ANALYTICAL REVIEW

What is the Function of Transferrin in Plasma?

By C.-B. LAURELL, M.D.

INVESTIGATIONS of iron metabolism during the past few years have given results, which among other things have improved our knowledge of iron transport in the body. It has been shown that the acid soluble iron in plasma, which is usually called serum iron, is practically quantitatively bound to a special plasma protein.[1–4] This protein is characterized among other things by its capacity of forming highly colored complexes with iron and copper.[5–8] This protein appears in the literature under the following names: iron-binding component (protein),[1, 2, 3, 5] β_1-metal-combining globulin,[7] siderophilin,[8] and transferrin.[6, *]

Indirect methods of investigation have generally been used in the study of the variations in the concentrations of transferrin under physiologic and pathologic conditions. The fundamental principle of these methods is that plasma (serum) can bind iron added in vitro or in vivo up to a certain limit (saturation limit) in a characteristic chemical bond. The quantity (μg) of iron which is bound in this specific way by 100 ml. serum is usually used as an expression for the iron-binding capacity of serum.[3, 9, 10]

The values of the iron-binding capacity of serum can be transformed into grams of transferrin per 100 ml. serum since the molecular weight of purified transferrin has been determined as 88,000–90,000[5, 7] and since it has been shown that each molecule of transferrin can bind two atoms of iron specifically.[5, 7] The mean values which are reported for the iron-binding capacity of serum vary between 300 and 360 μg per cent.[1, 3, 7, 10] These values correspond to 0.24 to 0.28 Gm. transferrin per 100 ml. serum. Jager[11] has succeeded in determining the concentration of transferrin by using the quantitative precipitin reaction. He obtained as a mean value 0.27 Gm. transferrin per 100 ml. serum. The good agreement of these results with those of the indirect methods show that the latter are reliable.

One molecule of transferrin can bind two atoms of iron, but the question whether both atoms are bound in exactly the same way independently of each other has not hitherto been discussed in the literature. Solutions of transferrin have been investigated spectrophotometrically after saturation with iron to different percentages. One unit of iron results in the same change of spectrum both if the iron is added to an iron-free solution of transferrin and to a solution of transferrin, which has previously been saturated to fifty per cent. This is true on the condition that the added quantity of iron does not increase the iron concentration in the solution above two atoms of iron per protein molecule. This fact supports the hypothesis that each transferrin molecule contains two identical iron-binding groups which have such a position that they do not

From the Department of Clinical Chemistry, University of Lund, Sweeden.

*In this paper the word transferrin is used.

influence each other. The possibility can not be excluded, however, that the iron atoms are bound in pairs, i.e., that the affinity of the transferrin molecule with iron increases when one atom has been bound. The assumption that both iron atoms are bound independently of each other is of significance for the following discussion of the intermediate iron metabolism.

The observations published to the present concerning the reaction between iron and transferrin can be summed up schematically in the following formula:

$$2\ Fe^{+++} + 2\ HCO_3^- + \text{transferrin} \rightleftharpoons$$
$$4H^+ + [(Fe^{+++}\ OH^-CO_2)_2\ (\text{transferrin} - 4H^+)]$$

This reaction is reversible in vitro.[3, 7, 8] The equilibrium moves to the left in acid and to the right in alkaline solution. The equilibrium in neutral and slightly acid solutions is highly dependent upon the presence of other ions in the solutions which change the activity of the Fe^{+++} ions. All substances which form complexes with Fe^{+++} in this pH region favor the dissociation of the iron-transferrin complex. A dissociation of this complex can at first be observed photometrically on the acid side of pH 6.0. An easily measurable dissociation, however, is observed already at pH 7.0, if a reducing substance and phenantroline (Fe^{++}-complex former) is added to the solution.[3] This may help us to understand how iron in vivo can be liberated from transferrin.

It has not been satisfactorily ascertained whether transferrin in vivo serves as a real carrier of iron just as hemoglobin is the carrier of oxygen, i.e., if transferrin takes up and delivers iron in the different organs of the body without being metabolized itself.

It has been generally accepted that transferrin takes up ions of free iron from the hemoglobin-destroying organs, the iron depots and the mucosal cells in the intestinal tract. This is easily understood since the equilibrium in the reaction given above is strongly displaced to the right in the physiologic pH region. The resulting Fe-transferrin is conducted with the blood stream to the bone marrow and to other organs where there is need of iron for the synthesis going on.

It would be very interesting to know in which form iron leaves the blood stream in the iron-consuming organs. Two different hypotheses have been put forward as explanations: (a) it is assumed that the capillaries are relatively permeable to transferrin and that the whole Fe-transferrin complex leaves the blood channel.[13, 14] This must be true of all capillaries, since all cells have a more or less pronounced need for iron. (b) It is assumed that an equilibrium exists in plasma between ionized iron, iron-free transferrin and Fe-transferrin. The ionized iron of plasma through simple diffusion is in equilibrium with the ionized iron of the extracellular fluid.[3] Only the iron ions leave the blood stream. When the blood passes organs where syntheses of iron-containing substances are going on the reaction given above goes to the left. The same is true if the pH in the blood decreases in any organ. In this way the transferrin molecules can serve as real transporters of iron.

Hypothesis (a) was advanced on the assumption that the Fe-transferrin complex is completely undissociated within the whole physiologic pH-range. Flexner

et al.,[13] who put forward this hypothesis, write: "The state in which iron crosses the vascular wall from the plasma to the extravascular fluid is unknown. There is no evidence to contradict the view that it escapes from the plasma as the $beta_1$-globulinate. . . . " They have injected transferrin labeled with radio active iron into animals and investigated the elimination velocity of iron from the blood stream. This velocity was accepted as the velocity with which the transferrin molecule penetrated the capillaries, i.e., an expression for the permeability of the capillaries penetrated by a globulin molecule. The correctness of this interpretation of the experimental results was questioned by Neuman and Michaelis.[14]

Hypothesis (b) was advanced as several observations have been made which went to prove that the reaction between transferrin and iron was reversible in vivo. The transferrin concentration in the plasma remains constant during a peroral iron tolerance test in spite of the great changes in serum iron concentrations that occur.[3] The same is true during intravenous iron tolerance tests (ionized iron).[1, 10] The transferrin concentration is not influenced during the rapid initial decrease in serum iron which is going on when liver preparations are injected into a patient with pernicious anemia.[3] The relation between the concentrations of iron-free transferrin and of Fe-transferrin shows regular variations under various physiologic and pathologic conditions.[3, 9, 10] It has been shown that the quotient between Fe-transferrin and iron-free transferrin is higher than normal when the depot iron increases and lower than normal when the depot iron decreases.[3] Under the last mentioned conditions iron is also absorbed more rapidly from the mucosal cells than is normally the case. All these facts may be explained if the function of transferrin is to establish an equilibrium between iron ion activities in the different organs of the body, i.e., that the law of mass action can be applied to the reaction given above between iron and transferrin. If hypothesis (a) is accepted, the significance of the regular variations of the concentrations of Fe-transferrin and of iron-free transferrin are incomprehensible.

Since 1948, further experimental data concerning intermediate iron metabolism have been collected. Some of these can be used to elucidate the problem we are concerned with. These data have not been analyzed earlier from this point of view. This will be done below.

In the course of studies on the anemia of infection, Cartwright et al.[10] injected transferrin intravenously in such quantities that the concentration of transferrin in the plasma increased between fifty and one hundred per cent. The transferrin was afterwards saturated with iron by an injection of ionized iron. Two cases are reported in detail. "In both individuals the serum iron returned to its previous low level within seven hours in spite of the increased total iron-binding capacity. The total iron-binding capacity returned to its previous low level in forty-eight hours in one patient and in ninety-six hours in the other." They also showed that the decrease in serum iron comes earlier than the decrease in transferrin when turpentine is injected intramuscularly. These experiments clearly show that the elimination of iron and transferrin did not proceed simultaneously.

The amount of iron required for the normal hemoglobin synthesis is usually calculated to about 25 mg. since the mean lifetime of the red blood corpuscles seems to be about one hundred and twenty days. This quantity of iron is about six times bigger than the entire amount of serum iron. That the turnover of serum iron is really in accordance with the theoretically estimated one has clearly been shown by Huff et al.[15, 16] by determining the turnover rates of radio iron in human plasma. The average turnover of the iron of the plasma in 5 subjects was 0.35 mg./Kg. body wt./day. The average value for the red cell iron turnover was 0.26. In polycythemia vera the average value was 1.81 mg./Kg. body wt./day and the highest value found was 4.2. They also studied the assimilation of radio iron into the bone marrow and concluded: "It appears that at least the iron from such a protein-iron compound [Fe-transferrin] is susceptible of removal by the bone marrow without further reaction, since the time concentration relationship of the radio iron in the marrow when compared to the plasma is for all practical purposes inversely identical."[16]

If the assumption is correct that the whole Fe-transferrin molecule leaves the blood channel, we can transform the values of the iron turnover to turnover values of transferrin. If this is true and if it is supposed that each transferrin molecule contains two atoms of iron, about 19 g transferrin are eliminated from the plasma per day in a normal individual (70 Kg.). The transferrin, however, is only saturated to about one-third with iron at a normal level of serum iron (100 μg per cent) and the probable distribution among transferrin molecules containing respectively one and two atoms of iron is four to one. This means that the normal metabolism of transferrin per day ought to be about 32 Gm. instead of 19. The turnover of iron in the plasma reaches values ten times higher than normal after acute loss of blood and especially in polycythemia.[16] The serum iron is greatly decreased under these conditions so that the values of transferrin turnover of hundreds of grams ought to be obtained!

If Fe-transferrin has to be proteolyzed to set iron free in vivo it seems reasonable to assume that the free amino acids obtained are used for the synthesis of hemoglobin. The isoelectric point of transferrin is however 4.4[5] (5.9),[7] and that of hemoglobin about 6.8, which means that their amino acid compositions are different. Each molecule of hemoglobin (molecular weight 68,000) contains four atoms of iron, so it should be necessary to proteolyze four Fe_1-transferrin molecules (molecular weight 90,000) to liberate enough iron for one molecule of hemoglobin. A great surplus of amino acid ought to be free.

Conclusions

The data thus far collected are difficult to reconcile with the assumption that iron leaves the blood stream as an iron-transferrin complex. They support, however, the hypothesis according to which the iron leaves the blood stream in ionized form. The theory that the iron-binding globulin in plasma is a real carrier of iron just as hemoglobin is a carrier of oxygen is supported by the data given above. For this reason, the name of transferrin has been proposed. The name "metal-combining protein" is somewhat misleading as copper in vivo is trans-

ported with another protein (coeruloplasmin)[17] and not with the metal-combining globulin as was assumed earlier.[18]

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