rapid transport pathway is the ability of the cell to synthesize ferritin and thus sequester the iron within the cell. Conrad *et al.* (1964) have shown that parenterally administered iron enters the intestinal mucosal cells from the serosal pole at the time of their emergence from the crypts of Lieberkühn. It is suggested that in iron-deficient states relatively little iron enters the cells this way and ferritin formation is minimal, thus allowing any ingested iron to traverse the cell rapidly and be absorbed. In cases of iron overload the epithelial cells take up an increased amount of iron from the plasma, leading to increased ferritin synthesis and consequent sequestration of food iron in the cell, preventing its transfer to the plasma. Smith *et al.* (1968) have demonstrated mucosal ferritin synthesis in response to both enteral and parenteral administration of iron. If this interpretation is correct then it would be expected that the mucosal iron content would be inversely related to the amount of iron absorbed from a given dose. There is conflicting evidence on this point which some authors have attributed to methodological variation (Balcerzak & Greenberger 1968). Other authors have suggested that the control of iron absorption is by means of a humoral factor but this has not been identified (Conrad 1969). The chemical form of the iron traversing the rapid transport pathway within the intestinal cell to reach the plasma is at the present time unknown.

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Iron Transport in the Blood

The main source of plasma iron is the breakdown of hæmoglobin in the reticulo-endothelial system; small amounts of iron also come from the intestine and storage sites. Plasma iron is delivered to the marrow for hæmoglobin synthesis and, in small amounts, to storage sites, mainly in the liver. During pregnancy there is also a considerable delivery to the placenta. All of this iron is carried by a single plasma protein, transferrin. Hæmoglobin synthesis requires 30–40 mg of iron each day, which is 6 to 10 times the total plasma iron. Transferrin is therefore vitally important in maintaining the supply of iron to the marrow for production of normal red cells. Transferrin, or siderophilin as it is sometimes called, has a molecular weight of about 80,000 and can bind two atoms of ferric iron per molecule. The normal plasma iron is between 60 and 150 μg/100 ml and represents about one-third of the plasma's total iron-binding capacity; that is, circulating transferrin is normally one-third saturated with iron.

Laurell (1951), who originally described transferrin, conceived of it as an iron carrier in the same way as hæmoglobin carries oxygen. This implies that, in sites where iron is released, there will be movement of free iron across a concentration gradient from the tissues to the plasma. Conversely, where iron is being utilized, the concentration gradient will be reversed and free iron released from transferrin. This concept became untenable when it was realized just how tightly iron is held by transferrin (Aasa *et al.* 1963). The binding is so firm that at the concentrations of iron and transferrin in the plasma there are no free iron atoms. This is not surprising as even tiny amounts of free iron are toxic. Instead, it seems that cells which require iron, in particular developing red cells, possess on or near their surfaces receptor sites which combine with transferrin molecules carrying iron (Jandl *et al.* 1959). After attachment of the protein, there is either a change in its conformation or reduction of the carried iron to the ferrous state which allows iron to pass from transferrin to an intracellular carrier, which may be ferritin. The iron-free transferrin is displaced from the cell surface by the next transferrin molecule carrying iron. Thus, there is a plasma to cell to plasma cycle of transferrin which results in the efficient delivery of iron where it is required (Jandl & Katz 1963). Free iron does not exist at any stage in the process. When hæmoglobin synthesis ceases the red cells lose their transferrin binding sites.

It is a basic assumption of many studies of iron metabolism that the plasma iron consists of a uniform pool. However, when iron is added to plasma *in vitro*, it combines at random at the two binding sites of transferrin to form a mixture of molecules carrying two, one or no iron atoms (Fletcher 1969). The proportions of these molecules are determined by the level of saturation. At high saturation most molecules of transferrin carry two iron atoms, some carry one and almost none are iron-free. Consequently when iron is added to plasma at high saturation it combines mainly with molecules already carrying one iron atom to form saturated molecules and, if the added iron is radioactive, then these will be labell-

ed saturated molecules. At low levels of saturation most molecules are iron-free and a few will carry one or two iron atoms. Consequently, added iron combines mainly with iron-free molecules to form half-saturated molecules and, again, by using radioactive iron, half-saturated molecules can be labelled. Half- and fully-saturated molecules differ in their affinity for the cellular receptor sites, probably because of differences in their shape or charge. This leads, at least *in vitro*, to fully-saturated molecules having a considerable advantage over half-saturated molecules when competing for attachment to the receptors, so that they are several times more efficient in the delivery of iron to reticulocytes. This can be shown, very simply, by following the uptake of tracer radioactive iron by reticulocytes from plasmas at different levels of saturation which, therefore, contain different amounts of fully- and half-saturated molecules.

To compare two plasmas, A and B, tracer quantities of radioactive iron are added to equal quantities of each. After allowing the iron and transferrin to combine, an equal amount of unlabelled plasma B is added to A and an equal amount of unlabelled A is added to B. The mixtures are then identical except that, in one, the radioactive label is attached to the transferrin of plasma A and, in the other, it is attached to the transferrin of plasma B. Iron is so tightly bound that there is no redistribution of the label. If plasmas A and B are, in fact, the same plasma adjusted to two different levels of saturation, for example 12% and 65%, the result of uptake by reticulocytes of the tracer iron is as shown in Table 1. Radio-iron is taken up much more rapidly from the plasma labelled at a higher saturation level. This is because more radio-iron is combined with saturated molecules in this plasma. The total uptake of iron is equal. If both plasma mixtures are completely saturated with an excess of unlabelled iron, so converting all molecules to the saturated form, then this difference disappears.

When plasmas of different patients are compared in this way, the uptake of the tracer iron is again greater from the plasma labelled at the higher level of saturation. This is because circulating transferrin also exists as a mixture of molecules and the proportions of these molecules are, at least partially, determined by the level of saturation.

Table 1

Uptake of ^{59}Fe by reticulocytes from two plasma mixtures labelled at 12% (A) and 65% (B) saturation

	Uptake of ^{59}Fe by reticulocytes from:		
	Plasma A counts/min	*Plasma B counts/min*	$\frac{\text{Plasma B}}{\text{Plasma A}} \times 100$
Incubation time:			
15 minutes	776	2,016	260
45 minutes	2,912	6,877	236
Plasma iron remaining after 45 min	45 µg/100 ml	47 µg/100 ml	

When plasma labelled with radioactive iron, as already described, is injected into animals, the uptake of the tracer by the liver behaves in the same way as the uptake by reticulocytes. This appears to be true for the small intestine as well. The liver, small intestine and erythropoietic marrow are the three organs involved in iron metabolism normally and each possesses a system of tissue receptors, specific for transferrin, which is the mechanism by which iron is taken up from transferrin. The amount of iron delivered is controlled by the number of receptors. Cells like developing red cells which need iron have many, while others like mature red cells not requiring iron have none.

Very little is known about the movement of iron from the tissues to transferrin. Following an acute infection, there is a rapid fall in plasma iron due to block to the release of iron from the reticuloendothelial system. This block to the release of iron from the RE system may be an effect of fever or circulating leukocyte pyrogen. In chronic infection, uræmia and carcinoma this block persists with accumulation of iron within macrophages. However, the plasma transferrin level also falls, so maintaining the level of saturation and, consequently, the supply of iron to the depressed erythropoetic marrow. Below a saturation of 15%, hypochromic anæmia develops (Bainton & Finch 1964). In iron deficiency and pregnancy, when iron must be mobilized from stores and absorption increased, the level of circulating transferrin rises and provides more iron-binding sites. The rise in iron-binding capacity takes place before the plasma iron falls but, in these circumstances, only a small decrease in plasma iron will drop the level of saturation below the critical 15% and lead to deficient hæmoglobin synthesis.

According to the hypothesis of Weintraub *et al.* (1964), the amount of iron absorbed from the lumen of the intestine is determined by the amount of iron already present in the mucosal cells on the surface of the intestinal villi. The cells receive this iron from the plasma when they are formed in the crypts. In the crypts they are primed with an amount of iron which must reflect the two controlling factors, the state of the body's iron stores and the level of erythropoietic activity. As already described, there appear to be receptor sites in the small intestine which behave like those of immature red cells in that they have a greater affinity for saturated transferrin molecules than for half-saturated molecules. The proportions of these molecules in the circulation will, therefore, affect the amount of priming iron the cells receive and amount of iron later absorbed. The state of the iron stores affects the levels of circulating trans-

ferrin and iron and, therefore, the saturation of transferrin. Saturation, in turn, affects the proportions of the different molecules and so may relate absorption to iron stores. In some circumstances the proportions of saturated and half-saturated molecules may also be affected by the activity of the erythropoietic marrow. For example the plasma of patients with ineffective erythropoiesis and a high plasma iron concentration appears to contain more half-saturated transferrin molecules than would be expected from the level of iron saturation (Fletcher 1969). This presumably reflects the affinity of developing red cells for the saturated form of transferrin. Since the developing mucosal cells also have an affinity for saturated molecules which are removed by the marrow, they will not receive so much priming iron and iron absorption will continue out of proportion to the body's stores. Certainly patients with thalassæmia, sickle-cell disease or hereditary spherocytosis may develop severe iron overload. Therefore, the plasma messenger may be the proportion of saturated and half-saturated transferrin molecules in the circulation and this may relate the rate of absorption to the body's iron stores and to the rate of erythropoietic activity.

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Storage Iron and Desferrioxamine

Desferrioxamine is the most effective and specific chelator of trivalent iron. The iron chelate, the red pigment ferrioxamine, has a stability constant of 10^{31} at physiological pH and thus exceeds the two constants of iron transferrin. After its isolation ten years ago (Bickel *et al.* 1960), its potential use for eliminating body iron in conditions of iron overload was soon recognized. In fact, therapeutic applications have been limited, but desferrioxamine (Desferal) is now the definitive treatment for inorganic iron poisoning, which still claims children's lives, and in this application it is almost certainly life-saving.

Interest in desferrioxamine has developed mainly in clinical and experimental investigation, especially in connexion with the assessment of storage iron. Until recently the quantitative measurement of storage iron has been possible only on an experimental basis. Comparatively crude histochemical assessment can be made on biopsy material of marrow, liver or skin, using the Prussian blue reaction. Desferrioxamine, in various test procedures, has been the nearest approach so far made under clinical conditions to the quantitative measurement of whole body storage iron.

Information on total storage iron in man has been meagre. Hynes (1949) subjected himself to repeated small venesections over a long period of time until he became anæmic and calculated that he had begun with 600 mg storage iron. This principle was modified by Haskins *et al.* (1952) using large phlebotomies over a short period. They estimated normal storage iron to be between 1,000 and 1,500 mg. Isotope dilution methods give lower results, attributable to incomplete exchange between isotope and storage iron.

The properties of ligand and chelate differ considerably. Desferrioxamine is a straight chain trihydroxamic acid (mol. wt. 597), a white solid, colourless in solution. Ferrioxamine is a red pigment, containing one-tenth of its weight of iron. Desferrioxamine enters cells easily and occupies the total body water: ferrioxamine occupies the extracellular space only and seems to be averse to an intracellular site. When ferrioxamine is formed intracellularly by the action of desferrioxamine on storage iron, it appears to move quickly out of the cell. When formed in liver parenchyma, for instance, it moves mainly into blood capillaries, but also to some extent into the biliary tract. Desferrioxamine is rapidly excreted in the urine and undergoes enzymatic degradation. As a result of these two processes its chelating activity after parenteral injection persists for only about thirty minutes. Ferrioxamine is far more stable *in vivo*: only 14–18% of its iron appears as hæmoglobin; it is excreted in the urine more slowly than desferrioxamine. Neither hæm iron nor transferrin iron (*in vivo*) give up iron to the chelator. The main source of body iron chelated by desferrioxamine is undoubtedly storage iron as ferritin; but there is also much evidence that hæm, during its catabolism, may give rise to a transient form

Table 1
The effect of age and hæmoglobin concentration on 6-hour ferrioxamine excretion

	$F = k$	$+ A$ (S.E.a)	$+ H$ (S.E.b)	R^2
Normals	38·4	− 0·19A● (0·06)	+ 0·57H (0·97)	0·10
Iron deficiency anæmias	43·0	− 0·54A● (0·10)	+ 1·92H● (0·65)	0·54

F = 6-hour ferrioxamine excretion. A = age.
H = Hb concentration. S.E. = standard error of slopes a and b.
R^2 = co-efficient of determination.
● Significant correlations with ferrioxamine excretion