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Serum iron in health and disease

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SERUM IRON
IN HEALTH AND DISEASE

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I. Iron Metabolism

A. Characteristics of Serum Iron and Iron Binding Capacity

The concept of a mechanism by which iron was transported from its site of absorption in the intestine to erythropoetic tissue and other areas began to receive attention in the 1930's. With the advent of radioactive isotopes in the early 1940's workers were given another tool in addition to the chemical and weight measurements previously used.

In 1944 Waldenstrom[25] using human volunteers gave intravenous iron tolerance tests. He found that by using greater amounts than 10 mgm. of iron the subjects experienced flushing of the face, nausea, sneezing and headaches. The natural conclusion drawn from this experiment was that the body had a protective mechanism by which approximately 10 mgm. of ionized iron was "bound" by some component in the blood stream.

Now native serum removed before any exogenous iron is given was found not to react with alpha, alpha dipyridyl as does a solution of just ionized ferrous iron. The reaction did not change even when sodium hyposulfite was added as a reducing agent to the serum. When, however, the native serum in vitro had added to it an amount of iron equivalent to greater than 10 mgm. per total body serum, then, a reaction did occur with the

alpha, alpha dipyridyl. Serum of the volunteers to which intravenous iron was given in toxic doses surprisingly did not react with alpha, alpha dipyridyl either. But when this serum in vitro was subjected to additional iron before reaction with alpha, alpha dipyridyl it required only small amounts of additional iron before the reaction took place. It was felt therefore that the reaction of flushing of the face, nausea, sneezing and headaches was caused after the serum was saturated and the ionized ferrous iron passed unimpeded into the body tissues. Waldenstrom found this limit to be 291 γ %.

Laurell[18] conducted extensive work published in 1947 showing that serum iron is bound to proteins in the serum having a molecular weight of approximately 88,000 and is carried principally by the alpha and beta globulins. Further work indicated the beta$_1$ globulin as the main carrier. Each molecule binds two iron ions.

Ammoniom sulfate was shown to precipitate serum iron with other proteins. Granick[13] writing in 1954 calls this iron binding protein transferrin or siderophillin and states that there is 10 grams of protein in the serum to which is attached (chelated) a total of about 4 mg. of iron. This represents less than 1% of the total iron in the body. Since from catabolism of

blood alone there is about 20 mg. of iron per 24 hours the serum iron must be in a constant flux. The question then arises does the protein carrier decompose after it has carried the iron to its destination? This would necessitate the making of rather large amounts of this protein per day. Laurell ahowed that the reaction Protein + Fe^{++} $\longrightarrow$ Fe protein complex was a reversible reaction at least in vitro. If this is true in vivo the protein can be compared to a conveyor belt transporting the iron from one site to another. Native serum iron is thought to be both ferrous and ferric as shown by dialysis but it is not known as a certainty.

The serum iron-protein complex is practically entirely undissociated on the alkaline side of pH 7.2, and that it begins to undergo dissociation on the acid side so that at pH 5 there is nearly complete dissociation.

Until now our discussion has been confined to the serum iron binding capacity. Do the red cells have an iron-binding component in addition to the hemoglobin? To find out Laurell hemolyzed blood and then both ferrous and ferric iron were added. The difference between the iron binding capacity of enriched and non-enriched blood was very small. Compared to serum the red cells have practically no iron-binding capacity.

B. Iron Excretion

Excretion of iron from the body amounts to about 1 mg. in 24 hours. Less than .1 mg. is excreted via the kidneys. A small amount is lost through the desquamation of epithelial cells of the gastro-intestinal cells. Sweat serves as another exit. There is no mechanism in the body for active excretion of iron such as for sodium. In other words once a given amount of iron is within the body it will be lost at the rate of only 1 mg. per 24 hours unless blood is lost. If the body had a reserve of 1 gram of iron the body could go nearly 3 years without further intake before an iron deficiency would be manifest.

C. Storage

According to Laurell iron in a 70 kg. man is estimated to be 4.3 gm. Of this 55% is said to be in hemoglobin, 10% in myoglobin, 30-35% in storage and about 1% in the serum transport system. Granick however, writing in 1954 gives the following figures on iron distribution.

Blood hemoglobin	3.0 grams	60-70% of total
Myoglobin	.13 grams	3-5%
Transferrin	.004 grams	.1%
Ferritin	.4-.8 grams	15%

Ferritin is a brown iron containing protein found chiefly in the liver and spleen and is the storage form of iron. Ferritin is somehow associated with irreversible shock and is known as VDM. This is beyond the scope of this paper however. Hemosiderin is a granular substance that also takes an iron stain and possibly is formed when the rate of iron deposition or the quantity is too great for the ferritin mechanism. It is noted that the ferritin iron is mobilized much more rapidly by the body in time of need as in a hemorrhage than is hemosiderin.

D. Absorption

Absorption has been deliberately deferred to the end of this general discussion of iron metabolism as opinions vary as to how iron is absorbed and what is the controlling factor or factors. One point of agreement is that salt iron is absorbed better than is dietary iron such as found in bread.

Granick and others have shown that HCl is of questionable importance in iron absorption whereas he supports ascorbic acid as a factor in facilitating iron absorption by reducing ferric to ferrous form. Bile and intestinal flora help replace the effect of HCl when it is not present. Holly[15] in 1957 shows the

increased absorption during pregnancy by the addition of cobalt to the preparation. Phosphatides on the other hand inhibit absorption of iron. For a short time there were those who thought this was the controlling mechanism. Then just the opposite was depicted as the cause of hemochromatosis, that is, the absence of phosphates allowed excess iron to be absorbed. Granick's theory of mucosal block is the most popular concept of intestinal absorption of iron. Ferrous iron enters the mucosal cells of the upper intestine. It has been shown however that the entire length of intestine can absorb iron but in practice the conditions are right only in the upper intestine. As the iron enters the mucosal cells they are stimulated to produce the protein apoferritin. Iron reacts with apoferritin to form ferritin. This increase in ferritin corresponds to the time at which iron is not absorbed by the intestine. Granick[13] further states that the movement of iron from the mucosal cells into the blood streams is independent of the extent to which transferrin is saturated with iron and also independent of the total concentration of transferrin. He was of the opinion that low oxygen tension such as in anemia had some control of the release of iron by the mucosal cells. This is supported by Colehour[7] et al, in rats where he

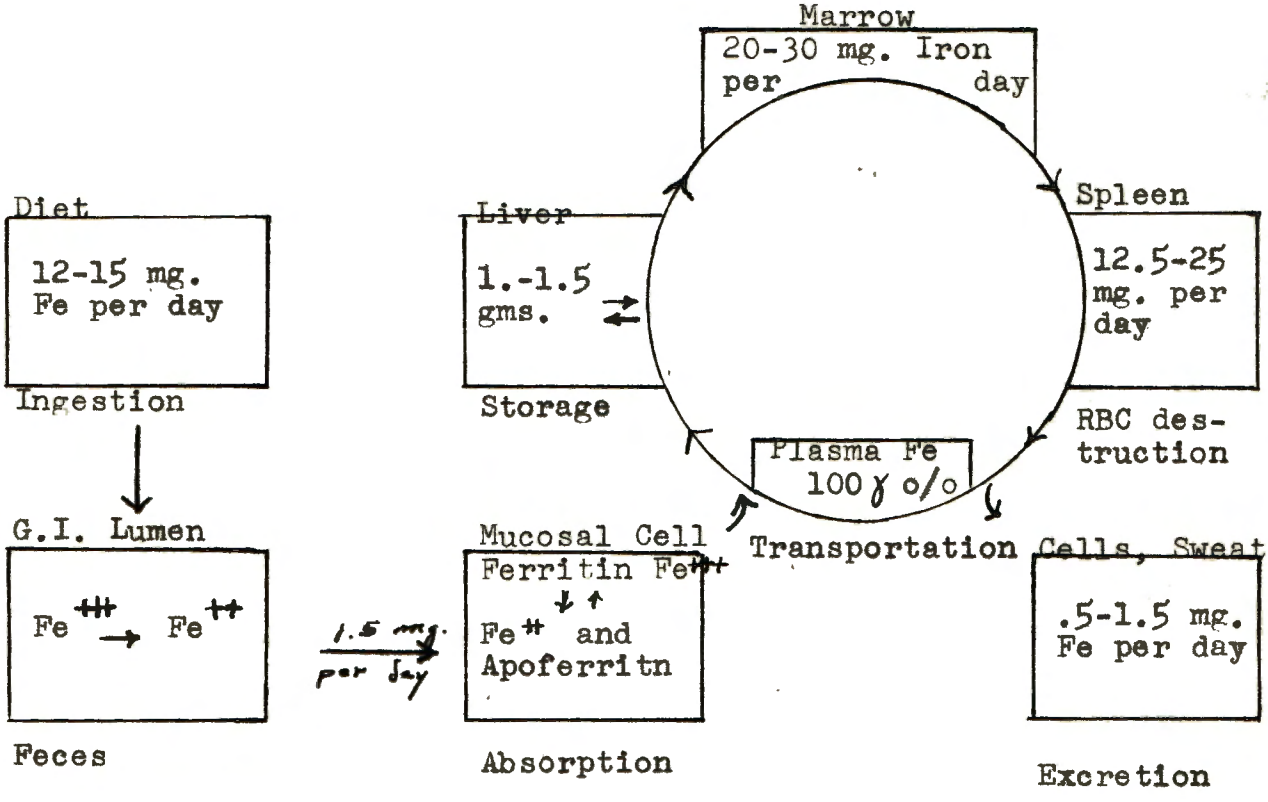
demonstrated a marked rise in the unsaturated iron-binding capacity, the serum iron decreased and the reticulocyte count rose after the rats had endured 10 days in ½ atmosphere of oxygen. Studies with radio-active iron by Yuile[28] in which the dogs were "saturated" with iron reached a similar conclusion for it was found that while saturation of the iron binding capacity may have modified absorption it certainly did not stop absorption. Balfour[1] writing in 1942 wrote that "the reserve stores of iron are of more importance than hemoglobin in ... iron absorption." Later this same author reported low absorption in pernicious anemia and in Hodgkin's Disease. A serious error was made in this experiment however. Radioactive iron was used and to check absorption the radioactivity of blood was checked. This assumes that all iron absorbed is utilized for hemoglobin. It is an obvious error when a disease such as hemochromatosis is considered. Haskins[14] found iron well absorbed during anemia but as soon as the hemoglobin rose close to normal absorption slowed.

Moore, et al, made dogs anemic by blood letting after which the iron was replaced by intravenous administration. Despite this replacement the dogs absorbed iron at a rate equal to control dogs receiving no intravenous iron.

Hutchison[16] as far back as 1937 by using balance studies showed storage had little effect on absorption but when the new research tools as radioactive isotopes appeared this work was brushed aside.

With all this contradiction it readily becomes apparent that very little is fact in iron absorption. Hugh Josephs[17] sums it up well when he stated "Iron is absorbed according to need .... As a matter of fact, we know nothing of the body's need for iron except what we make up in our own minds."

A diagram of the most commonly accepted metabolic pathways of iron is shown below.[19]



II. Normal Values For Serum Iron and Total Iron Binding Capacity

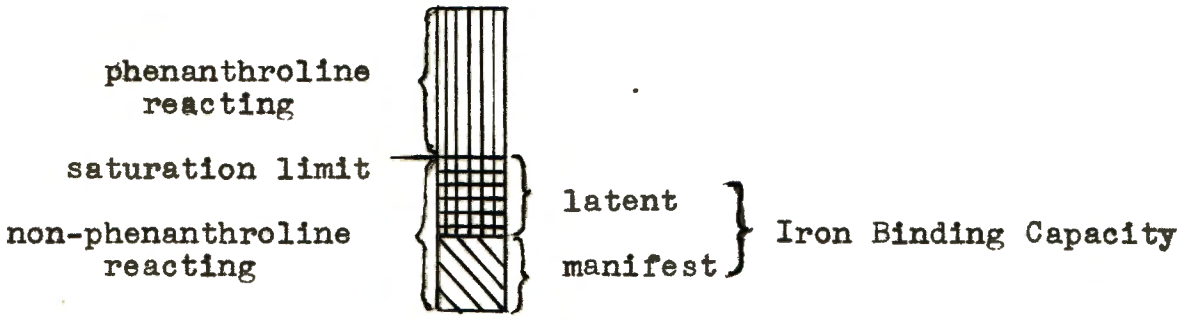
Determination of iron binding capacity is based on the fact that iron added to serum in excess of the iron binding capacity reacts with phenanthroline in the presence of hydrosulphite. If then the amount of iron added is known the unsaturated portion of the iron binding proteins is found. This is the latent iron binding capacity. Taking the saturated serum and releasing all the bound iron, for example by lowering the pH below 5, would give the iron present before saturation by merely substracting the latent iron binding capacity from the total previously calculated. This is known as the manifest iron binding capacity.

Laurell submits figures for serum iron determination from four investigators.

	Male	Female
Skouge	118 γ %	104 γ %
Vahlquist	142	123
Brocher-Mortensen	128	118
Hemmeler	132	103
Average	130	112
Range	68-263	53-210

Laurell determined the SI to be 124 γ % in males and 108 γ % in females. Total iron binding capacity was 315 γ % with no sex difference. Newborns had

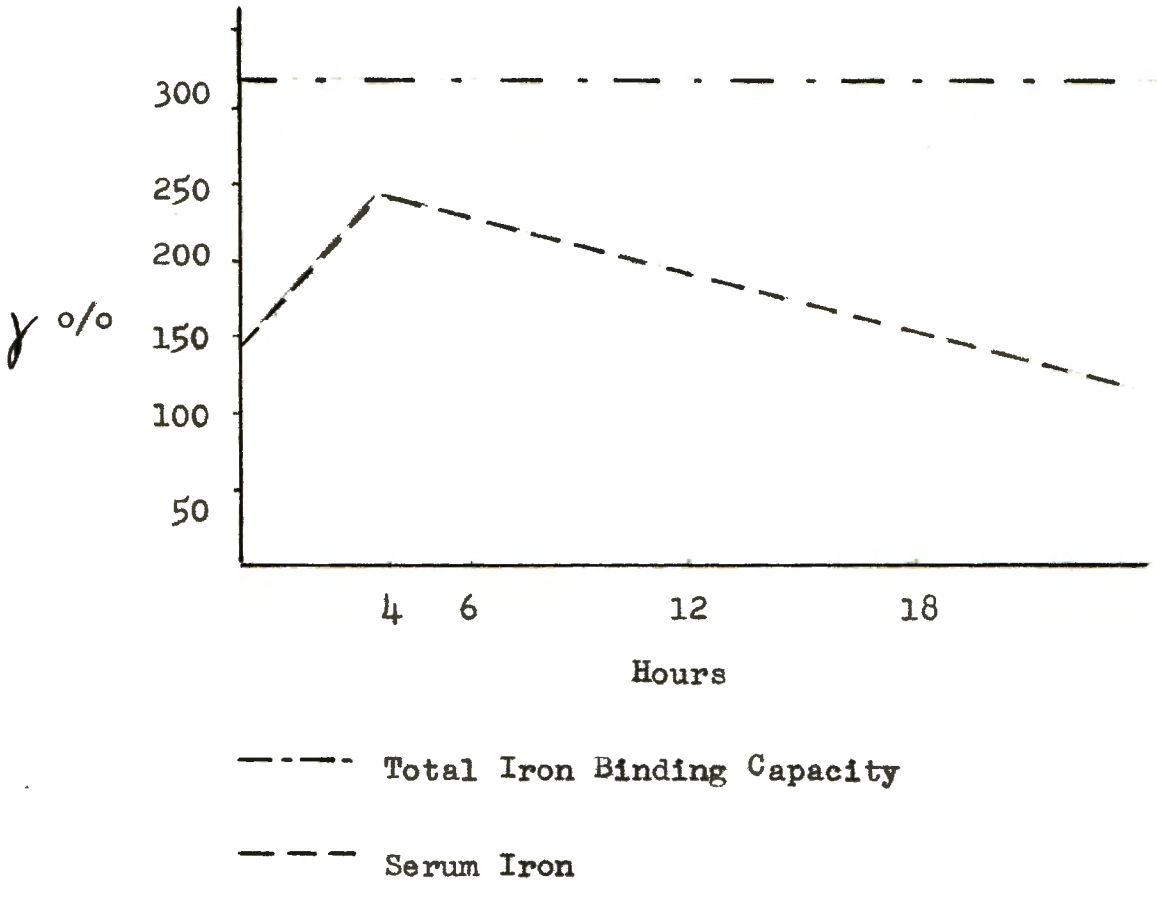
226 γ % total and 147 γ % serum iron.



There is a normal diurnal variation in serum iron with the high value after rest as in the morning and the low in the evening after activity.

Adult $\frac{\text{Manifest}}{\text{Latent}}$ = .77 in males .58 in females.

An example of change in the normal person after subject is given .55 gm. ferrous iron by mouth is shown on the following page.


300
250
200
150
100
50
γ %
4
6
12
18
Hours
Total Iron Binding Capacity
Serum Iron

III. Diseases of Iron Deficiency

Iron deficiency is usually thought of as a lack of iron intake in the diet. In this discussion we will consider iron deficiency to be low serum iron. If serum iron is considered to be a pool then decrease in supply-absorption, increase in utilization, or limited capacity of the pool would reduce the amount.

Pure iron deficiency in the diet is becoming more and more a rare occurence not only because of better diet but certainly the proprietary iron preparations are advertised and sold as panaceas to restore youthful vigor. Milk anemias in the newborn are an exception. In women, menstrual bleeding and pregnancy are a drainage on the iron pool. The average menstrual period blood loss is 50 cc. and pregnancy demands 350-500 mgm. of iron from the mother. How the iron is transferred from mother to fetus is one of the most prominent riddles in iron metabolism. The decrease in serum iron is not perceptable until the latter half of the pregnancy when the fetus requires nearly all its iron. A corollary to this is the rise of the saturation limit to about 200 γ % above the normal of 315 γ %. Iron absorption is increased two-ten times.

A. Relation of Hemoglobin to Absorption

Acute and chronic blood loss from a benign condition

is very similar to pregnancy. Immediately after acute loss the serum iron shows a slight rise during the first 24 hours then the value is subnormal until the hemoglobin returns to normal. The manifest and latent iron binding capacities go in divergent directions as in pregnancy.

Acute and chronic infections result in normochromic anemias. Both the total iron binding capacity and the serum iron are greatly reduced with the latter to a greater extent according to Cartwright and Wintrobe.[5] Therefore the percentage of saturation is relatively lower in infection anemia. In infections they found that the iron binding protein was decreased relatively more than other serum proteins. Intensive iron therapy by Schöfer[24] produced a rise in serum iron to about normal yet the anemia was unabated. On mice Schöfer even demonstrated that with infection the mouse absorbed more iron than his healthy counterpart.

Bone marrow examinations on patients with infection anemias reveal that the fault lies with depressed erythropoesis and not want of iron. Indeed Pratt and Johnson[22] found that iron stores in patients with rheumatoid arthritis were increased in most cases concurrent to anemia. Confirmation of this is given by failure of intravenous iron to increase erythropoesis in the face of infection. The iron must go into the body tissues supposedly to

fight the infection by some unknown mechanism. Brendstrup[3] states that the decrease in serum iron is due to the accumulation of iron in the reticuloendothelial system. Even less is known why the iron binding protein decreases. Is it because less protein is produced or does it too become diverted to a more pressing demand of the moment? Brendstrup found that all of his patients with rheumatoid arthritis had greatly reduced serum iron and iron binding capacity. He felt that the reduction of the latter had some part in the etiology of the anemia. Of interest is that Lyngøe and Lorenzen[20] treated a small series of rheumatoid arthritics with prednisone and noticed that the iron binding capacity increased. No mention is made to effect on anemia. An answer would tend to support or detract from Brendstrup's theory mentioned previously.

Ulcerative colitis is a chronic infection that reacts somewhat unusually. The serum iron is low but the saturation limit is normal. Added to this is the fact that iron therapy will often help an anemia in the patient with the disease.

Uremia patients are found to have low serum iron values but the anemia has long been thought to result from marrow depression. In malignant tumors affecting general health the serum iron is low even without anemia

probably through some toxic inhibition of the marrow. If the tumor does not affect general health the values are not altered. An anemia due to bleeding of a malignancy may benefit from iron therapy.

Three conditions with an increase in serum iron are pernicious anemia, hemochromatosis, and several liver diseases. In direct contrast to pregnancy, the patient with pernicious anemia has a high serum iron and a low saturation limit. Comparing pregnancy anemia with pernicious anemia it is seen that in pregnancy there is a deficiency of iron available whereas in pernicious anemia there is a relative excess of iron available since there is no utilization of the iron. The low saturation limit is much more characteristic of pernicious anemia than the low serum iron. After therapy the serum iron retreats to normal but for some unknown reason the saturation limit remains low.

Cirrhosis stands somewhat alone among liver diseases in that both the saturation limit and the serum iron are decreased, the former to a greater extent. During the necrotizing phase of cirrhosis the serum iron is found to be above normal.

Pratt and Johnson again substantiate the data reported by their assay of iron stores. They found that stores were normal or increased in pernicious anemia and

cirrhosis. Notable too is the fact that the iron stores decrease after treatment of the pernicious anemia.

At the other end of the scale viral hepatitis has the most persistently elevated serum iron. Christian[6] found the mean peak value to be 297 γ % with a range of 178-571 γ %. The serum iron gradually increases during hepatitis and peaks at about the fifteenth day. Christian stated that he could find no constant correlation between the height of the peak and clinical severity of the disease. Neither jaundice nor ascites had any significant effect on the values ascertained by Stone,[25] et al. Various authors agree that bilirubin peaks do not necessarily correspond to serum iron peaks. What is the cause of this rise in serum iron? Early studies by Waldenstrom dismissed increased absorption as a cause. Then reduced biliary excretion was suspected as the etiology. But post-hepatic jaundice has normal serum iron so biliary excretion could hardly be at fault. Brochner-Mortensen[4] states that liver cell disintegration is the reason for elevated serum iron. Reissman[23] used carbon tetrachloride a notorious liver devastating agent on animals and found the serum iron to increase greatly. Other hepatitides as abscess, fatty metamorphosis, neoplasm, passive congestion have values that vary in no consistent pattern.

Extrahepatic biliary obstruction does have values for serum iron that are usually within the normal range. If a patient has symptoms suggestive of either intrahepatic obstruction (hepatitis) or post hepatic obstruction then a value of over 300 γ % indicates hepatitis as the probable etiology of the jaundice.

IV. Diseases of Iron Excess

Hemochromatosis or bronze diabetes is a disease of iron storage in which large amounts of iron are found particularly in the liver, spleen and pancreas that results in diabetes, cardiac, and liver failure. It will be remembered that early experiments using radioactive iron may well have missed the recognition of the disease state known today as hemochromatosis since they measured iron absorption by measuring iron utilization for hemoglobin production. The amount of iron in a patient with hemochromatosis ranges from about 20 to 60 gm. as compared to the normal 4 gm. Since hemochromatosis is a disease of older people we see that in order to accumulate this amount the body need absorb only 2 to 4 mg. per day more than maintainence requirements in order to attain 20-60 grams over a period of 30 to 50 years. In patients receiving scores of blood transfusion, iron goes first to the reticuloendothelial tissues yet eventually the

pathological picture resembles hemochromatosis with parenchymal tissue destruction. The conclusion is that the iron itself inflicts the tissue damage. With normal hemoglobin and excessive stores another reason for excessive iron absorption just be found. However, at present the specific nature of the disease is unknown but thought to be some defect that enhances the ability of the mucosal cell to take up iron. The disease has a familial tendency. Hemochromatosis is then an iron absorption disease and not an iron storage disease.

V. Summary and Conclusions

An attempt has been made to learn the values of serum iron and iron binding capacities in the various disease states. In order to better understand the dynamics that occur with disease a resume' of the metabolism of iron in the normal has been given. Iron is found to be unique in the body in that it is the amount absorbed that determines the quantity in the body and not the amount excreted as this is a fixed property. Certain physical properties are known about the iron binding protein but the mechanism and control of absorption remains a mystery. Franick's theory of mucosal block is the most widely accepted explanation but this too leaves many questions unanswered. Apparently there are many controlling factors in iron absorption the most important of which is low hemoglobin.

In various disease states the serum iron is found to be elevated or depressed. It is only in recent years that these values have been investigated. Liver disease has been studied the most vigorously. Two conditions are likely to be present in liver disease. First, the necrotizing phase in which serum iron is elevated markedly and secondly the fibrotic state in which the serum iron is low. Hepatitis is an example of the former and cirrhosis of the latter.

Uremia has long been known to suppress erythropoesis and the serum iron. Malignant disease lowers serum iron and binding capacity only when the neoplasm interfers with the general state of health of the patient.

Special reference has been made to pregnancy and hemochromatosis as one is an iron deficient state and the other an iron excess condition. In pregnancy the total iron binding capacity is elevated and the serum iron is reduced. This is the standard response in iron deficiencies.

Acute infections present a peculiarity in that the erythropoesis is reduced, the serum iron is reduced and iron absorption is increased. What happens to this iron poses an interesting question in regard to the body's defence mechanism.

At present serum iron and total iron binding capacity values are of little practical value in diagnosis and prognosis as they supply minimal information that other more readily available tests can not provide. Iron therapy is indicated only in those conditions in which the body does not have adequate storage and still retains the ability to absorb and utilize the iron.

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