in accord with the macroscopic constants from the spectrophotometric data.

The effect of histidine modification on the macroscopic rate constants was investigated. As diferric transferrin was progressively ethoxyformylated, both rate constants decreased in proportion to the number of fast reacting histidines modified. The data for both sites plotted in the form of a Tsou Chen-Lu graph gave straight lines for $i=1$. These results suggest that a single histidine, presumably one in each iron binding domain, is involved in the release of iron from the protein.

It is well known that the rate of iron removal from transferrin is greatly accelerated below pH 7, following first-order kinetics in $[H^+]$. The effect of histidine modification on iron release was therefore investigated as a function of pH. Modification imparted increased kinetic stability to the protein in the pH range 5 to 7 with the effect greatest at pH 5. The kinetics at pH 5 were studied using citrate, pyrophosphate, orthophosphate, ATP GTP, and DPG as mediating chelators. In every case protein modification significantly slowed the rate by factors ranging from 2 to 10. Evidently the reduction in pK_a of histidine upon ethoxyformylation imparts kinetic stability to the protein in acid, perhaps by eliminating the formation of a protonated imidazole group near the iron. Such a group may be the site of binding of chelates and other anions to the protein.

The structural features of transferrin which play a role in the mechanism of iron release are largely unknown. Until now, amino acids which are not ligands have received little attention. The chemical modification studies presented here implicate the involvement of a key histidine, presumably not bound to the metal, in this process. We speculate that His-207 in the *N*-terminal domain of serum transferrin, which has counterpart residues in the *C*-domain and in both domains of ovotransferrin, is responsible for the effects reported here. Lactoferrin has unusual stability in acid and lacks histidine in this location in the *N*-domain [1-3]. The corresponding portion of the *C*-domain has not been sequenced.

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MS6.5 — FR

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TRANSFERRIN AND IRON TRANSPORT

The critical role of transferrin in the regulation of iron metabolism has long been appreciated, but the molecular mechanisms underlying the protein's activities are still largely obscure. Transferrin functions as an iron donor to cells requiring this essential metal for the biosynthesis of hemoglobin and other vital iron proteins, and as an iron acceptor from cells where the metal is absorbed, released from stores, or recovered from the hemoglobin of senescent red blood cells. Thus, both limbs of the metabolic iron cycle — plasma-to-cell and cell-to-plasma — hinge upon transferrin. Without functional transferrin, neither the maintenance of zero iron balance, nor the delivery of iron to cells, is successfully regulated by the organism.

Many of the processes underlying the interaction of transferrin with iron-requiring cells have been clarified in recent years [1,2]. Iron transfer from protein to cell is receptor-mediated, entails internalization of the receptor-transferrin complex to an acidic, pre-lysosomal compartment where the

iron-protein bond is disrupted and iron becomes available to the cell, and culminates with release from the cell of iron-depleted but otherwise intact transferrin. Relatively little is known, however, of the processes entailed by the return to transferrin of iron recovered from the hemoglobin of catabolized red blood cells. To approach these mechanisms, we have used an *in vitro* model in which immunosensitized red cells are phagocytosed by rat macrophages. In this model, more than half of the iron initially in hemoglobin can be processed for release to the medium, making it suitable for the study of iron delivery from cell to protein. When apotransferrin is present in the culture medium, from 40-70% of the iron released by macrophages is rapidly bound to the protein, with most of the remainder in a ferritin-like form. Macrophages induced by inflammation, or stimulated by preincubation with methemalbumin, release much less iron than non-stimulated macrophages, in accord with the clinical observation of hypoferremia in inflammatory states. Iron excreted by macrophages exhibits no distinct preference for either site of transferrin, so that our experiments offer no explanation for the observed differences in site occupancies of transferrin in the circulation [3,4]. The absence of transferrin in the culture medium depresses iron release only slightly, with much of the excreted iron then in a form that readily binds to apotransferrin *in vitro*. Treatment of macrophages with pronase, which largely abolishes their ability to bind apotransferrin [5], depresses iron release no more than 10-15%. It appears, therefore, that binding of apotransferrin to macrophages may not be essential for iron excretion by the cells [6]. The chemistry underlying sequestration of released iron by transferrin remains an enigma.

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COMPARATIVE CHEMICAL AND BIOLOGICAL STUDIES OF INVERTEBRATE FERRITINS

Ferritin has been isolated from the hemolymph of several molluscan species and characterised with respect to its protein composition, the nature of the iron cores present and to its biological function(s). In the case of the chiton *Clavarizona hirtosa*, ferritin is the major iron binding protein present in the hemolymph as determined by the chromatographic fractionation of hemolymph both according to its iron content and according to the distribution of presented Fe-59. The hemolymph concentration of ferritin is remarkably high, *ca.* 400 μmol/mL, a level that can be associated with its role of delivering Fe to the mineralising front of those tissues where magnetite,