INDEXED

PLASMA IRON AND THE TRANSPORT OF IRON IN THE ORGANISM

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INTRODUCTION

In 1925, Fontès and Thivolle found that iron regularly occurred in horse plasma and that this iron was not haemoglobin-iron (65). This observation was confirmed and extended by Barkan (11) and by Henriques and Roche (86), who found that human plasma and plasma from various animals contains 50 to 220 μg per cent iron. The same year Warburg and Krebs (212) arrived at the same conclusion by the use of a catalytic method (211) for determination of μg-quantities of iron. This plasma iron fraction (easily-split-off iron of plasma, non-haemoglobin-plasma iron) is now generally called serum iron or plasma iron.

The central position of the plasma iron in the intermediate haemoglobin metabolism is evident from the fact that the iron is transported with the blood as plasma iron from the organs where the red cells are destroyed to the organs where new red cells are formed, and that the main part of the plasma iron forms a link in a narrow cyclic process (haemoglobin → plasma iron → haemoglobin) rather independent of absorption, excretion and storage. In normal individuals about 75 per cent of the plasma iron is derived from the catabolism of haemoglobin while the remaining 25 per cent is derived from the catabolism of myoglobin, haeme-catalysts, storage iron and absorbed iron. More than a thousand articles have appeared treating with different aspects of serum iron in relation to

the intermediate iron metabolism of the body, but only those articles, which the author has considered to be of special importance, will be referred to here. A complete bibliography concerning papers on serum iron does not exist, but most of them can be found if the references in the numerous monographs (4, 9, 78, 80, 85, 113, 120, 150, 167, 182, 196, 198, 199, 202, 203) on serum iron are combined.

BIOCHEMICAL PART

Nature of serum iron

Barkan (11) showed that the iron in the serum was non-dialyzable at the pH of the blood, but after acidification iron appeared in the ultrafiltrate (12). The iron is thus protein bound and the iron-protein linkage is split on slight acidification. Starkenstein and Harvalik (185) fractionated sera to which iron salts had been added with ammonium sulphate and found that the iron was bound to the globulin fractions. With adsorption experiments Barkan showed (13) that the iron occurring in natural plasma was more firmly bound than the iron added to plasma. The precipitate obtained when saturating natural serum to 50 per cent with ammonium sulphate contained as much iron as the total serum. After partial electrophoretic separation of the serum proteins, Vahlquist (199) found that iron in serum was bound to both the albumins and globulins, but that the main part of the iron had a mobility corresponding to α- and β-globulins.

All these results were difficult to evaluate since very small traces of iron added as contaminants during the preparations could have influenced the results if iron added *in vitro* is not bound by serum in the same way as the iron originally existing in plasma, as indicated by Barkan (13). Yoshikawa *et al.* (218) therefore found it more satisfactory to work with natural plasma containing radioactive serum iron. They used plasma from dogs two hours after feeding the animals with radioactive iron. After saturation of the plasma to 50 per cent with ammonium sulphate, only about 15 per cent of the radioactive iron of the plasma was recovered in the precipitate, *i.e.*, in the globulin fraction. If the serum proteins were precipitated with trichloroacetic acid more than 90 per cent of the radioactive iron was recovered from the supernatant. When plasma was dialyzed against distilled water, about 5 per cent of the iron was found to be dialyzable. These results confirmed the earlier findings that iron in the serum is protein bound and acid soluble, but indicated that the iron in natural plasma is bound to some of the albumins.

Clinical experiments showed that the highest serum iron values obtained after peroral iron tolerance tests in healthy people was about 300 μg per cent (138) and that about the same values were obtained when ionized iron salts were injected intravenously. The subjects showed signs of intoxication if the intravenous iron injection was continued after this limit had been reached (207). This phenomenon made Holmberg propose the hypothesis that serum is only capable of forming a complex binding of iron up to a certain limit corresponding to about 300 μg per cent iron. Holmberg and Laurell (91) were able to show that the iron in the serum iron-protein complex at physiological pH does not react with $\alpha\alpha_1$-dipyridyl (a strong complex former for ferrous iron) in spite of the presence of sodium dithio-

nite. After addition of ferrous salts to serum the added iron is bound, up to a certain concentration (saturation limit), in non-dipyridyl-reacting form. On the other hand, all iron added in excess over the saturation limit reacts with dipyridyl. The mean value for the saturation limit in healthy persons corresponds to the binding of about 315 μg iron per 100 ml. serum, *i.e.*, normally the iron binding component is saturated only to about 30 per cent with iron as the normal serum iron is about 100 μg per cent. They also showed that when ferrous iron is added to serum a change in serum colour appears from yellow to yellow-red, easily determined in a photometer at about 5000 Å. This change of colour is developed successively with the addition of increasing small amounts of iron and seems to reach its maximum at the saturation limit. The same observation was independently made by Schade and Caroline (169). Earlier they had observed a similar change in the colour after addition of iron to hen's egg white (168). The component responsible for the latter reaction was identified as conalbumin (169). Schade *et al.* investigated the various protein fractions obtained by ethanol fractionation of plasma according to Cohn *et al.*, and found that the colour change occurs only when iron salts are added to fraction IV:3,4 (43). That the salmon pink colour appearing after addition of iron was due to the formation of a specific iron-protein complex was proved by a sensitive microbiological method (168).

Further evidence for the existence of a specific iron-binding protein component in serum was obtained by the observation that iron added to serum above the iron-binding capacity of serum (saturation limit) is dialyzable and can be adsorbed to AlC γ (120).

Terminology

Synonyms for the iron-binding protein of plasma are *transferrin*, *siderophilin*, *β_1-metal combining (pseudo) globulin*, and *iron-binding component of plasma*.

Synonyms for *serum iron* are *plasma iron*, *Fe-transferrin*, and *acid-soluble, non-haemoglobin iron of plasma*.

Increased concentration of serum iron is called *hyperferraemia* or *hypersideraemia*, and decreased concentration of serum iron is called *hypoferraemia* or *hyposideraemia*.

The transferrin concentration of serum (plasma) is generally expressed indirectly as the capacity of serum to bind iron specifically. Names such as *total iron-binding capacity (TIBC)* and *saturation limit* have been used.

The *total iron-binding capacity* minus the iron actually bound (serum iron) has been called the *latent iron-binding capacity*, the *unsaturated iron-binding capacity* (UIBC), or the *unbound iron-binding capacity*. Some authors have unfortunately used the term iron-binding capacity which elsewhere is generally used as an abbreviation for total iron-binding capacity.

Transferrin, the iron-binding component of plasma

Schade and Caroline have shown that fraction IV:3,4 of human plasma contained the iron-binding component (169). Further subfractionation of serum later showed that the main part of the iron-binding component could be further

purified and concentrated in fraction IV:7 (189). The iron-binding protein in iron-free state can be crystallized (112). The fractionation scheme of Cohn gives the iron-binding component in a mainly iron-free state. Laurell (119) saturated pig's plasma with iron before the fractionation, as the component is more stable when saturated with iron. Crystallization of the Fe-transferrin complex can easily be brought about by addition of ethanol to a salt-free solution of the pure protein at a pH about 6 until the first turbidity appears at 0°C. (122).

Iron-free transferrin shows no characteristic absorption of light in the visible region of the spectrum but, when saturated with iron, it has a strong red-orange colour with broad absorption maximum at 4700 Å (92, 188). In the ultraviolet, transferrin and Fe-transferrin have an absorption maximum at 2800 Å as do other proteins (188). The molecular weight of the protein is about 90,000 (188) (88,000 (119)). In electrophoresis (pH 7.5–8.8), transferrin moves with the group of β-globulins. It has therefore been called the β_1-metal combining globulin. Determinations of the iso-electric point have given varying results (5.9 (188), 4.4 (119)). The first value was obtained on analysis of human material, the latter on a preparation from pig. Moreover, different buffers and ionic strengths have been used. The investigated human protein was probably iron-free, but the protein from pig was saturated with iron. These differences may explain the diverging results. It has to be stressed that the mobility of this protein is markedly influenced by the kind and concentration of the inorganic ions in the buffer solution used. Further experimental work is necessary to elucidate this fact. The term "metal-combining *globulin*" used by Cohn is somewhat misleading as this protein is soluble in a solution half saturated with ammonium sulphate (119) and thus follows the albumins in this respect. Fe-transferrin is more soluble than iron-free transferrin on the alkaline side of its iso-electric point (44, 112). Solubility studies are presented by Koechlin (112).

Transferrin does not contain lipids (188). It seems to contain 1.8 per cent carbohydrate (188), but conalbumin, the corresponding protein from hen's egg white (213), is free of carbohydrate. The carbohydrate component is thus probably of no importance for the iron-binding capacity of transferrin. The nitrogen content is 14.7 per cent (112, 188).

Each molecule of transferrin can bind two atoms of iron (112, 119, 188). According to the present experimental facts, the two iron-binding groups of transferrin have such a position on the protein surface that there is no interaction between them, *i.e.*, that the energy content of the both Fe-protein linkages is the same (121).

The reaction between iron and transferrin is dependent on pH (63, 120, 188). If iron is added to a salt-free solution of transferrin at neutral or slightly alkaline reaction the added iron is bound to transferrin as is evident from the appearance of the typical change of colour. For complete binding of the iron practically no excess of iron has to be added. On acidification of the solution with dilute hydrochloric acid, the colour disappears at pH 4 and the iron can be dialyzed away. The dissociation of the complex and the loss of colour are readily reversed by readjust-

ing the system to pH 7 (63, 120, 188). A partial dissociation of the iron-transferrin complex can already be observed at pH 7 in the presence of serum (63, 120) or of phosphate ions (63), which means that the iron-transferrin complex is not completely undissociated within the physiological range of pH. The affinity constant has been determined to be approximately 10^7 at pH 7 (45). The data of the dissociation equilibrium are difficult to measure as the iron is rapidly hydrolyzed at neutral or slightly acid reaction. The iron-transferrin complex is stable up to pH 10, but is irreversibly destroyed at higher pH (63).

Transferrin can be acidified to pH 1.5 and the iron-binding capacity is nevertheless retained after neutralization (63), but it seems doubtful whether transferrin can be crystallized again after such a rough treatment since the crystallizability of conalbumin is lost if pHs below 4 are used during the preparation (213).

A yellow colour (light absorption maximum 4350 Å) appears on addition of copper ions to a metal-free solution of transferrin (92, 112, 188), but a more alkaline reaction is necessary for a complete binding of copper than for iron (188). That copper is bound by the same groups as iron is evident from the fact that the copper can be displaced by an equivalent amount of iron (92, 188). Within the physiological range of pH the copper is loosely bound to transferrin and the affinity of transferrin for copper is not higher than that of other serum proteins for copper (92). The main part of the serum copper is bound to an α_2-globulin (93). So far, no facts favour Cohn's hypothesis (43) that transferrin is of any importance for the copper transportation in the body, and this is also probably true for zinc, which is even more loosely bound than copper to transferrin (92, 188).

The same amount of iron is bound by transferrin (and serum) when either ferrous or ferric iron is added, and the change in absorption spectra obtained are identical (63, 64, 88). The reaction is more rapid with ferrous iron. The slower reaction with ferric iron may depend upon the lower solubility and the more rapid hydrolysis of ferric iron at neutral pH, since the autoxidation of ferrous iron was found to be a prerequisite to the binding (112). The result of magnetic susceptibility measurements performed by Michaelis on the iron-transferrin complex indicated that the iron is in the ferric state and that the bond is ionic (188).

It has been claimed that carbon dioxide (or bicarbonate) is necessary for the reaction between iron and transferrin (63, 64, 170). Based on comparisons between the complex iron compounds of hydroxylamine and carbon dioxide, and between iron and cyclic hydroxamic acids and the complexes formed between iron, carbon dioxide and transferrin, Fiala and Burk (63, 64) suggested that the groups responsible for the bond between iron and transferrin or conalbumin were hydroxamic acid groups. On the other hand, Fraenkel-Conrat (66, 67) found that bicarbonate is not necessary for the development of the coloured iron-transferrin complex, and he was unable to demonstrate the presence of hydroxylamino or alkylhydroxylamino groups of any kind in conalbumin and transferrin, even though sufficiently sensitive methods were available. The nature of the groups responsible for the high affinity of transferrin for iron is thus still obscure.

Other iron-protein compounds than transferrin in blood, of proposed importance for iron transportation

During the recent years some experiments with salting out and dialysis have been published (125, 126, 142, 143, 157, 191, 193), on the results of which the authors base the opinion that transferrin is not the only plasma protein of importance for the transportation of iron. Therefore Wallenius (209) reinvestigated the distribution of plasma iron between the different serum proteins by means of combined paper electrophoresis and autoradiography. He found no support for the assumption that more than one component is responsible for iron transportation. The activity was concentrated in the β-globulin, *i.e.*, in transferrin independently of whether the radioactive iron was given perorally or parenterally.

The deviating results of the salting out experiments can possibly be explained by the fact that addition of ammonium sulphate to unbuffered protein solutions will result in a pH of about 4–5.5, a range within which the natural serum-iron complex is partly dissociated. The conflicting results obtained with dialyzing experiments may be explained by the rapid hydrolysis of iron in solutions of pH higher than 5 in the absence of complex formers for iron.

The small amounts of *haemoglobin* found in plasma from normal subjects is probably a preparative artefact.

The iron in methaemalbumin (133) is not so firmly bound as that in haemoglobin, but since methaemalbumin in measurable quantities is only found in pathological sera (*e.g.*, from patients during haemolytic crisis) it is presumably an expression of an abnormal haemoglobin metabolism and probably does not play any role in normal haemoglobin catabolism. The plasma iron fraction observed by Plumier and Lambrechts (155) may possibly be methaemalbumin.

From a series of fractionated serum-iron determinations, Vannotti and Delachaux (202, 203) suggested that four different serum-iron fractions (A, B, C and D) occur in normal plasma. Their fractions A and B correspond to the usual serum iron fraction (transferrin-bound iron). Fraction C is obscure and fraction D seems to be haemoglobin iron. Their methods have, however, been severely criticized. (For literature, see Kooyman (113) and Hemmeler (85)). Normal plasma does not contain *ferritin* (the iron-storage protein) which has only been found in plasma in irreversible shock (72). *Red blood cells.* Barkan (12) adopted the same method for determination of acid-soluble iron in red blood cells as he had previously used for determination of plasma iron. The iron fraction obtained has since then been called "easily split off" blood iron. It was later (14) separated into two fractions E and E^1. Fraction E has been shown to be an artefact (74, 129). Grinstein and Moore (74) made a thorough study of the easily split-off iron with the aid of radio-iron and their data indicated that "easily split-off" blood iron as defined by Barkan *et al.* is an artefact and originates entirely or almost entirely from intact haemoglobin. Agner (1) has demonstrated *ferritin* in red blood cells from horse. If ferritin also exists in human erythrocytes (which is unproven), it will contribute to fraction E^1. Red blood cells contain no iron-free transferrin (120) but the possibility that they contain small amounts of Fe-transferrin can at the present not be excluded.

Nobody has investigated whether the *white blood cells* have any function in iron transportation or haemoglobin break down, in spite of the fact that leucocytes have a relatively high iron content.

Iron exchange between plasma and blood corpuscles

Barkan *et al.* (14, 15) showed an increase in serum iron when whole blood was left standing at 37°C. for 24 hours. In contrast thereto, Moore *et al.* (135) found no such increase. Lambrecht and Plumier (116) found an increase in serum iron after incubation, but this increase was highly dependent on the anticoagulants used and the degree of haemolysis during the incubation. There are no reasons to assume a passage of iron from vital red blood cells to plasma, since the haemoglobin degradation in the normal red blood cells seems to be negligible (74).

With radioactive iron it has been shown (130, 210) that *reticulocytes*, but not *mature erythrocytes*, can assimilate iron from plasma. The assimilation proceeds more rapidly when the cells are mixed with inorganic iron than with Fe-transferrin.

Taken as a whole, the exchange of iron between plasma and red blood cells seems to be negligible when compared with the rapid serum iron turnover.

Transferrin-bound iron also occurs in the *lymph* but in lower concentration than in the plasma (138). Preliminary experiments indicate that the transferrin concentration of the intestinal lymph (rat) is about one third of that of plasma (122). The transportation of iron via the lymphatic vessels during iron absorption seems to be negligible compared with the plasma transport (59).

Methods for determination of serum iron

A critical review of the methods used for serum iron determination (2, 25, 29 53, 78, 96, 110, 197, 199) has been written by Hemmeler (85) and only a few remarks on some discrepancies will be made here.

Most methods used are modifications of Heilmeyer and Ploetner's method (78). The proteins of the serum or the plasma are precipitated at acid reaction, the ferric iron in the filtrate is reduced by a suitable reagent to ferrous iron which is determined colorimetrically after addition of substances forming coloured complex iron compounds. Most modifications using this principle seem to be relatively reliable.

When analyzing sera from haemolyzed blood, incubation of the serum at a strong acid reaction for a short time gives more reliable values than incubation at a weak acid reaction for several hours.

There has been much discussion about the optimal pH for the determination of iron with phenantroline (85, 199). This discussion has been confusing since the main problem has not been stressed. The ferrous iron-phenantroline complex is stable with the same specific light absorption within the pH range 1.3–9. Therefore the pH suitable for the determination depends only on the reducing substances used. Hydrazin sulphate (197), thioglycollic acid (110) and sodium dithionite (91) rapidly reduce ferric iron in solutions of pH between 4.5 to 4.9. Hydroquinone is unsuitable for general use since it is only practicable within a

narrow pH range about 2 (199). Neutralisation of the acid serum extract to the pH wanted is most simply effected by addition of a suitable amount of a concentrated solution of sodium acetate (96, 197) or with a mixture of concentrated sodium acetate and ammonium hydroxide, to avoid titration of each sample as is described in most methods.

Most methods used for the determination of serum iron are not quite reliable when used to estimate the iron concentration of serum containing colloidal ferric oxides, *e.g.*, during the hours following an intravenous injection of saccharated ferric oxide. No critical study has been presented concerning this simple methodological problem.

Determination of the transferrin concentration of plasma (*saturation limit, iron-binding capacity*)

Two fundamentally different methods have been worked out for the determination of the transferrin concentration in serum. One is indirect and founded on the estimation of the iron-binding capacity of serum, *i.e.*, the amount of iron-reactive groups in serum with the same affinity for iron as those of transferrin (91, 169), while the other is direct and founded on the precipitation of the iron-binding protein with a specific antibody (101). The latter is complicated, but of great interest since with the aid of this method it has been definitely established (101) that changes in the iron-binding capacity of serum always run parallel with changes in the concentration of transferrin. Decrease of the iron-binding capacity of plasma thus depends upon a decrease of the concentration of transferrin and not upon blocking or destruction of the iron-binding groups of transferrin.

The total iron-binding capacity of serum (TIBC) can be determined in two different ways. One is founded on the fact that iron, when added to serum in excess of the iron-binding capacity, reacts with phenantroline (or $\alpha\alpha_1$-dipyridyl) after addition of a small amount of sodium dithionite (91, 120). The other is founded on the assumption that the whole colour-change of serum that occurs after addition of iron depends on the formation of the coloured iron-transferrin complex (169). The unsaturated iron-binding capacity (UIBC) can be computed from the change of the serum colour since the specific absorption of the iron-transferrin is known. The TIBC is then obtained by addition of the values for UIBC and for serum iron. This last mentioned method (169) has been employed by most investigators with minor modifications (37, 111, 158, 188) since it is extremely simple. It has, however, certain limitations. Lipaemic and icteric sera cannot, be analyzed (37, 158). False values are obtained if any coloured substance exists in serum which can be reduced by the ferrous iron generally used for the determination. That this simple method is not quite reliable even with normal plasma is evident from the values for TIBC recorded by some authors (18, 90, 180, 184) who have determined the TIBC of serum from the same person at short intervals and found variations which must be considered as extremely unlikely.

The phenantroline method (120) has not the limitations given above, but perhaps others. Concerning this method it has to be stressed that it is necessary to use sodium dithionite of highest quality (Merck pro analysi) and that the serum containing phenantroline and phenantroline iron has to be added drop by drop

to the hydrochloric acid with cautious shaking to effect a rapid decrease in pH of the serum added, since the iron liberated from the Fe-transferrin of the serum by the acid otherwise may be bound by phenantroline which results in a too low value for the TIBC of serum.

PHYSIOLOGICAL PART

Serum iron and transferrin in normal subjects

Adults. As mentioned later the serum iron shows pronounced diurnal variations with higher morning than evening values. Not every investigator has taken this into consideration when the materials have been gathered for analysis of the normal variation of serum iron. In general, however, the materials consist of samples gathered in the morning. The mean values recorded by different authors (85) show relatively good agreement and vary for adult men between 118 and 142 μg per cent and for adult women between 90 and 123 μg per cent. The sex difference is statistically significant (199). The statistical distribution has not been found quite binomial by most authors and the scattering differs considerably from material to material. From a practical point of view it can be stated that values above 220 μg per cent and below 70 μg per cent in men and above 200 μg per cent and below 60 μg per cent in women are rarely found, when blood is analyzed which has been withdrawn in the morning from fasting, thoroughly rested individuals.

The reason for the sex difference has been actively discussed, but no agreement has been reached (85, 200, 201). It has to be pointed out, however, that the mean value for the depot-iron fraction is greater per kg body weight in men than in women (117, 165). The contrary is found in rats (166, 215). It would therefore be of interest to investigate whether the mean value of serum iron in female rats is higher than in male rats.

The mean recorded values of the *transferrin concentration* of plasma, expressed as the total iron-binding capacity, vary between 300 and 360 μg per cent (37, 91, 120, 158, 204). The greatest material (100 cases) so far studied showed a mean value of 315 μg per cent (the standard deviation for a single observation was determined to be 33 μg per cent) (120). This value corresponds to a concentration of about 2.5 g transferrin per liter plasma. No statistically significant sex difference has so far been observed, but Cartwright (37) has found somewhat higher values in women than in men.

Children. The serum iron content of umbilical blood (about 150 μg per cent) is somewhat higher than in blood of normal individuals and of the mothers (50, 85, 89, 120, 199, 216). During the first two days of life a hypoferraemia of about 50 μg per cent develops. During the following 6 days there is a steady increase of the values to about 120 μg per cent. A new decline then begins and at an age of 8 months to 2 years a new minimum is reached with 50–60 μg per cent serum iron. The values thereafter again increase slowly up to the puberty when the mean values of adults are reached (4, 5, 46, 196, 199). The sex difference first appears after puberty (200, 201). No clearcut variation in serum iron is seen among the different age groups of normal adults (41).

No systematic study on the iron-binding capacity of serum during infancy and

childhood has been published. Laurell (120) found that serum from umbilical blood contained significantly lower amounts of transferrin (TIBC = 226 μg per cent) than serum from adults (TIBC = 315 μg per cent). Smith found higher iron binding capacity in children (aged 2½ to 10 years) than in adults (184).

The *diurnal variation* of the serum iron values was first observed by Vahlquist (199), and later analyzed by many authors (61, 76, 84, 97, 147, 177, 192, 208). Most authors find the serum iron values about 10 to 30 per cent higher in the morning than in the evening. Hemmeler (85) who has performed the greatest experimental work on this subject states that the diurnal rhythm with higher morning than evening values is valid only if the patients have slept during the night and that the increase of serum iron during the night is highly dependent on the duration of the sleep. The diurnal rhythm of the serum iron is found reversed in normal subjects working nights and sleeping during the day-time (85, 97, 177, 208).

No diurnal variation of the iron-binding capacity of serum has been observed (122).

With repeated determinations of serum iron in the same individual at intervals of *days, weeks* and *months*, some authors (78, 135, 182) have found only slight variations from time to time (30 μg per cent or less), others (85, 96, 113, 167) a considerably higher variation.

Repeated determinations of the TIBC in the same individual suggest that the transferrin concentration is rather constant over long periods in healthy individuals with normal blood status.

Seasonal variation is of little practical importance, as the small differences found (higher autumn than spring values) are not statistically significant (198, 199).

Meals. No common foodstuff contains so much available iron that the intake of meals can influence the serum iron (78, 182, 195), but as later shown, intake of absorbable iron compounds gives a sharp increase of the serum iron level.

Muscular work does not seem to influence the serum iron values (19, 55, 85).

The *menstrual cycle* is without influence on the serum iron values (25, 36, 97, 179). Dahl (51) and Powell (156) state, however, that the serum iron values are highest shortly before, and lowest during and immediately after menstruation.

Pregnancy. The extensive literature on the variations of the serum iron during pregnancy has been reviewed by Lundström (131) and Ventura (204). The results of earlier investigators diverge surprisingly, but authors of the more recent publications (50, 60, 89, 120, 131, 187, 204) agree that the serum iron values are unchanged during the first 20 weeks of pregnancy, after which a slight hypoferraemia develops. The lowest values are found during the two last months of pregnancy (the decrease is about 30 per cent). This decrease is greater than can be expected from the effect of the hydraemia of pregnancy.

During the hours following delivery a further decrease in serum iron appears and the normal serum iron level is not reached until some months after parturition (50, 144, 161, 199).

The total *iron-binding capacity* of plasma is unchanged during the first 4 months

of pregnancy, thereafter increases considerably, and reaches its maximum during the last two months. The increase found is about 50 per cent (60, 89, 120, 204). Rath *et al.* found only a slight increase (159).

During the two months following delivery the transferring level is gradually normalized (60).

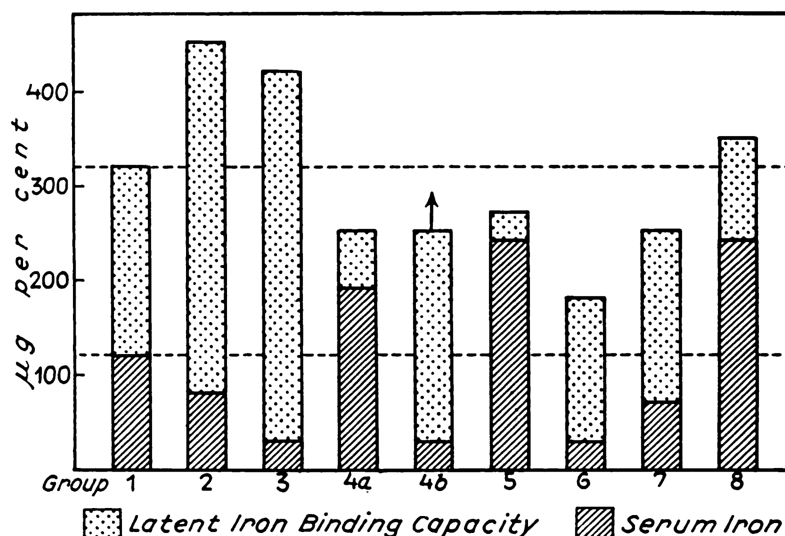

FIG. 1. Serum iron and total iron binding capacity of serum in various disturbances of iron metabolism.

1. Healthy adults (37, 120, 158).
2. Late pregnancy (60, 89, 120, 204).
3. Chronic iron deficiency (27, 28, 37, 78, 90, 103, 106, 120, 124, 158, 165, 183, 184); polycythaemia vera (28, 30, 36, 85, 95, 214).

4a. Haemolytic (8, 28, 36, 124, 153, 154, 171, 184), pernicious (30, 54, 120, 136, 158, 206, 207), aplastic (30, 36, 80, 120, 163, 199, 214), and myelophthisic (214), anaemias (fetus at parturition).

4b. Haemolytic and pernicious anaemias during remission.

5. Haemochromatosis (7, 10, 18, 44, 56, 68, 85, 100, 158) and transfusion haemosiderosis (158).
6. Acute infections (Hodgkin's disease) (34, 35, 37, 48, 57, 78, 80, 87, 120, 160).
7. Chronic infections (35, 37, 105, 109, 113, 120, 142, 147, 198), malignant tumours (85, 120), myelomatosis (120), uraemia (28, 36, 85, 120), leukaemias (23, 28, 36, 120, 158), cirrhosis of the liver (18, 120, 128), acute or subacute liver atrophy (18, 85).
8. Acute hepatitis (second to fifth week) (17, 20, 21, 26, 58, 83, 85, 120, 128, 181).

Serum iron and transferrin level in various diseases

The typical changes of serum iron and total iron-binding capacity of serum found in various diseases are schematically represented in figure 1. Groups 2 and 3 represent conditions with increased requirement for iron. These conditions are characterized by the following facts: i) the quotient between serum iron and latent iron-binding capacity of serum is considerably lower than normal; ii) in-

gested iron is utilized better than normally; iii) iron is mobilized from the iron depots. In most cases with iron deficiency the TIBC of blood is clearly increased as judged from direct determinations (37, 120, 158, 184) and from the degree of hyperferraemia attained after peroral iron tolerance tests (103, 164). In case of essential hypochromic anaemia, however, furthe1 studies on the TIBC of serum are necessary to elucidate whether this disease is accompanied by a disturbed regulation of the transferrin concentration of serum and the intermediate iron metabolism or whether it depends on poor utilization of ingested iron (82, 102, 190).

Groups 4 and 5 represent conditions with progressively increasing stores of iron. It is characteristic of these conditions that the quotient between serum iron and latent iron-binding capacity of serum is considerably higher than normal. During spontaneous remissions or following specific treatment of the diseases belonging to group 4, hypoferraemia develops and the TIBC increases (inversion of the quotient between serum iron and latent iron-binding capacity), and these changes are combined with a mobilization of iron from the storage organs.

Groups 6 and 7 represent the findings during infections and allied conditions. The hypoferraemia and the decreased TIBC are most pronounced during acute infections with high fever and during severe chronic diseases. The anaemia which often develops during these diseases does not, however, depend on an iron deficiency as earlier supposed. The retarded erythropoiesis seems to be caused by an inhibition of the maturation of the red blood cells at a stage before the incorporation of iron (34) and this anaemia is not influenced by peroral (172, 173) or intravenous iron therapy (114, 115). The hypoferraemia is supposed to depend on an increased accumulation of iron in the reticulo-endothelial system (38, 73, 80, 165). The form in which iron is bound by the reticulo-endothelial system in these diseases has not been explored. During the regression of the infections the iron is liberated and made available for haemoglobin synthesis at the same time as the transferrin concentration is normalized. A series of severe systemic diseases (malignant tumours, myelomatosis, etc.) show the same type of changes as found in infections, but less pronounced (Group 7).

The only disease which belongs to group 8 is acute hepatitis. Although the main storage organ for iron is attacked in this acute infection, this fact cannot explain the hyperferraemia sufficiently since there is no clear correlation between the severity of the disease and the degree of hyperferraemia. Marked hyperferraemia is often found in mild cases (85, 128), and in acute or subacute yellow atrophy hypoferraemia often develops after one or two weeks (18, 85). The reason for the hyperferraemia during acute hepatitis has been discussed, but no definite conclusion has been reached (85).

Effect of iron dosage

Iron given intravenously. A dose of iron salts corresponding to 5 to 10 mg Fe administered intravenously to human subjects may be sufficient to provoke slight toxic symptoms, especially when the iron is injected rapidly. The symptoms consist of flushing of the face. nasal stuffiness, sneezing, lacrimation, nausea,

vomiting and paraesthesias in the arm in which the iron is injected (70). They disappear, however, rapidly. This toxic reaction seems to be caused by an abrupt increase of the iron ion activity of plasma, which normally is extremely low. The amount of iron tolerated during slow injection depends mainly on the amount of iron-free transferrin in the blood which can de-ionize the injected iron (91, 207). Patients with a small amount of iron-free transferrin in plasma (*e.g.*, pernicious anaemia, haemochromatosis etc.) show symptoms of intolerance after injection of a few milligrams of iron and patients with high amounts of iron-free transferrin (iron deficiency, pregnancy) can tolerate about 20 mg. If the injection of iron is continued after the appearance of toxic symptoms the main part of the injected iron rapidly leaves the circulation, and the serum iron values rise only slowly. In animal experiments 70–80 per cent of the iron injected beyond the iron-binding capacity of plasma disappears within a few minutes (141), and it is reasonable to assume that a rapid distribution of the ionized iron between the blood plasma and the extracellular tissue fluid takes place. The iron concentration of plasma obtained some minutes after injection of 10 mg of iron (17, 18, 198, 207) is a good expression of the total iron-binding capacity of plasma except in cases with abnormally high transferrin concentration of the plasma.

The decrease of the serum iron during the hours following the injection varies. In normal individuals the serum iron decreases 0–80 μg per cent during the first two hours after the injection, and the initial serum iron values are not reached until after five to ten hours. In patients with iron deficiency (207), with pernicious anaemia during remission (207), and with acute infections (34, 198) a more rapid decrease has generally been observed. The increased elimination rate in these conditions can be explained by the increased bone marrow activity or, in the case of infections, by the increased affinity of the reticulo-endothelial system for iron.

The total iron-binding capacity of serum is not influenced during the change of serum iron following an intravenous injection of iron (37).

Intravenous iron therapy. Heath *et al.* (77) found that 96 per cent of intravenously injected iron was utilized for haemoglobin synthesis, but the toxic reactions were too severe to warrant its clinical use. The toxicity of the iron preparations depends on the rate with which iron ions are liberated from them. Solutions of iron cacodylate ($Fe[(CH_3)_2As_2O_3]_3$) (127) or sodium-bis-($\alpha\gamma$-dioxy-$\beta\beta$-dimethylbutyrato)-ferrate (194) have been used in the clinic since they form iron ions more slowly than simple inorganic iron salts, but nevertheless the tolerated dose of these compounds has often been found insufficient for general clinical use. Ferritin-iron can, however, be injected in the amounts desired (daily injection of 100–200 mg. Fe) without toxic manifestations, but has the disadvantage of being antigenic when not prepared from human material (3). Saccharated ferric oxide (148, 149) seems to be a rather valuable therapeutic agent. Daily injections of 100 mg and more of iron in this form are generally well tolerated. It is rapidly taken up by the reticulo-endothelial system and in this way eliminated from the blood (40, 178). The urinary loss is less than 10 per cent of the dose (178). After intravenous administration of saccharated ferric oxide, the iron from this com-

pound is so rapidly utilized that iron no longer need be the limiting factor in the blood regeneration of subjects with anaemias of iron-deficiency type (24, 94, 108). However, the rate of ionization does not exceed the rate at which ferritin formation and other iron ion detoxicating mechanisms of the body can prevent an increase of the iron ion activity in the tissues which causes toxic symptoms. The slow liberation of iron from the colloid is evident from clinical experiments; thus the plasma transferrin is not saturated with iron even 30 minutes after the intravenous injection of 100-200 mg (33, 90).

The high values for total iron-binding capacity of serum and the low values for serum iron in patients with iron deficiency are normalized during the course of intravenous treatment with saccharated ferric oxide (32, 90, 111).

Agner has developed another iron preparation in which the iron is linked to a soluble, high molecular carbohydrate, and built up in a manner similar to ferritin (9). Clinical experiments with this preparation have given results which seem to be as satisfactory as those obtained with various preparations of saccharated iron oxide (9).

No experiments have been published to determine whether the injected iron colloids circulate free or bound to protein in the plasma until they are taken up by the reticulo-endothelial system.

Iron administered perorally. The amount of iron absorbed from the intestinal tract in healthy people is regulated by the demand of the organism for iron. After oral loadings with large amounts of iron the serum iron level responds with a considerable increase and some iron is thus absorbed even in individuals who must be regarded as being in complete iron equilibrium. The serum iron curves after oral iron loadings are, however, difficult to interpret since the serum iron increase is influenced by different factors such as the rate of iron absorption, the rate of iron elimination from the blood, the plasma volume, the transferrin concentration, etc. The serum iron increase can thus not be used as a reliable expression for the amount of iron absorbed.

The dose necessary to influence the serum iron level in normal subjects by peroral administration of iron is about 50 to 100 mg. The height of the serum iron increase is roughly directly proportional to the quantity given up to a point at which intestinal irritation is great enough to interfere materially with intestinal motility (137). This seems to coincide with the point where the total iron-binding capacity of plasma is fully utilized and the disturbed intestinal motility which is manifested by diarrhoea may depend on a local irritation of the intestinal wall by the increased Fe ion activity obtained when the total iron-binding capacity of plasma is exceeded. The serum iron absorption curves are often higher following the ingestion of ferrous than ferric salts (88, 137, 139, 140).

The literature on serum iron curves after Fe-loadings in patients with different diseases has been comprehensively reviewed by Kooyman (113), Waldenström (207), Jasinski (103, 104) and Roth (164). The results of the different investigators are not quite comparable because varying doses of iron have been used. The average increase of the serum iron varies between 50 and 100 μg per cent after loading normal subjects with iron salts (82, 104, 132, 147, 164, 200). In patients

with iron deficiency after chronic loss of blood (haemorrhages from the gastro-intestinal tract or the uterus), the rise of the serum iron is about 200–250 μg per cent (79, 82, 105, 106, 132).

The high serum iron increase in these patients is followed by a rapid decrease probably depending on an excessive elimination rate, as also seen after intravenous administration. The same type of curve has been observed in children with iron defiency (107, 164) and in some cases of polycythaemia vera (151, 207). Patients with other iron deficiency states, such as essential (idiopathic) hypochromic anaemia, chlorosis etc., do not regularly react to iron ingestion with a supranormal serum iron rise, but a normal or subnormal rise may be obtained (79, 82, 102). Typical iron deficiency curves may, however, be obtained if the iron loading is repeated after some weeks of iron therapy (190).

During acute infections not only is the serum iron lowered, but the resorption curve is also depressed. In some cases the serum iron values may even be unchanged after the loading (34, 104, 173). The curves in subjects with chronic infections (34, 113, 147) and malignant tumours (105, 207) are similar to those in patients with acute infections, but the abnormality is less pronounced.

Subnormal serum iron increase is also obtained in untreated patients with pernicious anaemia (207), haemochromatosis (18) and other diseases with hyperferraemia. In such cases the probable explanation is that the transferrin is already almost or completely saturated with iron before the loading. A normal rise has been observed (190, 207) in acute hepatitis in spite of the hyperferraemia (corresponding to the increased transferrin concentration in the blood of such patients). However, most cases of acute hepatitis show a subnormal serum iron increase after the iron ingestion (17).

After gastrectomy a subnormal serum iron increase may be obtained, probably on account of impaired iron absorption (123).

Influence of vitamins, hormones and the central nervous system on the serum iron level

There is no connection between vitamin C and the intermediate iron metabolism (198). Peroral or parenteral administration of aneurin, nicotinic acid amide, pyridoxin, and pantothenic acid has no influence on the serum iron level in normal subjects (118, 145, 146), but the serum iron value decreases slightly after intravenous injection of large amounts of lactoflavin (145, 186). Pyridoxin deficiency in dogs is followed by an inhibited bone marrow activity and hyperferraemia (75, 134, 217). Folic acid and B_{12} as well as liver extracts supplied to patients with pernicious anaemia are followed by hypoferraemia during the period of blood regeneration.

Whereas the serum iron level seems to be uninfluenced by nicotinic acid amide, hyperferraemia and slight hyperbilirubinaemia are regularly produced in normal subjects 90 minutes after peroral or intravenous administration of 20–50 mg nicotinic acid (17, 122). This effect seems to depend on an increased catabolism of the erythrocytes. The transferrin level remains unchanged (122).

The serum iron values fall within the normal variation range in patients suffering from most endocrine disorders (85, 113, 128). A slight hyperferraemia has

relatively regularly been found in patients with pituitary hypofunction and after experimental hypophysectomy (47). In normal subjects the serum iron level is unchanged after injection of ACTH (39), testosterone, stilboestrol and desoxycorticosterone (128). In rabbits of both sexes testosterone and progesterone are without effect, but injections of oesterone preparations result in a rapid decrease of the plasma iron level (152). The presence of a hypothalamic centre for serum iron regulation has been proposed (62, 84, 85, 177, 190) This hypothesis is based on the existence of the diurnal serum iron variation and the fact that a slight serum iron decrease often comes about some hours after lumbar puncture, encephalography or electroshock (22, 85, 99, 177). Such treatment of the patient may, however, cause a stress and it is furthermore known that a reticulocytosis may appear after lumbar puncture (81). The observed serum iron variations may also be explained as secondary to changes in the functional state of the bone marrow or the reticulo-endothelial system.

Serum iron and the functional state of the reticulo-endothelial system

Dogs in which sterile turpentine abscesses were produced developed within 48 hours a pronounced hypoferraemia and a decreased transferrin level (35), followed by a slight anaemia (162). These changes are similar to those found during infection (80), and after injections of toxins (196) or species-foreign proteins (80, 205). This hypoferraemia is combined with accumulation of iron in the reticulo-endothelial system and with an increased elimination rate of iron from plasma (34, 147). It has therefore been assumed that the reticulo-endothelial system is of importance for the regulation of the serum iron level (78). Experiments to block the reticulo-endothelial system have given results which are difficult to interpret. Injection of electrocollargol (177) or colloidal thorium dioxide (38, 196) results within 24 hours in hypoferraemia in animals (rabbit, dog). On the other hand, if the administration of thorium dioxide to dogs is continued for 3 consecutive days, a considerable increase of the plasma iron (20 to 1500 per cent) is developed following the initial decrease (38). This increase generally begins on the first day following the last injection and reaches a maximum on the 3rd to the 7th day. This hyperferraemia is combined with an increase of the total iron-binding capacity of serum to more than double the initial values. Such hyperferraemic animals do not react with hypoferraemia and decreased total iron-binding capacity upon injection of turpentine (38).

An acute hypoferraemia (within 3–8 hours) can be produced in dogs by injections of large amounts of histamine (40, 174, 175), epinephrine (40, 42, 177), adrenal cortical extracts and ACTH (40). Administration of dibenamine to the animals before the injection of epinephrine does not prevent the hypoferraemia (40). The effect of these drugs on the transferrin level has not been reported.

That the effect of ACTH is mediated through the adrenal cortex is demonstrated by the fact that no hypoferraemia is produced by the injection of ACTH in adrenalectomized dogs (40). The hypoferraemic effect of epinephrine is significantly less in adrenalectomized dogs than in intact animals. Additional evidence of a connection between the adrenal cortex and the functional state of

the reticulo-endothelial system is afforded by the experiments on the rate of disappearance from the plasma of intravenously injected saccharated oxide of iron. As mentioned, the iron given in this form is taken up by the reticuloendothelial system during the first phase of metabolism (9). The colloidal iron is removed more rapidly from the plasma of intact dogs given ACTH, and more slowly from the plasma of adrenalectomized dogs and from dogs previously given thorium dioxide (39, 40).

Serum iron turnover

In man, the amount of iron required daily for haemoglobin synthesis, calculated from the mean life time of the red blood cells, is about 20 to 25 mg. If the main part of the aged red blood cells is destroyed outside the bone marrow, this implies that an amount of iron about six times greater than the total serum iron quantity of about 4 mg. is daily transported to and consumed by the bone marrow. The actual serum iron turnover has been determined in human subjects after injection of tracer quantities of highly radioactive iron (95) and of transferrin labeled with radioactive iron (214). The disappearance of the radioactive iron from the circulating plasma was exponential and the half-time of the disappearance in the normals was about 100 minutes (95, 214). From this figure the average value for plasma iron turnover was calculated to be 27 mg. per day (95). From the amount of radioactive iron found in the haemoglobin of the red blood cells a certain time after the administration the average value for the fraction of red blood cell iron renewed per day was estimated to be 0.85 per cent, which corresponds to about 75 per cent of the calculated total plasma iron turnover. If the iron stores were filled up the rate of removal of radioactive iron from the serum was found higher than normal in patients with excessive bone marrow activity (*e.g.*, polycythaemia vera, haemolytic anaemia, untreated pernicious anaemia and occasionally in leukaemias), and lower than normal in patients with subnormal bone marrow activity (*e.g.*, aplastic anaemia and uraemia) (214). This suggests strongly that the rate of removal of radioactive iron is a true index of the production of red blood cells in the body (214). The influence of the iron stores on the disappearance rate of injected radioactive iron is small in comparison with the influence of the bone marrow (214). Thus, in a patient with polycythaemia, Wasserman found that the half-life time of radioactive iron in plasma increased from 30 to 145 minutes after treatment with radioactive phosphate (214). Feeding experiments performed with normal foodstuffs labeled with radio iron and fed to normal subjects indicated that probably less than one per cent of the total plasma iron turnover was concerned with absorption (95).

Previously Cruz *et al.* showed that the newly liberated iron from destroyed red blood cells in normal dogs is very effectively utilized in blood regeneration even though ample storage iron is available (49). This means that the exchange between the plasma iron and the storage iron is slower than between the plasma and the iron newly liberated from catabolized red blood cells. The relatively slow exchange between plasma iron and storage iron is also evident from the already mentioned fact that the increased serum iron level following an injection of some

milligrams of iron is only slowly normalized in normal subjects in spite of the rapid iron turnover. The hypoferraemia which remains for weeks following an acute haemorrhage in subjects with normal iron stores may also be explained by a relatively slow exchange of iron between the plasma and the stores, *i.e.*, a slow mobilization of iron from the depots.

In this way all experimental evidence indicates that the main part of the red blood cells is normally destroyed outside the bone marrow and that the rate of erythropoiesis is the principal factor determining the serum iron turnover.

In consequence, the relatively stable serum iron level in normal subjects must mean that the formation and destruction of the red blood cells are going on during the whole day at approximately the same relative rate. It is reasonable to assume that the diurnal serum iron variation depends on a somewhat lower rate of incorporation of iron in the immature erythrocytes during the night when compared with the rate of liberation of iron from destroyed cells.

The turnover of transferrin has not been determined but it is reasonable to assume that it is of the same magnitude as that of other serum proteins.

DISCUSSION

The data thus far collected concerning the metabolism of transferrin and serum iron support the theory that the transferrin in plasma is the carrier of iron and that the iron leaves and enters the blood stream in ionized form (121). The mechanism regulating the transferrin concentration is a little known as the mechanism regulating the concentration of other serum proteins. We only know that the transferrin concentration increases during enhanced requirement for iron and that it decreases during haemolytic and pernicious anaemia, during infections and haematologically allied conditions (see page 381). The diseases which are followed by a decreased transferrin level are on the whole those in which there is a decreased level of serum albumin. The regular increase of the transferrin concentration of the blood during iron deficiency and pregnancy strongly supports the assumption that the transferrin level is of importance for iron mobilization and absorption.

The only likely explanation of the proposed influence of transferrin on iron metabolism is based on the following reversible reaction: Fe^{+++} + Fe-free transferrin $\rightleftharpoons$ Fe-transferrin. The corresponding equilibrium is expressed by the equation: $\frac{[\text{Fe-transferrin}]}{[\text{Fe-free transferrin}]} = K \cdot [Fe^{+++}]$. The influence of the transferrin level on the concentration of ionized iron in plasma can be comprehended from this formula and further it is clear that the plasma in this way may mediate an equilibrium between the iron ion activity in the different organs of the body. This hypothesis explains why iron is mobilized from the iron stores during increased iron demand in the bone marrow and during pregnancy when the iron is lost to the fetus. Under these conditions the iron ion activity decreases in plasma and a concentration fall is established between the body cells and the plasma.

The hypothesis is purely schematic and factors such as pH and "redox" levels

in plasma and the different cells are certainly of great importance for the actual iron ion activity. We do not know whether only ferrous iron or both ferrous and ferric iron can pass the cell membranes. The solubility of ferrous iron is much higher in the physiological range of pH than that of ferric iron, but even if the ferric iron concentration in plasma is extremely small this does not speak against the hypothesis since the equilibrium reaction between iron and transferrin is very rapid. We know that blood regeneration is increased during the weeks following an acute haemorrhage and during polycythaemia in spite of hypoferraemia. The average half-life time of plasma iron in patients with markedly increased serum iron turnover (*e.g.*, in polycythaemia vera) can be computed to be about 10 to 30 minutes from the figures given by Huff *et al.* (95); even in this case the main part of the administered iron is assimilated by the bone marrow, which is evident from the amount of radioactive iron appearing in the red blood cells. These results must signify that during increased haemopoietic activity more than 10 per cent of the total amount of the plasma iron passing through the bone marrow is retained even when the calculation includes a rather high minute volume for the bone marrow. This forms a biological proof for the rapid dissociation of the iron-transferrin complex in the body since no facts have been present which favour the theoretical possibility that the whole iron-transferrin complex is consumed in the bone marrow. The definite proof or disproof of the latter possibility can only be obtained by measuring the transferrin and the plasma iron concentration in the arterial and the venous blood from red bone marrow.

The equilibrium between the iron ion activity in plasma and different organs is probably established rapidly. The variations of the serum iron can be explained by the assumption of a relatively slow exchange between ionized iron and the storage iron (ferritin). This is further supported by Granick's finding (71) that the maximal ferritin concentration in the intestinal mucosal cells after peroral iron loading is obtained after about 7 hours, but the highest iron ion activity is probably reached during the two first hours after the loading as seen, for example, from the early maximum of the serum iron values after peroral intake of iron.

All these points of view can explain the observed variations of the serum iron during health and disease as being caused by a series of factors of which the variations of the transferrin level, the amount of available storage iron, the relative rate between the formation and the destruction of the various iron containing porphyrine compounds, and the iron demand of the reticulo-endothelial system are the most important. The low serum iron during infections and allied conditions can be understood if it is assumed that the iron temporarily is accumulated in the reticulo-endothelial system in a form nonavailable for intermediate iron metabolism.

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