hypoferrremia in swine. The administration of bacterial endotoxin to swine was followed by a reduction in the plasma iron (Fig. 1). The plasma iron level reached minimum values 6–9 hr after the administration of the endotoxin and then returned to normal over the next 10 hr. There was no significant change in plasma copper, ceruloplasmin ferroxidase activity, or nonceruloplasmin ferroxidase activity during this 18-hr period. In contrast to the other measures of ceruloplasmin, pPD oxidase activity decreased and increased in parallel with the plasma iron.

This discrepancy between pPD oxidase activity and other measures of ceruloplasmin was studied further. Since iron is an intermediate in pPD oxidation (6, 7), it seemed possible that alterations in plasma iron concentration could have an effect on the assay. Accordingly, pPD oxidase was measured in the usual manner and in the presence of EDTA (0.004 mM final concentration) in order to eliminate the effects of iron. With EDTA present, the change in pPD oxidase paralleled plasma copper and ceruloplasmin

ferroxidase curves and not the plasma iron curve (Fig. 2).

Influence of ceruloplasmin on endotoxin-induced hypoferrremia in swine. Porcine ceruloplasmin was administered to a pig to determine if additional ceruloplasmin would prevent the development of endotoxin-induced hypoferrremia. The endotoxin and ceruloplasmin were given at zero time. The amount of ceruloplasmin given was sufficient to increase the plasma copper from 204 to 305 $\mu\text{g}/100\text{ ml}$, the pPD oxidase from 0.46 to 0.92, and the ceruloplasmin ferroxidase from 122 to 290 $\mu\text{mole}/\text{ml}/\text{hr}$. Ceruloplasmin did not prevent the development of hypoferrremia (Table II).

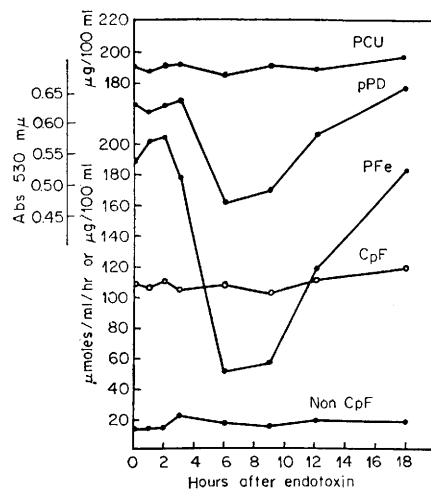


FIG. 1. Alterations in plasma iron (PFe), plasma copper (PCu), paraphenylenediamine (pPD) oxidase activity, ceruloplasmin ferroxidase activity (CpF), and nonceruloplasmin ferroxidase activity (non CpF) in the plasma of a pig following the administration of bacterial endotoxin.

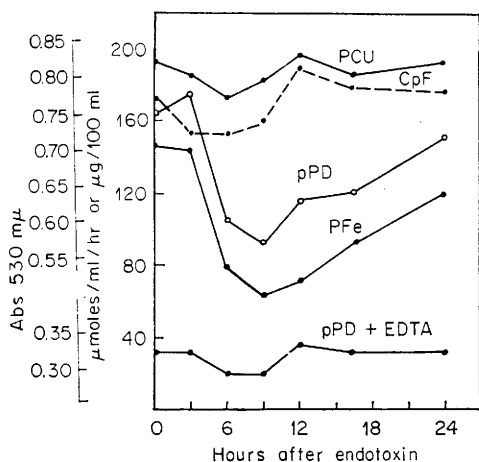


FIG. 2. Alterations in plasma iron (PFe), plasma copper (PCu), paraphenylenediamine (pPD) oxidase activity, paraphenylenediamine oxidase activity in the presence of ethylenediaminetetraacetate (pPD-EDTA), and ceruloplasmin ferroxidase activity (CpF) in the plasma of a pig following the administration of bacterial endotoxin.

Influence of endotoxin on the plasma iron response to ceruloplasmin in copper deficient swine. The administration of ceruloplasmin to copper-deficient pigs is followed by a prompt increase in the plasma iron concentration (4). Previous studies (4) have indicated that the ceruloplasmin functions at the cell-plasma interface rather than intracellularly. The influence of endotoxin on the plasma iron response to ceruloplasmin was investigated in a copper-deficient pig. Six hours after endotoxin injection, at which time the plasma iron concentration was decreased, ceruloplasmin was given iv, and the increase in plasma iron was compared with the increase observed following the administration of the same amount of ceruloplasmin to a copper-deficient pig not given endotoxin.

The ceruloplasmin-induced increase in plasma iron was greatly impaired in the copper-deficient, endotoxin treated animal compared with the copper-deficient animal not given endotoxin (Fig. 3).

Discussion. The mechanism by which the flow of iron from reticuloendothelial cells to transferrin is impaired (RE iron block) in the anemia of chronic disorders is unknown. This could be due to (1) a defect in the intracellular movement of iron to the membrane-bound iron pool, (2) interference with the access of ceruloplasmin to the membrane-bound iron, or (3) to inhibition of the ferroxidase activity of ceruloplasmin. The studies reported herein indicate that the block cannot be explained by inhibition of the ferroxidase activity of ceruloplasmin. This conclusion is supported by the following observations: (1) There was an increase in ferroxidase activity in the plasma of patients with rheumatoid arthritis, (2) no inhibition of ceruloplasmin ferroxidase was observed following the administration of endotoxin to swine, and (3) administration of ceruloplasmin did not prevent endotoxin-induced hypoferrremia in swine. The ability of ceruloplasmin to increase the plasma iron in copper-deficient swine was blocked by the administration of endotoxin (Fig. 3). Although this experiment suggests that the "RE iron block" induced by endotoxin may be at a step which precedes the site of action of ceruloplasmin, it does not distinguish between the first two possibilities mentioned above.

In pigs given endotoxin, the pPD oxidase activity decreased and increased in a pattern similar to that of the plasma iron, while the plasma copper and ceruloplasmin ferroxidase activity remained unchanged (Fig. 1). This discrepancy between various measures of

TABLE II. Failure of Ceruloplasmin to Prevent Endotoxin-Induced Hypoferrremia in a Pig.

Determination	Hours after endotoxin			
	0	3	6	9
Plasma iron ($\mu\text{g}/100\text{ ml}$)	110	102	51	35
Plasma copper ($\mu\text{g}/100\text{ ml}$)	204	305	266	257
pPD oxidase ($A, 530\text{ m}\mu$)	0.46	0.92	0.70	0.67
Total ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	138	311	253	240
Ceruloplasmin ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	122	290	233	220
Nonceruloplasmin ferroxidase ($\mu\text{mole}/\text{ml}/\text{hr}$)	16	21	20	20

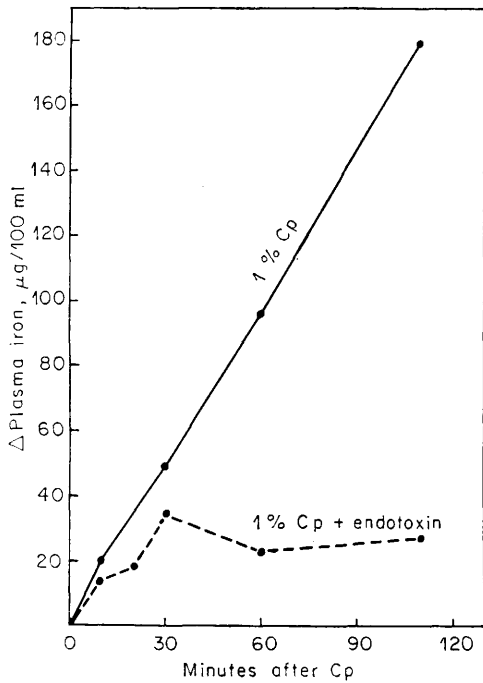


FIG. 3. Increase in plasma iron following the administration of ceruloplasmin (Cp) to a copper deficient pig as compared with a copper deficient pig given bacterial endotoxin. The dose of ceruloplasmin was 1% of the total amount in the circulation of a normal animal.

ceruloplasmin may be explained by the observation of others (6, 7) that *p*PD is oxidized not only by ceruloplasmin but also by ferric iron generated via the ferrous iron-ceruloplasmin coupled reaction. The measurements of *p*PD oxidation were performed at pH 5.5, a pH at which there is dissociation of iron from transferrin. Under such circumstances, *p*PD oxidation is a function not only of the ceruloplasmin concentration but also of the concentration of ferric iron. The available ferric iron is a function not only of the amount of iron present but also of the rate of regeneration of ferric iron by ceruloplas-

min. Additional evidence that this is the case is the observation that *p*PD oxidase activity of pig plasma in the presence of EDTA was decreased and that it paralleled the plasma copper and ceruloplasmin ferroxidase activity (Fig. 2).

Summary. Impaired reticuloendothelial outflow was studied in patients with rheumatoid arthritis and in swine given endotoxin. In both of these situations, ceruloplasmin and ceruloplasmin ferroxidase activity either remained the same or increased. Administration of ceruloplasmin did not prevent or overcome the endotoxin-induced block. Thus, the "RE iron block" of the anemia of chronic disease cannot be explained by inhibition of the ceruloplasmin dependent step in iron metabolism.

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Received May 25, 1972. P.S.E.B.M., 1973, Vol. 142.