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On the biological function of transferrins, coeruloplasmins and haptoglobins

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Transferrins

It is generally accepted that the complex between ferric iron and transferrin (Tr)—in clinical language designated serum-iron—is the chemical form which the body utilizes for the transport of iron between the different organs of the body.

Conclusive evidence of Tr being a true transporter of iron was produced when it was shown that normally the half life of iron in the Fe_2Tr complex was about 90 minutes against 12 days for the protein part [1].

At physiological pH and normal bicarbonate concentration the iron-transferrin complex (Fe_2Tr) is extremely stable. The slow rate at which Fe_2Tr dissociates seems to be independent of the concentration of iron chelating substances in plasma [2].

Each Tr-molecule can bind 2 ferric ions and two bicarbonate ions [3]. Measurement of the magnetic susceptibility of the complex suggests that the two ferric ions are linked in such a way that interaction between them is highly probable [4].

Under physiological conditions the plasma Tr is saturated to about one third with iron. This may in principle mean that plasma contains transferrin molecules without any or with one or two bound ions of iron. Table 1 shows the alternatives available when the plasma transferrin is half-saturated with iron. It is of importance to know which of these alternatives represents the truth, since the calculation of the plasma iron turnover is based on the half-life of a tracer dose of iron injected i.v. and on the serum iron level. Calculation of the total turnover is made on the assumption that the iron administered is bound in exactly the same way as the native plasma iron and that this linkage is independent of the degree of saturation of the transferrin. Indirect evidence argues for alternative 3 in table 1 [2, 5]. The situation has become more complicated during the last year since with the aid of starch gel electrophoresis *Smithies* and later investigators have so far found

Table 1

Distribution frequency of transferrin molecules at half saturation with iron

Assumed strength of linkage at the two sites	Tr	FeTr	Fe_2Tr
Both identical	1	2	1
First strongest	0	4	0
Second stabilizing	2	0	2

that 8 different, genetically determined Tr-types have been traced in the population of the world [6] and that more than one type may exist in the plasma of one and the same individual. However, *Turnbull* and *Giblett*'s preliminary results suggest that at least those two transferrins with the most pronounced physico-chemical difference seem to have iron-binding groups with identical Fe-affinity [6]. On empirical grounds it has long since been stated that regular changes in transferrin as well as serum iron level reflect different types of intermediary iron metabolism. Few attempts have been made to ascertain whether these regular changes in the plasma homeostasis of transferrin are secondary to the changed iron metabolism and/or whether these changes directly influence the body economy and partition of iron between the metabolic pools.

Recently *Hallberg* and *Sölvell* [7] refined the technique for determining Fe^{55} and Fe^{59} in one and the same plasma sample. *Hallberg* and *Sölvell* used this technique to study the rate of iron absorption and the iron exchange between different body compartments at different Tr levels and at different levels of serum iron, i.e. at different degrees of iron saturation of the transferrin. The plasma iron turnover was continuously measured by following the turnover rate of a tracer amount of Fe^{59} i.v. when the iron administered by mouth was tagged with Fe^{55}. From their results it is evident that the iron and transferrin levels in plasma influence the absorption rate of iron—at high degree of saturation it was slow and at low degree it was faster [8–11]. The Tr-level in plasma thus seems to be one of the variables determining how efficiently the body may utilize iron administered orally. It has long been known that persons with iron deficiency, which is accompanied by increased Tr and decreased iron content in the plasma, utilize oral iron more efficiently than normal persons. A causal relationship thus seems to exist between iron absorption and plasma level of iron-free transferrin.

High—even hypernormal—erythropoesis may be seen in association

with moderately subnormal serum iron levels in patients with normal or increased Tr. In such cases it can be calculated from the minute volume of the blood in the bone marrow and from the serum iron values that every time the transferrin-bound iron passes through the bone marrow a considerable percentage must be retained by the erythropoetic cells [12]. The first suggestion how this may occur was presented by *Paoletti* et al. [13], who showed that reticulocytes utilize Tr-bound iron more efficiently than ionized iron. They also showed that immature red cells utilize the iron without consuming the transferrin. This has been confirmed in elaborate studies by *Jandl* et al. [14] and by *Schade* and *Woodworth* [15]. The latter further showed that the rate at which the reticulocytes absorb iron from the FeTr complex is independent of the Tr concentration in the test system and that their release of iron from the FeTr complex in contact with the erythrocyte membrane appears to be enzymatically catalyzed. From *Jandl* et al. immunological studies [16] it is probable that the immature erythrocytes in contrast to mature cells either have Tr bound to the membrane or contain a structural protein immunologically similar to Tr. An important, recent contribution by *Schade* and *Woodworth* [15] is the development of two techniques for measurement of the rate of iron incorporation into reticulocytes. With these they could show that most methods utilized for purification of Tr result in products from which iron is not adsorbed as efficiently by the reticulocytes as from the native plasma Tr, even though no denaturation could be shown by conventional physico-chemical methods. Minor changes in the tertiary structure of the protein molecule may thus impair the biological activity of transferrin. This finding must be borne in mind when analysing the literature in the field and will be of fundamental importance for future studies on Tr-metabolism which will concern pool sizes of Tr and of FeTr turn-over, synthesis and destruction in health and in disease. In all these studies tagged, isolated Tr will be used and the reliability of the results will depend on the intactness of the transferrins used.

The main biological significance of the transferrins is summarized in table 2.

Table 2

Main biological significance of transferrin

1. Transports iron in de-ionized form
2. Favours transportation of iron to the immature erythropoetic cells
3. Facilitates iron absorption from the mucosal cells
4. Iron-free transferrin inhibits bacterial growth in plasma by depriving the pathogens of iron

Steinbuch 1958 [17] and *Scheinberg* 1960 [18] have reviewed the literature on coeruloplasmin (Cp). The progress during the last years concerns mainly methods for purification of Cp [19–22] and its assumed molecular heterogeneity [23, 24]. The published methods for purification by chromatography represent valuable simplifications, but those methods which use chromatography below pH 5.2 are inadvisable since below this pH Cp will incur an irreversible loss of copper.

Turning to the physiology of Cp it may be stated that its biological function is still obscure.

Why is there than any reason to suspect that it may serve any biological function ?

1. The homeostasis of Cp in plasma is maintained within rather narrow limits [25].

2. Regular changes in the plasma concentration of Cp occur during disease. This regular variation was well described in 1941 by *Heilmeyer* et al. [26]. The regular Cp increase observed in various disorders might fit in well with *Heilmeyer*'s early assumption that serum copper (Cp) represents one of the substances constituting the general defence mechanisms of the body.

3. Cp homeostasis in plasma is under endocrine control. Administration of oestrogens is regularly followed by considerable increase of the plasma concentration—a finding which is probably related to the marked Cp-increase in plasma found during late pregnancy [18].

4. These three empirical observations have also been verified in higher animals.

No experimental data favour the hypothesis that Cp is important for copper transportation [27].

The observation that Cp is subnormal in Wilson's hepatolenticular degeneration has repeatedly been confirmed but some well established exceptions have been found [25, 28–30].

The low Cp content seems in Wilson's disease to depend on decreased synthesis in contrast to other conditions with low Cp content which generally depends on increased urinary or intestinal loss or on amino-acid deficiency. The nature of the basic defect in Wilson's disease remains uncertain. That the subnormal Cp-level found may merely be a secondary and not an essential feature must not be discounted [29].

Scheinberg has suggested that Cp influences the copper absorption directly or indirectly. This is an interesting working hypothesis [18].

It has long been known from in vitro studies that Cp has weak oxidase

properties of the type found for laccases but nobody has found any substrate of biological interest which is oxidized so rapidly in plasma in vivo that Cp can be suspected to be of importance for its metabolism.

During the last years investigators in the field of biochemical psychiatry suddenly got very excited about Cp and a series of controversial papers have appeared. A well balanced report was first presented by *Özek* on the Cp content in plasma from patients with schizophrenia [31]. This and later critical investigators have confirmed that no relationship seems to exist between schizophrenia and the Cp content of plasma measured as serum copper or as serum oxidase [18].

Relatively favourable therapeutic results have been reported by *Mårtens* et al. [32] after repeated injections of heavy doses of Cp in cases with acute or subacute schizophrenia, but the number of cases studied have been small and the Cp used has not been pure. The results can as yet not be accepted as any support for the idea of a dysregulation of plasma Cp in schizophrenia.

Haptoglobins

The haptoglobins (Hp)—the hemoglobin (Hb) binding plasma-protein —represent an other group of genetically determined proteins of very similar structure but of different molecular size. The different types of haptoglobins have been purified, but the chemical basis for the differences is not yet understood [12, 33]. This protein group is, therefore, of special interest for research workers in the field of genetic control of protein synthesis.

The biological function of the haptoglobins is not yet properly understood. Its high affinity for hemoglobin is well known. The fact that the complex HbHp is rapidly eliminated from the blood-stream explains the anhaptoglobinemia seen in hemolytic disorders. The individual variation in the so-called renal threshold for hemoglobin has been explained as a phenomenon secondary to the haptoglobin level in plasma, since in contrast to free Hb, the complex HpHb cannot pass the glomerulus membrane. It may, however, be questioned whether plasma Hp has any important biological function. In this connection, two facts are of interest: 1. Lifelong anhaptoglobinemia, e.g. in congenital spherocytosis, is not followed by any symptoms or any complicating disease reasonably ascribable to the plasma anhaptoglobinemia. 2. The normal variation of the plasma Hp level is much wider than is usually found for substances of fundamental biological importance. The variation range found is of the magnitude generally noted for substances under transport either

as precursors or as catabolites from metabolic processes. If Hp was a precursor, anhaptoglobinemia would probably be accompanied by deficiency symptoms. If it is a substance with a biological effect on the cellular level or a breakdown product, no deficiency symptoms need occur in anhaptoglobinemia since anhaptoglobinemia seems to be only secondary to increased elimination of Hp from plasma. If the site for biological activity is within or at the surface of the cells where Hp is synthetised, plasma haptoglobin is not necessarily missing at the functional sites even in anhaptoglobinemia.

Since haptoglobins now can be relatively easily isolated, the time is mature to tackle more basic problems than simply the Hp-level in the plasma:

Place and rate of synthesis, distribution space and turn-over have to be explored in health and disease. The variation of plasma Hp in disease is well known [34], but the increased plasma level found in all diseases with inflammatory reactions, such as tumors, infections, aseptic necrosis, antigen-antibody reactions, is most probably an expression of increased passage of Hp into the plasma. It is not known whether this depends only on increased synthesis or possibly also on changed distribution or on both. No evidence for decreased catabolism is available. In general, experience suggests that the plasma Hp level follows the intensity and the extent of inflammatory reaction, particularly the degree of involvement of the connective tissue.

One other observation that may be of interest is that treatment of women with massive doses of androgens may result in plasma Hp-levels 10 times the normal, but female dogs, in which the trauma induces a more striking increase in the plasma Hp than in human beings, do not respond to the androgens which are very effective in human beings.

Summary

A brief review is presented of recent advances in research concerning physiological function and plasma homeostasis of transferrins, coeruloplasmins and haptoglobins.

The concentration and degree of iron saturation of the serum transferrin seem to influence the iron absorption from the gut and the iron exchange between plasma and depots. The immature red cell seems to have special receptors for the iron-transferrin complex favouring their iron uptake. Better methods have been worked out for purification of coeruloplasmins and haptoglobins but the physiological functions are not yet properly understood in spite of regular changes in the plasma homeostasis found in health and in disease.

Zusammenfassung

Es wird ein kurzer Rückblick gegeben über neue Fortschritte in der Untersuchung der physiologischen Funktion und der Plasmahomöostase von Transferrinen, Coeruloplasminen und Haptoglobinen.

Die Konzentration und der Eisensättigungsgrad des Serumtransferrins scheinen die Eisenresorption aus dem Darm und den Eisenaustausch zwischen Plasma und Depot zu beeinflussen. Der unreife Erythrocyt scheint besondere Rezeptoren für den Eisen-Transferrin-Komplex zu besitzen, die seine Eisenaufnahme begünstigen. Es sind bessere Methoden zur Reingewinnung von Coeruloplasminen und von Haptoglobinen ausgearbeitet worden, aber die physiologischen Funktionen sind noch nicht völlig geklärt, trotz der regelmäßigen Schwankungen in der Plasmahomöostase, wie sie bei Gesundheit und Krankheit gefunden werden.

Résumé

L'auteur fait une courte révision des dernières recherches concernant les fonctions physiologiques et la teneur dans le plasma en transferrines, coeruloplasmines et haptoglobines.

La concentration et le degré de saturation en fer de la transferrine sérique semblent exercer une influence sur la résorption du fer, à partir de l'intestin, et de l'échange de fer entre le plasma et les dépôts. L'érythrocyte encore immature semble avoir de récepteurs tous particuliers pour le complexe transferrine-fer, afin de devenir capable d'assimiler le fer. De meilleurs procédés ont été mis au point pour purifier les coeruloplasmines et l'haptoglobine, mais leur rôle au point de vue physiologique n'a pas encore pu être nettement défini, bien que l'on ait constaté des différences typiques de l'homéostase du plasma chez les gens en bonne santé et chez les malades.

Riassunto

Si fà una breve rassegna dei progressi recenti nello studio delle funzioni fisiologiche e dell'omeostasi plasmatica della trasferrina, della ceruloplasmina ed aptoglobina.

La concentrazione ed il grado di saturazione in ferro della trasferrina sierica sembrano influenzare l'assorbimento intestinale del ferro ed i suoi scambi tra plasma e depositi. Le emazie immature possiedono presumibilmente ricettori speciali per il complesso ferro-trasferrina che permettono loro di immagazzinare il ferro. Metodi migliori sono stati elaborati per la purificazione della ceruloplasmina e dell'aptoglobina, ma le

loro funzioni fisiologiche non sono ancora state propriamente comprese nonostante che lo stato di salute e quello di malattia presentino variazioni regolari dell'omeostasi plasmatica.

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