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# Hemoglobin and its derivatives Iron

*General Medicine*

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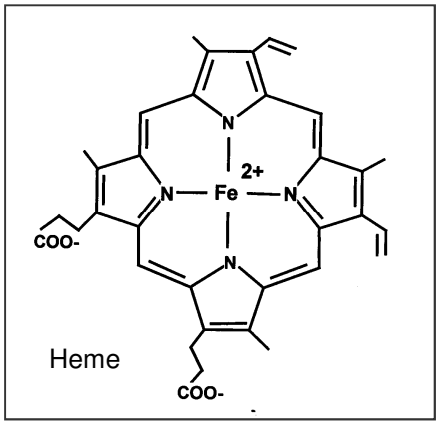
# 1 Hemoglobin

Hemoglobin is the red pigment in blood that provides transport of oxygen from lungs to tissues, and transport of $CO_2$ and protons from peripheral tissues to the lungs. It is a **tetrameric protein** (made of four subunits); always two and two subunits are identical; and each subunit contains one **heme**. In physiologic types of hemoglobin, four kinds of polypeptide subunits occur: α, β, γ, and δ, which differ in number and sequence of amino acids. The hemoglobin tetramer always contains two subunits α, and two of a different kind that determine the character of the whole hemoglobin molecule. In adults, the prevailing form is hemoglobin A that consists of two α subunits (141 amino acids), and two β subunits (146 amino acids).

The heme structure is based on **protoporphyrin**, made of 4 pyrrole rings connected with methenyl bridges, holding an iron atom in the centre. The heme iron is hexavalent in total: four coordination bonds connect it to the pyrrole nitrogens; fifth bond attaches to the imidazole amino group of a histidine in the globin chain; and the sixth valence is available for molecule of oxygen ($O_2$).




## *1.1 Hemoglobin in blood*

**Principle of estimation:**

The photometric assay is based on **oxidation of ferrous iron** in hemoglobin to ferric iron with **potassium ferricyanide**. The resulting **methemoglobin** is converted in the next reaction with **potassium cyanide** to a very stable **cyanmethemoglobin** with a broad absorption maximum at 540 nm (Fig. 1).

**1. Oxidation of hemoglobin to methemoglobin:**

$$\underset{\text{Hemoglobin}}{HbFe^{2+}} + [Fe^{3+}(CN)_6]^{3-} \rightarrow \underset{\text{methemoglobin}}{HbFe^{3+}} + [Fe^{2+}(CN)_6]^{4-}$$

**2. Conversion of methemoglobin to cyanmethemoglobin:**

$$\underset{\text{methemoglobin}}{HbFe^{3+}} + CN^- \rightarrow \underset{\text{cyanmethemoglobin}}{HbFe^{3+}\,CN}$$

**Fig.1: Principle of the estimation of hemoglobin in blood**

**Evaluation:**

Estimation of hemoglobin in the blood is one of the most essential laboratory examinations. When hemoglobin and/or number of erythrocytes below the lower limit of reference values are found, the term **anemia** is used. It is a common finding in clinical practice. Anemia leads to low transport capacity for oxygen and impairment of tissue respiration.

Anemia in principle results from any condition in which erythropoiesis does not match the body demands for red blood cells. It develops due to:

- Blood loss
- Increased destruction of red blood cells
- Insufficient production of red blood cells

**Table 1: Some causes of anemia:**

| | |
|---|---|
| ***Anemia due to loss of blood*** | • Acute bleeding<br>• Chronic bleeding |
| ***Anemia due to increased destruction of erythrocytes (hemolysis)*** | • Auto immune hemolytic anemia<br>- auto-antibodies against erythrocytes<br>• Defects in erythrocyte membrane<br>- altered composition of erythrocyte membrane<br>• Inborn errors in erythrocyte enzymes<br>- pyruvate kinase<br>- glucose 6-phosphate dehydrogenase<br>• Unstable hemoglobins (hemoglobinopathies)<br>- e.g. hemoglobin S in sickle cell anaemia |
| ***Anemia due to insufficient production of erythrocytes*** | • Deficit of factors essential for erythropoiesis:<br>- iron<br>- vitamin $B_{12}$<br>- folic acid<br>- erythropoietin (chronic renal disease)<br>- other substances (e.g. vitamins $B_1$, $B_6$)<br>• Anemia due to chemical, physical or radiation damage<br>• Anemia in chronic inflammatory, infectious, and tumor diseases |

Increased values of hemoglobin in blood may result from dehydration, chronic respiratory insufficiency, or (rarely) genuine over-production of red blood cells in polycytemia vera.

**Reference values for hemoglobin in blood:**
**Healthy adult man: 130-180 g/l**
**Healthy adult woman, children: 120-160 g/l**

## *1.2 Hemoglobin in urine*

### Principle of estimation:

Hemoglobin catalyses decomposition of hydrogen peroxide; the nascent oxygen is able to oxidize suitable substrates, e.g. derivatives of benzidine such as ortho-tolidine. Unlike action of peroxidases, this activity is resistant to heat denaturation and represents no true enzyme activity (catalysis is caused by the heme iron). This **pseudoperoxidase activity** is used for sensitive, albeit unspecific detection of hemoglobin or trace amount of blood. The reaction mixture must contain a chromogenic substrate and hydrogen peroxide (Fig. 2).

The **diagnostic strips** for detection of blood in the urine are based on the same principle. The reagent zone contains chromogen together with a stabilized hydrogen peroxide (e.g. cumene hydroperoxide). In the presence of free hemoglobin (**hemoglobinuria**) the test zone turns evenly green. If red blood cells are present in the urine (**erythrocyturia**), blue-green dots or spots develop on the strip.

In addition to hemoglobin, also **myoglobin** released into urine due to destruction of skeletal muscles (rhabdomyolysis, the crush syndrome) gives the pseudoperoxidase reaction. The test can become positive also due to presence of peroxidases from leukocytes, bacteria or yeast, which can all occur in the urine. In order to exclude this false positivity due to cellular peroxidases, the test should be performed with boiled urine sample. Contamination of the sample container with strong oxidants (disinfectants) can bring about a false positivity as well. On the other hand, presence of reducing compounds (e.g. ascorbic acid) may slow down or block the pseudoperoxidase reaction and so produce false negative results.

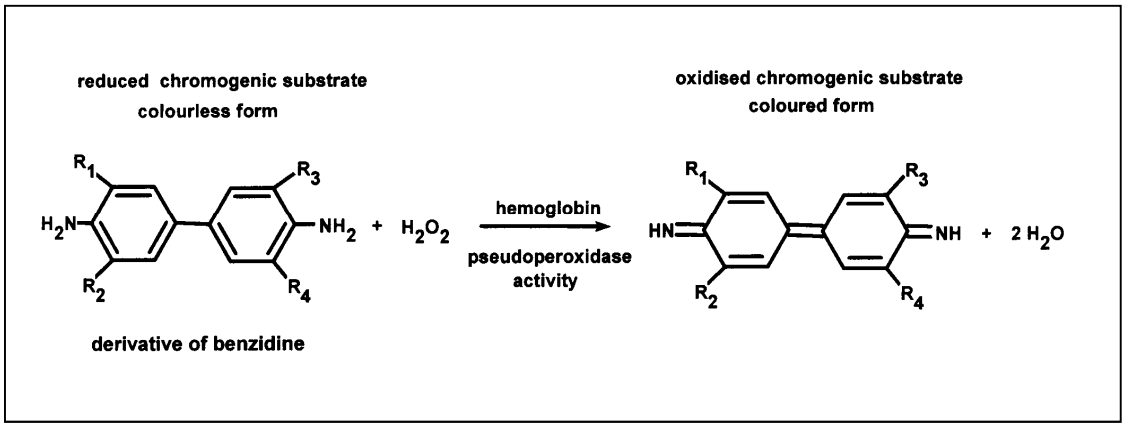



**Fig. 2: Principle of the test for blood in urine**

### Evaluation:

Hemoglobin can occur in urine either free (**hemoglobinuria**) or bound inside the red blood cells (**hematuria, erythrocyturia**). From quantitative point of view we can distinguish macroscopic and microscopic hematuria. The **macroscopic hematuria** is visible by bare eyes, since it gives the urine sample pink or red color[1]. For detection of the **microscopic hematuria** chemical and microscopic examination of urine is needed. Some red blood cells (about 2-3 erythrocytes/ µl of urine) are present even in the urine of entirely healthy individuals. This tiny amount is not detectable by common chemical tests.

[1] The macroscopic hematuria is seen if 1 liter of urine contains at least 1 ml of blood

Hematuria is a finding that should always be given an adequate attention, because it can indicate a severe disease of the kidney or urinary tract. In general the blood in urine can be either of **renal** or **extrarenal** origin. It is a sign of **inflammatory kidney diseases** such as glomerulonephritis or pyelonephritis. Another source of erythrocytes in the urine can be rupture of small blood vessels or bleeding into urinary tract due to **tumors** or **traumatic injury to kidney** or other parts of the uropoetic system; and also **urolithiasis**.

**Hemoglobinuria** occurs in intravascular hemolysis in hemolytic anemias, transfusion of incompatible blood, or paroxysmal hemoglobinuria. Chemical tests cannot differentiate between the presence of erythrocytes and free hemoglobin in urine; rather, examination of urinary sediment provides the answer, since in case of hemoglobinuria no erythrocytes are seen in the sediment.

### *1.3 Hemoglobin in feces: Occult bleeding*

Occult bleeding means presence of trace amounts of blood, detectable with chemical tests, in stool of otherwise normal macroscopic appearance. Its detection serves to **early diagnosis of colorectal cancer**, when a radical and effective treatment is still possible. Next, it can point to presence of **precancerous states in the large intestine**, especially adenoma polyps.

The techniques for detection of occult bleeding utilize the **pseudoperoxidase activity** of heme iron, described above. The patient receives a set of three test cards. The reagent zone of the cards contains guajac resin[2] or benzidine derivative as the indicator. Patient takes samples from three consecutive stools, applies them on the test zones on the cards, and sends to the laboratory. There, a detection reagent, which consists of stabilized hydrogen peroxide, is applied on the cards, and the resulting color is evaluated. Two positive results from three consecutive samples are suspect of colorectal cancer. Other possible sources of blood in stool, however, must be excluded, such as bleeding from gingiva or duodenal ulcer.

The test is affected by presence of vitamin C and hemoglobin in food (meat, blood). That is why it is performed following three days of meat-free diet with exclusion of vitamin C. Plant peroxidases in some kinds of root vegetables (cauliflower, broccoli, radish, horse-radish) can cause false positive results as well.

There are other tests based on the **immunochemical detection of hemoglobin** using an antibody against human hemoglobin. These techniques are more specific and sensitive than the pseudoperoxidase reaction; and no diet is necessary before the examination.

[2] Guajac resin is extracted from wood of plant Guaiacum officinale, guajacol is chemically methoxyphenol.

# 2 Derivatives of hemoglobin

## *2.1 Oxyhemoglobin and deoxyhemoglobin*

Hemoglobin bearing oxygen is called **oxyhemoglobin (oxyHb)**. Following release of oxygen it becomes **deoxyhemoglobin (deoxyHb)**. Iron remains in the ferrous state in both these hemoglobin forms since only hemoglobin containing $Fe^{2+}$ is able of reversible binding and transporting oxygen. Oxygenation of hemoglobin changes the electron configuration of the heme-$Fe^{2+}$ complex that manifests as the color change from dark red venous blood to the bright red color typical for arterial blood.

| | | | |
|---|---|---|---|
| **Hb** | **+ 4 $O_2$ ⟶** | **Oxyhemoglobin ($O_2$-Hb)** | **bright red** |
| **$O_2$-Hb** | **– 4 $O_2$ ⟶** | **Deoxyhemoglobin (Hb)** | **dark red** |

## *2.2 Methemoglobin (hemiglobin)*

**Methemoglobin (metHb)** is characterized by **presence of ferric iron**; it originates by oxidation of ferrous iron in hemoglobin. The sixth coordination bond of $Fe^{3+}$ is occupied by molecule of water instead of oxygen and so methemoglobin is no longer able to transport oxygen. Color of methemoglobin is chocolate brown.

There is a constant production of small amounts of methemoglobin in the erythrocytes (about 3 % of total hemoglobin daily). Its reduction back to hemoglobin must be provided by enzymatic as well as non-enzymatic reactions. The enzyme-catalyzed reduction is performed by **NADH-dependent methemoglobin reductase**, while the non-enzymatic mechanisms involve **glutathione and ascorbic acid**.

Increased concentration of methemoglobin in blood is called **methemoglobinemia**. It can appear due to various causes. **Inherited methemoglobinemia** usually results from inborn error of NADH-dependent methemoglobin reductase or from presence of abnormal hemoglobin M. **Acquired methemoglobinemia**, however, is the most common. It can be produced by action of oxidants:

- poisoning by some substances (e.g. nitrobenzene, aniline and its derivatives – some stains)
- effects of some drugs (e.g. local anesthetics, phenacetine, sulfonamides)
- high amount of nitrites/nitrates in food and water.

Especially newborn are sensitive to these substances due to immaturity of reducing systems and high proportion of fetal hemoglobin that undergo oxidation fairly easy.

**Symptoms of methemoglobinemia** include mainly cyanosis with characteristic grayish-brown hue and hypoxia.

**Table 2: Symptoms of methemoglobinemia:**

| ***Methemoglobin in %*** | ***Symptoms*** |
|---|---|
| 0 – 2 | Physiological value |
| > 10 | Cyanosis |
| > 35 | Cyanosis and other global symptoms (headache, dyspnoea) |
| 70 | Lethal concentration |

Therapy of acquired methemoglobinemia is based on administration of some reducing agents: **methylene blue** or **ascorbic acid**.

## *2.3 Carbonyl hemoglobin*

**Carbonyl hemoglobin (CO-Hb)** originates from binding of carbon monoxide, CO, to hemoglobin. Carbon monoxide binds to hemoglobin 250 – 300 times more strongly than oxygen. The carbonyl hemoglobin is no longer available for transport of oxygen and low ability of blood to deliver oxygen results in tissue hypoxia. In an excess of oxygen the binding of carbon monoxide to hemoglobin is reversible. Therefore, in case of CO poisoning the most important therapy is inhalation of oxygen.

Small amount of CO-Hb can occur even in the blood of healthy persons. People living in cities have values about 2 %, but in strong smokers it can be as high as 10 %. Few minute-stay in the air containing merely 0.1 % CO can increase carbonyl hemoglobin up to 50 %.

Carbon monoxide originates from incomplete combustion of fossil fuels; it is contained e.g. in car exhaust gases, or smoke from wildfires in closed rooms.

**Table 3: Symptoms of poisoning by carbon monoxide:**

| ***Concentration of CO-Hb in %:*** | ***Symptoms:*** |
|---|---|
| 10 | Breathlessness during physical exercise |
| 20 – 40 | Headache, dyspnoe, fatigue, vomiting |
| 40 – 60 | Hyperventilation, tachycardia, syncope, seizures |
| 60 – 80 | Coma, death |

Carbonyl hemoglobin is colored crimson red; persons severely intoxicated with carbon monoxide often have ‘healthy’ pink complexion. In comparison to hemoglobin the carbonyl hemoglobin is rather resistant to various chemical agents; some classical test tube tests for carbonyl hemoglobin are based on this fact (e.g. reaction with tannin).

## *2.4 Spectrophotometry of hemoglobin derivatives*

Hemoglobin and its derivatives display characteristic absorption spectra in the visible range, which can be used for their **analysis and rapid identification** (see Fig. 3, table 4).

All hemoproteins possess typical strong absorption maxima at 400 – 430 nm (the Soret's band). The further absorption peaks are much lower. **Oxyhemoglobin** is characterized by two incompletely separated maxima at **540 and 578 nm**, while **deoxyhemoglobin** has only one maximum at **555 nm**. Spectrum of **methemoglobin** shows maximum at **630 nm**, and another inconspicuous peak at **500 nm** that is dependent on pH. When methemoglobin reacts with cyanide the maximum at 630 nm disappears, since cyanmethemoglobin originates. Decrease of absorbance at 630 nm is proportional to the concentration of methemoglobin. The **cyanmethemoglobin** has a broad band at **540 nm** that is used in estimation of hemoglobin in blood. Finally, spectrum of **carbonyl hemoglobin** resembles that of oxyhemoglobin, but **both peaks are red-shifted**.

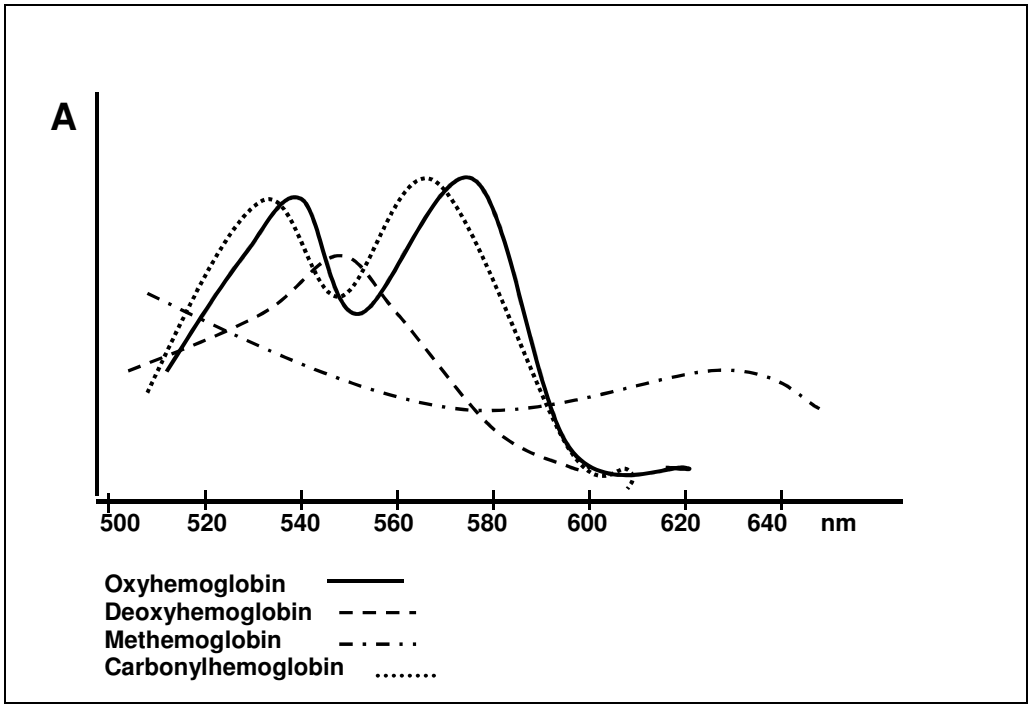



**Fig. 3: Absorption spectra of hemoglobin derivatives**

| *Derivative of hemoglobin* | *Absorption maxima (nm)* |
|---|---|
| Deoxyhemoglobin | 431, 555 |
| Oxyhemoglobin | 414, 540, 578 |
| Methemoglobin (acidic form) | 404, 500, 630 |
| Carbonyl hemoglobin | 420, 538-540, 568-569 |
| Cyanmethemoglobin | 421, 540 |

**Table. 4: Absorption maxima of hemoglobin derivatives**

### *2.5 Estimation of carbonyl hemoglobin*

Estimation of CO-Hb in blood is one of the basic toxicological examinations, invaluable for assessment of acute as well chronic poisoning by carbon monoxide.

Carbonyl hemoglobin can be quickly estimated spectrophotometrically by measuring the shift of the absorption maximum of diluted blood with respect to the absorption maximum of didymium filter at 586 nm.

The procedure is simple: absorption spectrum of diluted blood in the range 500 – 700 nm is recorded, together with spectrum of the didymium filter. Distance between filter peak (586 nm) and peak of the blood sample in the region 568 – 577 nm is measured. There is a linear relationship between distance between these maxima and CO-Hb content in the sample, so the CO-Hb concentration can be read from a calibration curve with CO-Hb in % on the x-axis and distance of the maxima in nm on the y-axis. See Fig. 4 for an example.

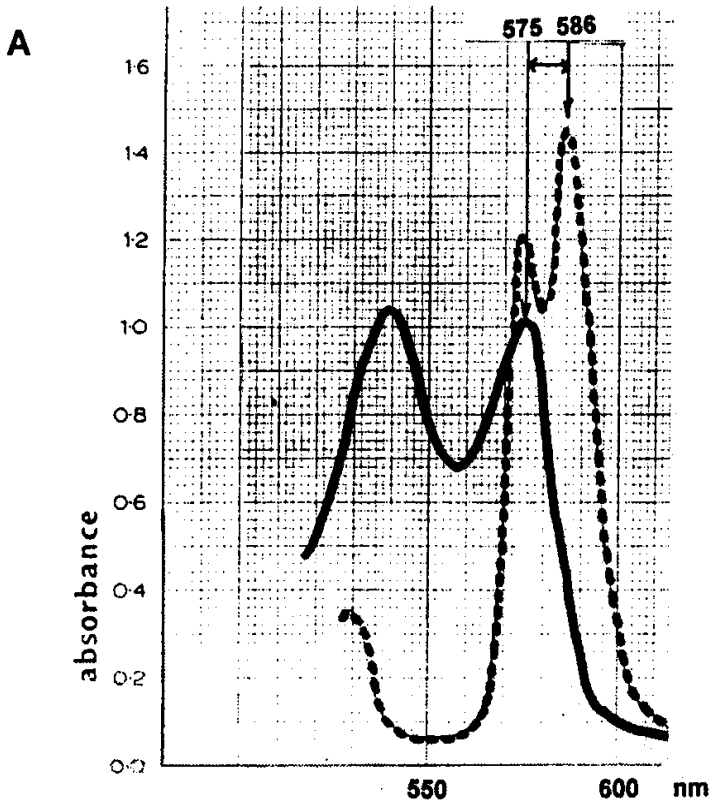



**Fig. 4: Estimation of carbonyl hemoglobin**

There are also simple tests for carbonyl hemoglobin, such as the reaction with tannin or Ajatine. They can detect CO-Hb when it exceeds about 10 %. Tannin forms with carbonyl hemoglobin a strawberry-red precipitate, whereas in the absence of carbonyl hemoglobin the precipitate is brown-grey.

Photometric analysis of the derivatives of hemoglobin that are the most significant in a toxicological sense, methemoglobin and carbonyl hemoglobin, is nowadays also built into the sophisticated **analyzers of the acid-base status**.

# 3 Iron in the body

Iron is one of the most important elements in the human organism. A healthy adult human body contains over 70 mmol (4.0 – 4.5 g) of iron; majority of it is built in hemoglobin.

**Table 5: Distribution of body iron:**

| *Form and function* | *Protein* | *Amount in g* |
|---|---|---|
| ***Active iron*** | | |
| Transport of oxygen | Hemoglobin<br>Myoglobin | 2.5 – 3.0<br>0.3 |
| Transfer of electrons | Cytochromes, cytochrome oxidase | 0.2 |
| Decomposition of hydrogen peroxide | Catalase, peroxidase | |
| ***Storage iron*** | Ferritin, hemosiderin | 0.8-1.0 |
| ***Transport iron*** | Transferrin | 0.003 |

Presence of iron is necessary for function of the cells. Iron in hemoglobin participates in transport of oxygen; and iron in cytochromes enables transfer of electrons in the respiratory chain. On the other hand, iron as a transitional and fairly reactive element participates in unwanted radical reactions, producing the 'reactive oxygen species', which can subsequently damage cellular membranes, proteins and DNA.

Iron is absorbed as $Fe^{2+}$ by active transport in duodenum and oral part of ileum. Some of the absorbed iron is incorporated into storage protein – **ferritin** – in the cells of intestinal mucosa; while another part enters the blood plasma where it circulates bound to **transferrin**. The uptake of plasma iron by peripheral cells is mediated by **receptor for transferrin**; inside the cells the iron is either used for synthesis of hemoproteins, or stored in ferritin. The involvement of specific transport protein transferrin and storage protein ferritin in iron handling represents protective mechanisms that aim at preventing the toxic effects of redox active iron.

## *3.1 Biochemical examination of iron metabolism*

Laboratory examination of disorders of iron utilization includes estimation of:

- iron in serum
- transferrin in serum /iron-binding capacity in serum
- ferritin in serum
- receptor for transferrin in serum

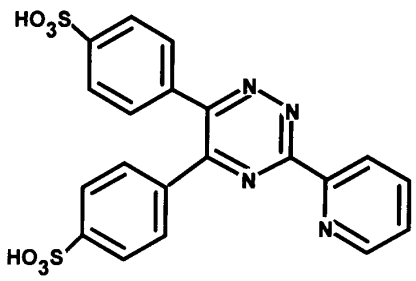
### 3.1.1 Iron in serum

**Principle of estimation:**

For estimation of serum iron colorimetric methods, atomic absorption spectrophotometry, and other specialized techniques can be used. The photometric methods, based **on reaction of iron with a complex-forming reagent**, are used most widely.

All these assays encompass the following steps (Fig. 5):

- Release of $Fe^{3+}$ from complex with transferrin by action of acids (such as HCl) or detergents
- Reduction of $Fe^{3+}$ to $Fe^{2+}$, necessary for the next reaction with the chelating agent. As a reductant, e.g. ascorbic acid can be used.
- Chelation of $Fe^{2+}$ by the complex-forming agent containing –N=C–C=N– groups, yielding a colored complex. Two chelators are nowadays used: bathophenanthroline and ferrozine [3-(2-pyridyl)-5,6-bis-(4-sulfophenyl)-1,2,4-triazine, registered name FerroZine]. The latter has higher absorption coefficient and is more soluble in water.



Ferrozine

**1)** **Transferrin $(Fe^{3+})_2$ + HCl → 2 $Fe^{3+}$ + transferrin**

**2)** **$Fe^{3+}$ + reducing agent (ascorbic acid) → $Fe^{2+}$**

**3)** **$Fe^{2+}$ + ferrozine → colored complex**

**Fig. 5: Principle of estimation of serum iron**

**Evaluation:**

Levels of serum iron fluctuate with circadian rhythms and are affected by many other factors. Accordingly, diagnostic significance of this marker is limited. Serum iron poorly reflects status of tissue iron stores and must be always assessed together with serum transferrin and iron-binding capacity.

Low levels of iron in serum are observed in iron deficiency due to e.g. big or repeated bleeding, low iron intake or impaired iron absorption; but serum iron is low also in acute infections and chronic inflammatory diseases (sequestration of iron in tissues). On the other hand, high serum iron is found in hemochromatosis, iron overload or iron intoxication, increased destruction of erythrocytes and some liver diseases.

**Reference values (S-iron):**
**Men: 9 – 29 µmol/l**
**Women: 7 – 28 µmol/l**

### 3.1.2 Serum transferrin and iron-binding capacity

Iron is transported in blood bound to a specific protein with $\beta_1$ electrophoretic mobility called **transferrin**, which is synthesized in the liver. Its production is inversely proportional to body iron stores: it increases in lack of iron and decreases when iron is abundant. Transferrin readily forms non-toxic complexes with iron and transfers iron absorbed in the small intestine to the bone marrow or places of iron storage (ferritin or hemosiderin). One molecule of transferrin binds two atoms of $Fe^{3+}$ (1 g of transferrin binds 25.2 μmol Fe).

Transferrin can be estimated either directly using immunochemical methods, or indirectly as ability of transferrin to bind iron - the iron-binding capacity. **Total iron-binding capacity (TIBC)** is defined as the amount of iron bound to transferrin in case all the iron binding sites are occupied. It corresponds to transferrin concentration. Usually, however, only about one third of circulating transferrin is saturated with iron. Free transferrin without iron represents **free iron-binding capacity** (two thirds of transferrin), available for transport of iron when an extra demand for it arises in the body.

Conversion between transferrin concentration and total iron-binding capacity:

**Total iron-binding capacity (μmol/l) = transferrin (g/l) × 25.2**

**Reference values:**
**Concentration of transferrin in serum (S-transferrin): 2.0 – 3.6 g/l**
**Total iron-binding capacity: 50 – 70 μmol/l**

### 3.1.3 Saturation of transferrin

From values of serum iron and serum transferrin it is possible to calculate degree of **transferrin saturation (TfS)**, defined as the ratio of serum iron concentration to serum concentration of transferrin. It is a sensitive parameter for detection of a latent iron deficiency.

$$\text{Saturation of transferrin (\%)} = \frac{\text{S-iron (μmol/l)}}{\text{S-transferrin (g/l)} \times 25.2} \times 100$$

**Values of transferrin saturation:**

- physiological: 20 – 50 %
- low saturation in iron deficiency: < 15 % (supplementation therapy must start)
- high saturation in iron overload: > 50 %

### 3.1.4 Ferritin and hemosiderin

**Ferritin** is the most important storage iron protein. Molecule of ferritin is adapted for binding a high amount of $Fe^{3+}$ in a soluble and non-toxic form. It consists of a protein shell made of 24 subunits (apoferritin, MW 440,000) forming a central cavity that can gather as many as 4,500 iron atoms in the form of ferric oxo-hydroxide $[FeO.OH]_n$ in micro-crystalline form together with phosphate ($FeO.OPO_3H_2$). Entry and release of iron atoms proceeds through pores that are among the particular subunits of the ferritin shell. Normally the ferritin iron storage capacity is used from about 20 %. Ferritin is stored mainly in the liver, spleen and intestinal mucosa cells.

Concentration of ferritin in blood plasma is very low. Serum level of ferritin is a **marker of status of body iron stores**. Low levels indicate depletion of body iron reserves and can reveal anemia of iron deficiency still in pre-latent phase. On the other hand, high levels of serum ferritin accompany high tissue stores of iron. They are also found in many patients with liver diseases, some malignancies, or inflammatory diseases (ferritin is a positive acute phase reactant).

Another storage protein for iron is **hemosiderin**, originating from aggregation of denatured ferritin with other components. It forms particles of size 1 to 2 μm, visible under light microscope when staining for iron is used. Hemosiderin contains more Fe than ferritin, but the iron in hemosiderin is poorly soluble in water and difficult to mobilize. It is formed in the body when the amount of iron in the organism exceeds the storage capacity of ferritin.

**Reference values:**

**Concentration of ferritin in serum (S-ferritin):** **Men: 30 – 300 μg/l**
**Women: 20 – 120 μg/l**

### 3.1.5 Receptor for transferrin

Iron transported in blood bound to transferrin is taken by cells through a specific receptor for transferrin (TfR). It is present on the surface of all cells at some stage, but its expression is highest on the red blood cell precursors in bone marrow. TfR is a transmembrane protein consisting of two identical subunits, connected with a disulfidic bond. Cleavage of the extracellular receptor domain releases a **soluble fraction of transferrin receptor** (sTfR) into circulation.

Cells respond to low iron stores by increased expression of transferrin receptor. Elevated sTfR is a **reliable indicator of deficiency of iron for hemopoiesis**. Increased levels of STfR are found in **anemias of iron deficiency** or in **hemolytic anemias**. Estimation of STfR is valuable in anemic patients who have simultaneously elevated ferritin due to the acute phase reaction. Estimation of STfR is also useful in patients following bone marrow transplantation for monitoring recovery of erythropoiesis.

For sTfR estimation immunochemical methods are used.

## *3.2 Disorders of iron metabolism*

### 3.2.1 Iron deficiency (sideropenia)

Lack of iron in the body is usually due to its insufficient absorption in the intestine or chronic bleeding. It can result in **sideropenic anemia (anemia of iron deficiency, hypochromic microcytic anemia),** which is one of the commonest hematologic diseases. However, anemia occurs rather late in the course of slowly developing sideropenia. It appears in the blood count only after almost all body iron has been depleted. Therefore, it is necessary to reveal sideropenia at an early stage not yet accompanied by anemia.

Based on estimation of the main parameters of iron metabolism, three stages of iron deficiency can be distinguished (Table 6):

- **Pre-latent iron deficiency** denotes a condition of gradual depletion of iron stores, but the delivery of iron to bone marrow erythroblasts is not yet affected. About half of the patients have serum ferritin levels below 12 μg/l.

- In **latent iron deficiency** the body stores of iron are essentially exhausted. Serum ferritin falls below the low reference limit; at this stage it is accompanied by low serum iron and impaired delivery of iron to the bone marrow. Iron-binding capacity increases. Sensitive marker of latent iron deficiency is a decrease of transferrin saturation below 15 %. Anemia is still absent at this stage.

- In **manifest iron deficiency** anemia develops with hemoglobin level under the low reference limit. In anemia of iron deficiency typically a low serum iron together with high transferrin (iron-binding capacity) is found. In contrast, in hemolytic anemias or in iron overload the serum iron is high and simultaneously the total iron-binding capacity is low.

**Table 6: Laboratory findings in iron deficiency:**

| ***Pre-latent*** | ***Latent*** | ***Manifest*** |
|---|---|---|
| • decrease in iron stores: low serum ferritin | • lack of iron stores: low serum ferritin<br>• low serum iron<br>• transferrin saturation below 15 %<br>• total iron-binding capacity increases<br>• STfR increases | • lack of iron stores: low serum ferritin<br>• low serum iron<br>• transferrin saturation below 10 %<br>• total iron-binding capacity increases<br>• STfR increases<br>• decrease in hemoglobin concentration: ***anemia*** |

### 3.2.2 Iron overload

There is no effective excretion pathway for iron in the organism, and so under certain circumstances the surplus iron can accumulate in tissues. Early diagnosis can prevent organ damage by excess of iron. Iron overload usually develops very slowly. Three stages are distinguished (Table 7):

- In **pre-latent iron overload** its contents in organs increases, but without exceeding their iron storing capacity
- In the stage of **latent overload** storage capacity of cells is exceeded, but organ function is still intact. Serum ferritin as well as serum iron increases. Transferrin saturation rises above 35 %.
- In the **manifest iron overload** damage of some organs occurs.

**Table 7: Laboratory findings in iron overload:**

| *Pre-latent iron overload* | *Latent iron overload* | *Manifest iron overload* |
|---|---|---|
| • increase in iron stores: ferritin rises | • increase in iron stores: ferritin rises above 300 μg/l<br>• increase in serum iron<br>• transferrin saturation above 55 % | • increase in iron stores: ferritin rises (above 2,000 μg/l in severe cases)<br>• high increase in serum iron<br>• transferrin saturation high (may be above 90 % in severe cases) |

**Hemochromatosis** is a disease associated with accumulation of iron in tissues. **Primary hemochromatosis** is a hereditary disease caused by increased absorption of iron in the intestine. The surplus iron accumulates in parenchymatous organs such as liver, heart, pancreas, and adrenals. Iron is toxic in the affected organs since it can catalyze generation of free radicals. The major clinical signs are skin hyperpigmentation, hepatosplenomegalia, and diabetes mellitus.

**Secondary hemochromatosis** can develop as a result of repeated transfusions, excessive intake of oral iron supplementation, or hemolytic anemia. The biochemical findings include rising ferritin and iron in serum, also saturation of transferrin increases while its concentration decreases.