

The study of Klomp et al. (1) in this issue of *The Journal* is the latest in a recent series of papers that have yielded new insights into the physiology and pathology of transition metals. Earlier studies showed that swine made copper-deficient by dietary restriction developed severe plasma hypoferrinemia and an associated anemia (2). Introduction of iron into animals by diet or injection showed that iron could be absorbed by cells of the intestine, liver, or spleen. Once absorbed, however, iron could not be released into plasma. The inability to transport iron out of cells was the direct result of the absence of the copper-containing protein ceruloplasmin (Cp); the nutritional deficiency of copper resulted in the inability to assemble and secrete Cp. Further studies indicated that Cp had ferroxidase activity and suggested that this activity was critical for loading iron onto transferrin (Tf) (3).

In the ensuing years since the elaboration of this hypothesis, progress on understanding the physiological role of Cp was at best moderate. New insights into the role of Cp have resulted from the analysis of mammalian genetic diseases as well as analysis of specific mutations in yeast (4). Of particular interest is the recent analysis of individuals with aceruloplasminemia (5, 6). Due to a variety of mutations, these individuals do not synthesize ceruloplasmin, and they exhibit systemic siderosis, neural and retinal degeneration, and diabetes. The observation that these individuals show no defect in copper accumulation, but show excessive iron deposition in selected tissues, indicated that ceruloplasmin plays little role in copper transport but has an essential role in iron metabolism. Tissue injury is associated with excessive iron accumulation, most probably related to the generation of toxic oxygen radicals.

One of the features that make the pathophysiology of this disorder interesting is that the pattern of tissue iron loading is different than that seen in other iron-loading disorders such as hereditary hemochromatosis or atransferrinemia. While both aceruloplasminemia and hemochromatosis also result in a systemic hemosiderosis and diabetes, only aceruloplasminemic patients present with neural and retinal degeneration. It is well recognized that the brain and retina are separated from the general circulation and comprise "sanctuary sites." These tissues are bathed by fluids in which the macromolecular constituents are generally sequestered from the systemic circulation. In the other iron-overload diseases there is no identifiable iron loading in brain or retina. Tissue iron loading is a consequence of increased gut absorption of iron and the clearance from plasma of absorbed iron by the Tf-independent transport system present in parenchymal tissue. That there does not appear to be iron loading in either brain or retina indicates that the endothelial cells that separate these tissues from the body can regulate iron entry and egress.

These observations suggest that there must be metal transport systems from the general circulation to the sanctuary site, and from the sanctuary site to the cells within. Insight into how metals are transported to the cells within these sites has been provided by Klomp et al. (1). Using *in situ* hybridization, they demonstrated that a variety of cell types including those of the brain and retina had Cp mRNA. They further demonstrated that cultured brain and retina cells secreted and appropriately processed Cp.

This study, in concert with others that have demonstrated that brain, retina, and testes secrete Tf, indicates that tissues isolated from the general circulation, i.e., those in sanctuary sites, contain at least two of the proteins required for iron transport. The iron deposition seen in these tissues has been interpreted by Klomp et al. (1) to result from the absence of Cp. Given that statement, it is again instructive to compare the effects of atransferrinemia and aceruloplasminemia. Both disorders give rise to plasma hypoferrinemia and iron-overload disease. Yet there is no evidence that atransferrinemia affects iron overload in retinal or neural tissue (7). In aceruloplasminemia, increased iron stores can be detected in many tissues, although the most severe pathology is seen in brain and retina. In the absence of Tf there is selective iron deficiency within specific tissues, the Sertoli cells of the testes and the erythron. Other cell types show iron excess resulting from increased absorption. The tissue iron-overload seen in aceruloplasminemia reflects an inability to mobilize absorbed iron. While it is reasonable to implicate the ferroxidase activity of Cp in iron transport, the mechanism by which this soluble protein can affect iron egress is unknown. Indeed, one of the major unresolved issues in iron metabolism is the mechanism by which iron is released from cells.

The best available evidence indicates that there is only one copy of the gene for either Cp or Tf. The selective tissue expression of these genes implies the existence of tissue-specific enhancers and transcription factors. The expression of these proteins has been demonstrated to be regulated both developmentally and by soluble molecules, i.e., cytokines. These observations suggest the possibility that genetic defects in either *cis* or *trans* elements that regulate these genes may give rise to selective and localized areas of metal accumulation or deficiency and tissue injury, particularly in sequestered areas such as sanctuary sites.

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