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# The valency state of absorbed iron appearing in the portal blood and ceruloplasmin substitution

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**Summary.** (1) Attempts to determine the redox-state of the absorbed iron, which appeared in the portal blood when the free iron-binding capacity was previously saturated, indicate that about 30–90% of this iron was in the ferrous state. This effect was particularly prominent after luminal administration of ferrous iron, but was also seen when iron was given in the ferric state. (2) Total iron absorption is significantly higher in ceruloplasmin-substituted copper-deficient animals as compared to copper-deficient controls. (3) The appearance rate of absorbed iron in the portal blood of copper-deficient animals increased several times immediately after the intravenous infusion of ceruloplasmin. (5) The distribution of absorbed iron was changed due to the ceruloplasmin substitution: it was increased in the reticulocytes (+66%), plasma (+400%) and the body (+112%), whereas in the liver it was decreased by about 78%. (5) In iron-deficient rats intravenously injected ceruloplasmin did not increase iron absorption. (6) The conclusion was drawn that, as for the entrance into the mucosa from the luminal side, also for the release at the contraluminal side into the portal blood, the ferrous state of iron is favoured and that ceruloplasmin accelerates the release into the portal blood by catalyzing the oxidation of ferrous iron due to its high Fe(II):oxygen oxidoreductase (EC 1.16.3.1) activity.

**Key words:** Ceruloplasmin - Iron-absorption - Rat small intestine - Copper-deficiency - Portal blood

## Introduction

In a previous publication, it was shown that reduction of ferric iron is a pererequisite for iron uptake into the intestinal mucosa of the rat (Wollenberg and Rummel 1987). Inside the mucosal cell, iron is, at least in part, converted into its ferric form and is either stored in mucosal ferritin or is bound to mucosal transferrin. The latter is supposed to constitute a transport compartment from which iron is released into the body (Huebers et al. 1971; for additional references see Huebers and Rummel 1984).

In contrast to mucosal uptake, little attention has been paid to the mechanism of iron release from the mucosal cells at the contraluminal side of the epithelium, although its control is probably of pivotal importance in the regulation of iron absorption. The involvement of copper in the absorptive mechanism of iron was postulated, since copper deficiency has been shown to impair iron absorption in rats and pigs (Chase et al. 1952; Gubler et al. 1952). By analogy to the role of ceruloplasmin in the release mechanism of iron from the perfused dog liver, Osaki and Johnson (1969) hypothesized that iron which is released in the ferrous form to the contraluminal surface of the intestinal epithelium is oxidized to the ferric form by ceruloplasmin, the copper-containing ferrous iron oxidase of plasma, and bound to transferrin simultaneously. This hypothesis, however, has been questioned by others (Brittin and Chee 1969), who failed to observe an influence of changing ceruloplasmin levels on iron metabolism. Recently, Coppen and Davies (1988) reported that addition of ceruloplasmin to the vascular medium in an in vitro rat intestinal perfusion system failed to influence the iron metabolism of this particular preparation.

All these considerations are based on the hypothesis that iron must be converted to the ferrous form prior to, or during the transport out of, the mucosal cell at the contraluminal side. This hypothesis was originally put forward, based on indirect evidence, by Manis and Schachter (1964), who measured mucosal ferric and ferrous iron pools in isolated intestinal loops.

The aim of our study was to gain information about (a) the valency state of absorbed iron appearing in the portal blood, (b) the process of mucosal iron release at the contraluminal side, particularly in copper deficiency, and (c) the role of ceruloplasmin. The isolated gut loop technique of Ochsenfarth and Winne (1969) was used as an experimental model since it allows the analysis of the mesenteric venous blood directly, at the

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same time maintaining the natural arterial supply and excluding recirculation of the perfusate.

## Materials and methods

*Chemicals.* $^{59}Fe$ was purchased as ferric chloride in 0.5 M HCl (0.75 TBq × $g^{-1}$) or ferrous sulphate (0.63 TBq × $g^{-1}$) from Du Pont (NEN, Dreieich, FRG). Ficoll 70 and Sephadex G-50 were from Pharmacia Fine Chemicals. Analytical grade chemicals were from Merck (Darmstadt, FRG).

*Radioactive solution.* Acidic iron solutions or iron bound to rat albumin were used as test doses. The acidic iron solution contained 300 nmol Fe ($FeCl_3$ or $FeSO_4$) in 1 ml 1 mM HCl, 0.9% NaCl, and was labelled with $2 \times 10^6$ cpm of $^{59}Fe$. The iron-albumin solution was prepared according to Huebers et al. (1983) except that human albumin concentrate was replaced by purified rat albumin. The final solution contained 10 µg Fe × $ml^{-1}$ at pH 7.5. Human erythrocyte concentrate was generously supplied by the local blood bank.

*Animals.* Female Sprague-Dawley rats (40–60 g; Charles River, Wiga, Sulzfeld, FRG) were made copper-deficient by feeding them a low-copper diet (C 1041; Altromin, Lage-Lippe, FRG, containing 1.405 mg Cu × $kg^{-1}$; as specified by the supplier). They were used when they had a mass of 140–160 g. In these animals, plasma ceruloplasmin had dropped from 0.06 ± 0.004 unit × $ml^{-1}$ in control animals ($n = 8$) to less than 0.004 unit × $ml^{-1}$, which is the detection limit (for definition of the unit see below), and plasma copper had dropped to 155 ± 28 ng × $ml^{-1}$ ($n = 26$). Iron deficiency was induced by feeding a low-iron diet for six weeks (Diet C 1038; Altromin, Lage-Lippe, FRG, containing 6.338 mg Fe × $kg^{-1}$; as specified by the supplier). After this time, plasma iron had dropped from 1550 ± 380 to 400 ± 120 ng Fe × $ml^{-1}$ and the free iron-binding capacity was increased from 2100 ± 550 to 5200 ± 500 ng × $ml^{-1}$. Animals were starved overnight before the experiment, having free access to tap water which was rendered free of Cu and Fe ions by passage through Chelex.

*Ceruloplasmin.* Human ceruloplasmin buffered in 0.25 M NaCl, 0.05 M sodium acetate, 50 mg × $ml^{-1}$, 50–100 Curzon units × $mg^{-1}$ (= 0.065–0.13 Schosinsky units × $mg^{-1}$), copper content 2.2 mg/g ceruloplasmin protein (as specified by the supplier) was obtained from Sigma (Deisenhofen, FRG).

*Absorption experiments.* To study the body distribution of absorbed iron, animals were anaesthetized by intraperitoneal injection of ethylurethane solution (250 mg × $ml^{-1}$, 4.5 ml × $kg^{-1}$ body mass). The animals were laparotomized and 20 cm of the upper jejunum, beginning at the ligament of Treitz, was ligated. A solution of $^{59}Fe$-labelled iron was injected into these tied off jejunal loops and the abdomen was closed with clips. The animals were maintained for 1 h in a thermostatted chamber (38° C), the abdomen was reopened thereafter and the animals were exsanguinated by puncture of the abdominal aorta. Residual blood was removed from the body by two perfusions of the vascular bed with 10 ml warm saline. The exposed gut loop and liver were removed and their radioactivity counted. The gut loop was opened and the residual luminal fluid was washed out with 20 ml ice-cold buffered saline (0.9% NaCl, 1 mM phosphate pH 7.4) and collected together with the perfusion fluid.

*Kinetics of absorption.* This was studied with a modification of the *in situ* gut loop technique (Ochsenfarth and Winne 1969; Winne and Remischovsky 1971). Animals were anaesthesized as desribed above. The jugular vein was exposed and ligated twice. The cranial ligature was closed immediately and a heparinized polyethylene tube was introduced through a short longitudinal incision into the vein caudal to the ligature. The cannula was fixed by closing the second ligature around it. The animal was laparotomized along the linea alba and a proximal gut loop was selected (length 4–6 cm) with a single draining vein. This loop was spread out to the right of the animal on a thermostatted plate (38° C) which was covered with teflon foil and the loop covered with a piece of wet gauze after its ends had been ligated The gut loop was kept moist by saline solution. A cannula (18 gauge), the lock of which was removed and replaced by a short (5-cm) piece of polyethylene tubing filled with heparin solution (20 mg × $ml^{-1}$ in 0.9% NaCl), was carefully introduced into the mesenterial vein draining the loop. In our hands it was not necessary to fix the cannula in the vessel as described by Ochenfarth and Winne, because the diameter of the cannula is somewhat larger than that of the vein, thus sealing the vessel efficiently. All blood draining from the exposed loop was collected at 1-min intervals. In order to compensate for the blood loss of the animal, 1 ml heparin solution, followed by a continuous infusion of blood replacement medium, was pumped into the animal through the jugularis catheter. At time zero, 0.1 ml [$^{59}Fe$]iron solution/cm gut loop was injected into the ligated loop. The dose was adjusted to the length of the loop because the instillation of too large a volume inevitably resulted in a decrease of blood perfusion, hemorrhage and low iron absorption. The medium for the replacement of blood was prepared from washed human erythrocytes, Ficoll 70, and a modified Krebs-Ringer buffer as described earlier (Wollenberg et al. 1983). The medium was used as a substitute for whole rat blood, because results did not differ from controls obtained with whole rat blood as described in the original publication. The sacrifice of six blood-donor rats can be avoided by this modification in each experiment.

*Analytical methods.* Serum iron and copper were determined with commercial kits (Merck, Darmstadt, FRG).

*Gel-filtration.* High- and low-molecular-mass iron compounds were separated in portal plasma by applying 1 ml pooled plasma from the peak phase of absorption to a 50-cm column with an internal diameter of 0.9 cm, packed with Sephadex G-50 and equilibrated with 5 mM Tris/HCl pH 7.4. The flow rate was adjusted to 0.25 ml × $min^{-1}$. Fractions of 1 ml were collected and counted for radioactivity.

*Determination of radiolabelled ferric and ferrous iron.* Due to the experimental conditions, the redox state of the small amounts of newly absorbed radiolabelled iron has to be determined in the presence of a high excess of non-labelled iron in the portal blood. Therefore, the prerequisite for the application of direct spectroscopic methods was not fulfilled and an indirect chemical method was used to determine the amount of iron in the ferric and ferrous state. This method is a modification of the method described by Manis and Schachter (1964) and is based on the binding of the respective iron species either to citrate or 2,2′-dipyridyl. Both of these ligands have high affinities to their respective iron species. It must be emphasized that the actual ratio between the two species is conserved and remains stable after the formation of the complexes. If only one ligand was used, the change in the actual redox potential of the free ferric/ferrous iron couple would result in a complete conversion of the iron to the species (ferric or ferrous) which is sequestered by the ligand. In the presence of ligands for both species, however, any redox reaction involving unbound iron is slowed down, the rate constant being first order with respect to the free iron. Proper precautions were taken and controls were made in order to confirm that the results obtained were not seriously biased by the method. With respect to the experimental conditions, it could be assumed that an oxidation of ferrous 2,2′-dipyridyl-iron was more probable than a reduction of ferric iron citrate. Therefore the tubes in which the portal blood was collected contained an excess of ferrous iron-2,2′-dipyridyl together with the free ligands. In order to validate this procedure, trace amounts of radioactively labelled ferrous or ferric iron were added to freshly drawn rat blood and processed in the same man-

ner as the actual samples under the epxerimental conditions. No indication was found for any change in the redox state of either species. The recovery remained unchanged for at least 30 min of incubation at room temperature and pH 4 which is far in excess of the time needed for processing of the samples. Modifying the method of Manis and Schachter, the concentration of the acetate buffer was increased to 2.5 M and 0.4 g, instead of 0.2 g, Amberlite CG-400 I was used in order to improve the separation of the two iron species. Manis and Schachter were not aware that, in the presence of plasma, the selectivity for ferric iron is diminished as revealed in our control experiments. This was due to the binding of ferric iron to high-molecular-mass compounds (of about 80 kDa, not transferrin), which resulted in only 60% binding of ferric-citrate iron to the ion-exchange resin, as compared to 10% binding of the ferrous iron-2,2′-dipyridyl complex. When the supernatant (unbound fraction = 90% in the case of ferrous, 40% in the case of ferric iron) was chromatographed on Sephadex G-50, all the ferric iron was recovered in the high-molecular-mass peak, whereas the ferrous iron-2,2′-dipyridyl complex was eluted with the low-molecular-mass fraction. A linear correlation was obtained between the amount of iron which was not bound to Amberlite and the amount of iron in the low-molecular-mass fraction ($r^2 = 0.965$). Therefore, chromatographic separation of the supernatant was included in the experimental protocol in order to achieve complete separation of the ferric and ferrous iron complexes. The amount of ferric iron was equal to the sum of the fraction which was bound to Amberlite and the fraction in the high-molecular-mass peak, the remaining activity being equivalent to ferrous iron.

*Assays.* Radioactive iron was determined in a Packard Instruments series 9001 multichannel analyzer, set at a counting window of 0.86–1.2 MeV. A Packard Armac small-animal whole-body counter was used to determine the radioactive iron remaining in the carcass. The amount of absorbed iron in the samples was calculated after background correction, using a reference standard of known specific activity. Ceruloplasmin was quantified due to its *o*-dianisidine oxidase activity according to Schosinsky (Schosinsky et al. 1974); 1 unit is the amount which catalyzes the oxidation of 1 nmol *o*-dianisidine/min.

*Statistical evaluation.* Mean and standard error of the mean were calculated and differences between means were evaluated for significance by the *t*-test. Differences with *P* values lower than 0.05 were considered significant.

## Results

### *Rate of iron absorption in iron-deficient animals*

As can be seen from Fig. 1, iron appears within 1–2 min in the portal blood after injection of the test dose into the ligated loop. Within 6–8 min a peak is reached with an absorption rate of 250 ng Fe × $min^{-1}$ in the case of ferrous and 100 ng Fe × $min^{-1}$ in the case of ferric iron. The mean blood flow during the experiment amounted to $0.4 \pm 0.03$ ml × $min^{-1}$. Therefore the portal concentration of absorbed iron in the effluent blood was up to 625 ng × $ml^{-1}$ (11.7 nmol × $ml^{-1}$), which is about one order of magnitude less than the free iron-binding capacity of anemic rats ($5200 \pm 500$ ng × $ml^{-1}$).

### *Valency state of iron in the portal blood*

Iron-deficient animals (plasma iron $400 \pm 120$ ng Fe × $ml^{-1}$; $n = 21$) were pretreated intravenously with 6

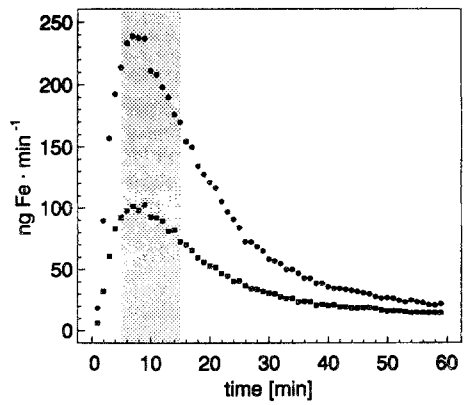


**Fig. 1.** Kinetics of iron absorption in iron-deficient rats. Appearance of absorbed iron in the portal blood as calculated by means of the measured $^{59}Fe$ activity after the injection of ferric and ferrous iron solution labelled with $^{59}Fe$ (0.1 ml × $cm^{-1}$; 300 nmol × $ml^{-1}$, pH 2) into a gut loop (for details see Methods). A transient rise in iron absorption is observed, which is more than twice as high in the case of ferrous iron (●) as compared to ferric iron (■). The dotted area represents the time interval in which samples were drawn for the determination of the valency state of iron appearing in the portal blood. One out of ten similar experiments

μg Fe(II) × $ml^{-1}$ blood at the beginning of the sampling period in order to saturate transferrin and thereby to prevent rapid binding of absorbed iron to transferrin. Portal blood was collected in precooled vials containing 0.5 ml 40 mM 2,2′-dipyridyl in 0.9% NaCl solution, and 20 μl 1 mM ferrous ammonium sulphate. Samples were taken 5–15 min after the acidic iron solution had been placed in the ligated loop, since absorption was maximal in this time period (Fig. 1). The pooled supernatants of the samples were further processed as described in Methods.

In 11 iron-deficient rats, $63.5\% \pm 22.4\%$ of the absorbed iron was in the ferrous state (range: 28–93%) when it appeared in the portal blood after administration of ferric iron into the lumen. When ferrous iron instead of ferric iron was offered at the luminal surface, the mean value for the fraction of ferrous iron in the portal blood was higher and amounted to $86.6\% \pm 6.2\%$ ($n = 10$; range 79–96%) of the total absorbed iron.

### *Iron absorption and distribution in ceruloplasmin deficiency caused by copper deficiency*

The amount of absorbed iron from iron-albumin and its distribution between various organs in ceruloplasmin-deficient rats with and without substitution of ceruloplasmin is seen in Fig. 2. Due to the substitution of ceruloplasmin (0.15 unit, equivalent to 0.015 unit × $ml^{-1}$ blood), uptake of iron into the mucosa (+28%) and its transfer into the body (+61%) were significantly increased. Mucosal uptake amounted to $1.5 \pm 0.09$ μg in control rats vs $1.9 \pm 0.13$ μg in ceruloplasmin-treated rats ($P < 0.05$, $n = 6$). The values for the transfer to the contraluminal side were $0.7 \pm 0.06$ μg and $1.1 \pm 0.13$ μg respectively ($P < 0.05$, $n = 6$). The dis-

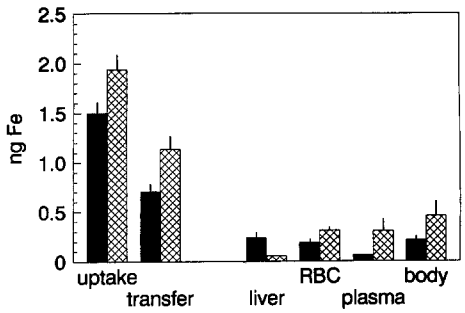


**Fig. 2.** Influence of intravenously injected ceruloplasmin on iron absorption and distribution in copper-deficient rats (140–160 g). A test dose of 10 μg Fe labelled with $^{59}$Fe bound to albumin was injected into a tied-off jejunal loop (20 cm). Ceruloplasmin (0.15 unit) was injected into the jugular vein immediately before iron was given. In animals of the control group the same volume of isotonic saline was administered. After 1 h iron distribution was measured. Uptake: iron in the mucosa; transfer: sum of iron in the total body minus luminal and mucosal contents. Less absorbed iron is incorporated into the liver and more into the other compartments after ceruloplasmin. Body: transfer minus iron in liver and blood. Mean values ±SEM, $n=6$; all differences are significant ($P<0.05$). ■ Controls, ▨ ceruloplasmin

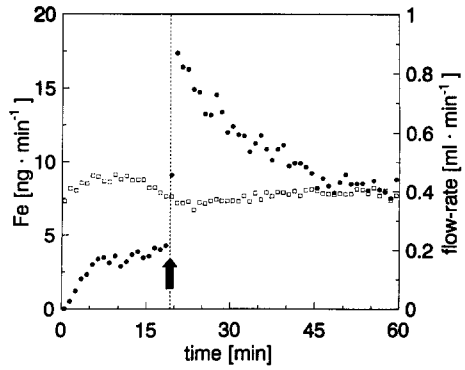


**Fig. 3.** Appearance of absorbed iron in the portal blood after substitution of ceruloplasmin in a copper-deficient rat. A test dose of 1 μg Fe × cm$^{-1}$ labelled with $^{59}$Fe bound to albumin was injected into a tied-off jejunal loop; 19 min after the beginning of the iron absorption, ceruloplasmin (0.15 unit) was injected into the jugular vein. (●) The amount which appears during the 1-min sampling interval in the portal blood; a steep rise in iron absorption rate is observed which decays to a new steady state without a change in flow rate (□). One out of 10 similar experiments

tribution of absorbed iron was also changed: whereas uptake into the liver was decreased (−78%), increased amounts of absorbed iron were found in all other compartments investigated.

This result must be interpreted cautiously taking into account the influence of ceruloplasmin on iron turnover of the various compartments, since at the same time, the total plasma iron was increased from 53 ± 10 ng × ml$^{-1}$ in controls to 376 ± 82 ng × ml$^{-1}$ in ceruloplasmin-substituted animals ($P<0.05$, $n=6$) i.e. sevenfold. The total amount of absorbed iron in the plasma, measured by means of [$^{59}$Fe] iron, was increased from 61.4 ± 9.7 to 306 ± 97.7 ng ($P<0.05$, $n=6$) i.e. fivefold. In the red blood cell fraction, 312 ± 24 ng of absorbed iron were measured in ceruloplasmin-treated vs 187 ± 47 ng in control rats ($P<0.05$, $n=6$) i.e. 1.5-fold. The value of this fraction represents, as generally accepted, mainly the uptake of iron into reticulocytes. If the isotopic dilution in the plasma is considered, the total iron uptake into the reticulocytes was increased by a factor of 7–10, which is within the same range as the increase in total plasma iron. It may be concluded, therefore, that iron uptake into the reticulocytes was roughly proportional to the iron pool in either experimental group. As far as the liver is concerned, the interpretation of the observed values is still more complex, since the liver takes up and releases iron at the same time and $^{59}$Fe will distribute in the turnover pool of the organ. In contradistinction to copper-deficient animals, no change in iron uptake, absorption or distribution was observed in iron-deficient animals after the injection of ceruloplasmin (data not shown).

### *Kinetics of iron absorption in copper-deficient animals*

When ceruloplasmin was injected intravenously into copper-deficient rats 20 min after the administration of iron-albumin into the ligated gut loop, an instantaneous rise in the rate of iron absorption was observed in the portal blood without any change in blood flow (Fig. 3 shows one out of ten similar experiments). This rise decreased gradually and finally reached a new steady state which was higher than the initial value (range: 1.2–2-fold). It must be underlined that this effect of ceruloplasmin was only observed in severe copper deficiency, when the ceruloplasmin activity drops below 0.004 unit × ml$^{-1}$ plasma. At residual ceruloplasmin levels above this value, no change in iron absorption was observed despite a remarkable degree of deficiency (data not shown).

One might argue that ceruloplasmin does not act upon iron release by itself, but rather by supplying copper to any copper-dependent enzyme which might be linked to iron absorpion. Furthermore, ceruloplasmin is

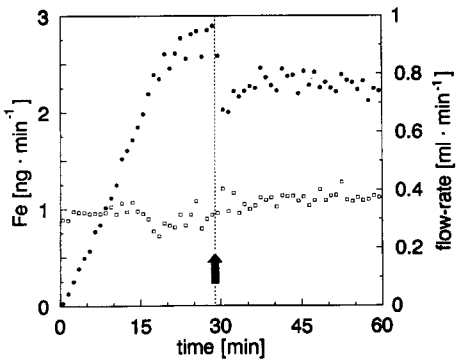


**Fig. 4.** Appearance of absorbed iron after injection of copper bound to albumin in a copper-deficient rat. Iron absorption was measured by means of $^{59}$Fe (see legend of Fig. 3); 29 min after the beginning of the iron absorption, copper (4.5 μg) bound to albumin was injected into the jugular vein. The appearance of iron (●) decreases without a change in flow rate (□). One out of 10 similar experiments

generally contaminated by free copper (McDermott et al. 1968; Lovstadt 1979). In order to rule out that the observed effect was caused merely by copper weakly bound to proteins, the influence of albumin-bound copper on iron absorption was investigated. When copper bound to albumin in equimolar amounts to ceruloplasmin-copper was injected, quite in contrast to the effect of ceruloplasmin not an increase but a small drop in iron absorption was seen (Fig. 4). This was not due to changes in the blood perfusion of the preparation, as can be seen by tracing the vascular flow rate, which remained unchanged after the injection of copper-albumin.

## Discussion

Redox changes are widespread and crucial in the metabolism of iron. Reduction of ferric iron is a prerequisite for intestinal uptake (Wollenberg and Rummel 1987) as well as for the uptake into the liver (Morley et al. 1985; Thorstensen and Romslo 1984). Reduction is supposed also to precede the release of iron from the liver into the blood (Osaki and Johnson 1969).

The early observation of Lintzel (1933), Moore et al. (1939), and others (for references see Forth and Rummel 1973), that ferrous iron is more efficiently absorbed than ferric iron, was explained, apart from the markedly different availability of ferrous and ferric iron, by a higher mucosal uptake as well as transfer of the reduced iron to the contraluminal side; however, there was no direct experimental evidence for this assumption. Later, Manis and Schachter (1964) suggested that conversion of ferrous iron to ferric iron is a means of controlling the absorption process. They again, upon indirect evidence, assumed that it is ferrous iron which is released from the mucosal cells.

Not only after addition of ferrous iron, but also after addition of ferric iron into the lumen, up to 90% of the iron is released from the mucosal cells in the ferrous state and can be identified in the portal blood under conditions in which premature oxidation and binding to transferrin are prevented. Although the time between iron release from the basolateral cell membrane and the trapping of ferrous iron by the 2,2′-dipyridyl in the test tube is rather short, oxidation of ferrous iron will occur at least partly. Since under our experimental conditions the absorption rate of ferrous iron is about two times higher than that of ferric iron, the fraction of ferrous iron recovered will be much higher after the administration of ferrous than after that of ferric iron, as was actually observed.

The results discussed here were obtained by a modification of the method described by Manis and Schachter (1964). Controls have been made to ensure that the results are not biased by the method as might be suspected since the equilibrium between ferrous and ferric iron may be influenced by the presence of ligands for either species. Because no evidence was found for such a change during a period of experimental relevance, the data seemed to be at least of indicative value for the redox state of absorbed iron appearing in the portal blood (see Methods).

With respect to these results, it seems justified to assume that iron is reduced to the ferrous state before leaving the mucosa at the contraluminal side. This agrees with results obtained with vesicles of basolateral membranes. As demonstrated by Eastham et al. (1977), ferrous iron is transported across basolateral membrane vesicles. The transport shows a saturable component and this component is dependent on SH groups in the membranes, whereas the diffusing fraction is not.

A model (Fig. 5) in which the ferrous valency state is a prerequisite for iron to be transferred across the basolateral membrane of the enterocytes, similarly as already postulated for crossing the brush-border membrane (Wollenberg and Rummel 1987), can also explain

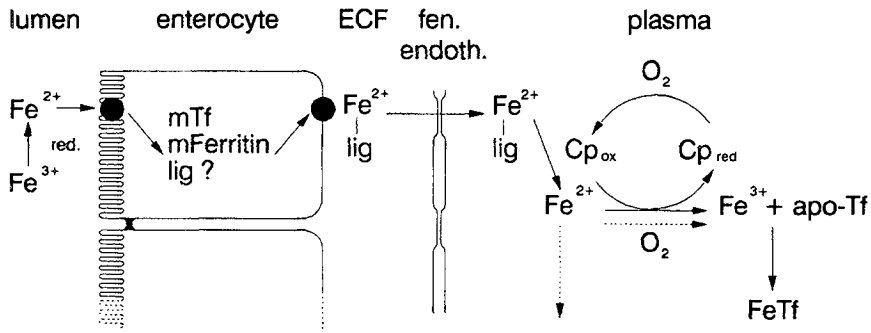


**Fig. 5.** Schematic presentation of the release of iron at the contraluminal side of the mucosa. Reduction of iron to the ferrous state in the lumen is a prerequisite for uptake into the mucosal cell (Wollenberg and Rummel 1987) via a transporter of ferrous iron (Marx and Aisen 1981; Muir et al. 1984; Stremmel et al. 1987). Inside the mucosal cell, iron is bound to mucosal transferrin (mTf) and ferritin (mFerritin) (Huebers et al. 1971; for additional references see Huebers and Rummel 1984). From mucosal transferrin, iron is released across the basolateral membrane in the ferrous form. Transfer of ferrous iron across basolateral membrane vesicles was shown by Eastham et al. (1977). The appearance of ferrous iron in the portal blood can be demonstrated, if binding to transferrin is prevented. Under physiological conditions the transmucosal transfer of iron to the contraluminal side is associated with the transport of ascorbic acid and of degradation products of nutrients with chelating properties like amino acids etc. into the extracellular fluid (ECF). In the presence of ceruloplasmin and apo-tansferrin ferrous iron is oxidized and bound to transferrin (Osaki et al. 1971; this study). In the absence of ceruloplasmin the velocity of the appearance of iron in the portal blood after crossing the capillary wall with its fenestrated endothelium (fen. endoth.) is decreased

observations which showed that hypoxia increases the epithelial iron transfer (Osterloh et al. 1987; for further references see Forth and Rummel 1973) and abnormal cellular oxidation processes like those induced by endotoxin (El Shobaki and Rummel 1985) which decrease iron absorption. Despite the open questions, this model will be useful at least as a working hypothesis.

The question of whether a low-molecular-mass ferrous iron chelator might be involved as part of the cellular transport compartment, in order to protect the enterocyte against the cytotoxic properties of ferrous iron, remains unanswered. Until now the only naturally occurring low-molecular-mass chelator of ferrous iron with a high stability and specificity was isolated from *Pseudomonas* GH and identified by Shiman and Neilands (1965).

A link between iron and copper metabolism was suggested by Chase et al. (1952) and Gubler et al. (1952) and was confirmed by Lee et al. (1968), who all found decreased iron absorption in copper-deficient animals. This link was explained by Osaki et al. (1971) who had observed that ceruloplasmin strongly enhances iron release from the perfused dog liver and its binding to apo-transferrin. Analogously, they assumed that, in the small intestine, the iron released from the contraluminal side of the epithelium is in the ferrous state as well; it was supposed to be bound to, an oxidized by, ceruloplasmin before binding to transferrin (Fig. 5). The physiological relevance of their observation was questioned by Brittin and Chee (1969) and by Coppen and Davies (1988), who failed to find an influence of ceruloplasmin on iron absorption.

Therefore, an attempt was made to reinvestigate these aspects of iron absorption. In severely copper-deficient animals, iron absorption was higher in animals substituted with ceruloplasmin in comparison to a non-substituted group. The distribution pattern of absorbed iron differs considerably between ceruloplasmin-treated and non-treated animals. Absorbed iron is preferentially directed to the liver in copper-deficient, i.e. ceruloplasmin-deficient, animals, whereas in ceruloplasmin-substituted animals the distribution takes place preferentially in favour of the other compartments (Fig. 2; Lee et al. 1968). If the increase in plasma iron from 50 to 400 $ng \times ml^{-1}$ is taken into account, it may be suspected that this is not a primary effect of ceruloplasmin on tissue uptake of newly absorbed iron, but instead is a consequence of the increased plasma-iron pool after ceruloplasmin. This is in agreement with earlier observations on the changes in iron metabolism in copper deficiency (Lee et al. 1968).

A direct effect of ceruloplasmin was seen when the appearance of absorbed iron in the portal blood was measured with a relatively high time resolution in a preparation which allows the quantification of iron transfer in 1-min intervals. Immediately after intravenous injection of ceruloplasmin, there was an initial rise in iron absorption which levelled off to an increased steady state of absorption. The peak of absorption was reached within 1–2 min after the injection of ceruloplasmin. Within this short time period, the ceruloplasmin must have reached its site of action. If it is assumed that ceruloplasmin acts at, or close to, the basolateral membrane, it must have left the capillary bed quite fast and sufficient free iron-binding capacity must have been available in the interstitial fluid to bind the newly formed ferric iron, but for quantitative reasons this seems highly improbable. As has been shown by Morgan (1980), the rate of transferrin and albumin diffusion out of, as well as back into, the capillary bed is not sufficiently high to account for the transport of iron from the mucosa to the blood and a substantial amount of absorbed iron can not be bound by the transferrin, which is available in the extracellular space. Therefore, it is more plausible to assume that ferrous iron leaves the mucosal cells, diffuses to the capillaries bound to low-molecular-mass ligands, and is oxidized by ceruloplasmin upon its entry into the capillary lumen (Fig. 5). It should be taken into account that, during absorption of nutrients, a large number of iron chelators, like amino acids and ascorbic acid etc., pass the mucosa, actively transported at least partly, and appear in the extracellular space at the contraluminal side, before crossing the capillary wall and entering the portal blood (for references see Hopfer 1986). Due to the relatively high concentration of these chelators the effective stability is sufficiently high to bind the released iron. The concentration of vitamin C, for instance, amounts to 36 $nmol \times ml^{-1}$ in human plasma and to 129 $nmol \times ml^{-1}$ in gastric juice (Sobala et al. 1989).

The role of ceruloplasmin, due to its low $K_m$ for the oxidation of Fe(II) (Osaki et al. 1966), would be to maintain a steep concentration gradient for ferrous iron between the site of its release from the enterocytes into the capillaries. Since ferrous iron in the systemic blood is a very toxic metal, due to its ability to form active oxygen species during oxidation (Frieden 1980) which will initiate lipid peroxidation as well as oxidation of catecholamines with a concomitant drop in peripheral vascular resistance, a mechanism which efficiently removes this dangerous species from the portal blood is required. Ceruloplasmin, together with the free iron-binding capacity of apo-transferrin, is able to inactive free ferrous iron very efficiently, thereby preventing the formation of activated oxygen species (Frieden 1980), and to trap it immediately. This mechanism functions even in copper-deficient animals until the ceruloplasmin level drops close to the detection limit. Therefore, the primary role of ceruloplasmin in iron absorption seems to be that of a protective agent against the toxicity of Fe(II), which it binds and oxidizes. The ferric iron which is formed is safely bound to transferrin. In addition it is an accelerator of the iron transfer from the contraluminal side of the mucosa to the portal blood.

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