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Pathogenesis of iron deficiency anemia in pregnancy

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The definition of a true anemia in pregnancy is somewhat confused by the decrease in hemoglobin and red blood cell count which may normally be experienced due to the so called physiologic anemia of pregnancy. This phenomenon has been attributed to a dilutional effect by an expanding plasma volume. Thus, measurements such as hemoglobin and red blood cell counts that are made by unit volume may decrease without a dimunition of the total of the hemoglobin or red cell masses. The degree of anemia that should be attributed to this process is a source of controversy. It is the author's opinion that too often, for the purpose of convenience, an arbitrary lower limit is set for the parameter of hemoglobin and red blood cell count beyond which the anemia is no longer considered to be physiologic but becomes pathologic. No doubt this is not a fixed point but varies in each individual case. In other words, to determine the point at which an anemia surpasses the physiologic state and becomes truly iron deficient is best evaluated by measuring iron stores or total red cell and hemoglobin masses rather than exceeding a lower limit such as 10 grams % or 3.5 million R.B.C. per cubic centimeter as has been set by many investigators.[1]

Other defining factors of a true iron deficient anemia of pregnancy which come closer to the truth are an abnormal decrease in the size of the red cells or a decrease in hemoglobin content. However, it must be remembered that these values are subject to normal variation.

The purpose of this thesis is to account for the mechanisms which produce a significant iron deficiency during pregnancy. Most people tend to over simplify the issue by assuming that the increase in iron demand for the fetus during pregnancy may cause a woman to become iron deficient. However, when one notes that the pregnant woman's excretion of iron decreases due to absence of menstrual loss, additional causative factors must be considered.

History:

While the concept of an iron deficient anemia secondary to pregnancy is relatively modern, man has long suspected the curative properties of iron. There is some evidence that iron was used therapeutically in the Mediterranean area as early as 1500 B.C. One interesting example of such evidence is related by the legend of Iphyclus. Prince Iphyclus of Thessaly, a province of Greece in 1200 B.C., was successfully treated for infertility with a mixture of wine and rust scrappings from a knife.[2]

The Egyptions and Sumerians called iron, "the metal of the heavens". There is documented proof of its theraputic use in the Ebers Papyrus,[3] dated 1500 B.C., which includes two perscriptions calling for the use of iron. One was for alopecia and called for iron, red lead, onions, alabester and honey. The other was an iron preparation in the form of a paste to be applied for pterygriun. For many years iron preparations enjoyed popularity in the treatment of various eye disorders. Babylonian physicians applied

an iron paste mixed with the perinephric fat of a black ox to treat photophobia. As late as 1000 A.D. Arabic physicians used iron in the treatment of trachoma.

Nicholas Monarde,[4] a sixteenth century physician of Seville, reviewed the medical uses of iron in the Roman Empire:

> "The iron and steele do serve in medicine with great effects and marvellous workes, by curing and heeling diver's diseases, and so Plinie in his booke of the natural historie, treting of this matter of iron, after he wrote great things of it, as well in that which doth profit in the service of man, as other curious thinges, hee treateth of the vertues and workes which it doth in medicine, showing first the qualities of it, saying: The yron hath vertue to drie up, to retayne and to holde faste; it is good for such as dooe lacke their haire, that it may growe, beeing prepared and mingled with some licour prepared and made for the same purpose, it taketh away the roughnesse of the cheekes, mingled with the vinegar and being made in an oyntment with oyle of Myrtiles and waxe, it taketh away the blisters of all the bodie; the pouder of it mingled with vinegar, doeth heal the disease called Saint Anthonies fire, as also al manners of skabbers: it healeth the little sores between the nailes and the finger, the pouders thereof being applied thereunto with a linen cloth. It healeth also the flux of women of what sorte soever it be, being put thereunto after the maner of a tent in the lower partes: the pouder being mingled with and put to sores or wounds new hurt, doeth soder them and heale them: and beeing mingled with vineger and put upon the piles, it dissolveth them. It is a great remedie for such as are growtie, being applied with thinges made for the purpose upon the grief: it stencheth the blood of such as are wounded, which is for the most part made of iron.
>
> "It is given to bee drunke to such as are diseased of the lungs, for it consumeth the disease, and healeth him that is sicke, it stayeth any manner of fluxe and the Piles, and doeth remedie the sores of them. It healeth sore cheekes, casting the pouders upon them it is a great remedy and worthy of estimation. He that doeth cause it to be made and doeth profit to take away and make cleane the soares and to take away the Fistula and to eate away the Braunches, and too cause that the sores bee filled with fleshe: all this is of Plinie in the Chapter of Iron. Galen seclareth much the necessitie of iron, for the life of mankinde and for the service of man, and dooeth account it for a most excellent remedy, for to dry up the moistures and tears of the eyes. In that of continuall dissolution he sayeth: that

peeces of burning yron cast into milke, by taking away the waterishness which the milke hath, is good for over much stooles, and especially for the bloodey flix (amebiasis?). And in the tenth of the simple medicine, he commaundeth that milke be given wherein peeces of yron have beene quenched, and saith that such kinde of milke dooeth good unto them which have the bloody flix.

Dioscorides in the chapter where he treateth of the rust of yron, saieth, that the water or the wine, that hath quenched a peece of burning Iron, is good for them that have the fluxe of the stomach, and the bloody fluxe, it dissolveth the hardnesse of the lungs, and serveth in cholerike stooles, and in the looseness of the stomache. Aecio, treating of certaine rawles which are verre excellent for the opilations (purification) of the inner partes, sayeth, that it is a moste convenient remedie for the Lunges, and inner partes of the Body, that the water that hath quenched whotte Iron bee taken for a long time; but such as have a whotte disease, must use of the water, and such as colde if they be weake, of wine that hath quenched Yron. Scribonio, an auncient Phisition sayth, that the water which hath quenched whot steele is a great remedie for such as are swollen, and for such as have sores and griefs of the bladder and chiefly if they use it continually."

In modern terminology, Monarde considered iron, in one form or another, effective treatment for alopecia, acne, vesicules, bullous and crusting eruptions, erysipelas, paronychia, vaginal discharge, wounds, hemorrhoids, gout, tuberculosis, diarrhea, peri-anal fistulas, excessive lacrimation, amebiasis, vomiting, weakness, edema and cystitis.

While others had hinted at the possibility, it was not until 1713 when Lemery and Geoffroy[5] established the presents of iron in the ash of blood.[6] Meghini made the next important contribution to our knowledge of iron and blood when, in 1746, he showed that the iron content in blood could be increased by feeding animals iron rich food. This observation has since been confirmed by Rouelle and Bacquet in 1747, by Von Forke in 1779, and Lichtenstein in

1899 and by George Whipple in this century.[7]

In 1831 Pierre Blaud discribed his treatment and cure of thirty cases of chlorosis by administering tablets composed of 320 milligrams of ferrous sulfate and 320 milligrams of potassium carbonate, giving 64 milligrams of elemental iron per tablet. He believed that there would be a fine dispersion of the insoluble ferrous carbonate, and that potassium sulfate would have a direct action on the lymphatics and peristalsis of the gastrointestinal tract enabling better iron absorption. Now, we know that ferrous sulfate, alone, would be more efficiently absorbed, and that his cures were probably due to the large quantity of iron that was given.

Blaud expressed his thoughts with the following statement:

> "But in all these cases, it comes from a vicious sanguinification, a result of which is an imperfect fluid, where the serosity predominates, where the coloring principle is lacking and which is no longer adequate to excite suitably the organism and carry on the regular exercise of its function---the treatment is ferruginous preperations, modifiers of the organism, which return to the blood the exciting principle which it has lost, that is to say the coloring substance, When one knows the importance of the blood and role which it plays in the organic scene of life, when one knows that this fluid is the exciting agent of all our parts, and prime mover of all their functions, one is little astonished at the trouble manifested when the conditions necessary to its influence no longer exist in its composition and that it lacks some one of the elements. Here the coloring matter is lacking. It is a clinical fact, which we know to be beyond doubt and it is this which gives birth to all functional disorders."

After the clinical value of iron had been established there was much speculation as to the mechanism of action. In 1893 Stockman[9] demonstrated that chlorotic women would respond with in-

creased hemoglobin levels on iron citrate, iron peptonate and oral ferrous sulfate. He also assayed the iron content in various diets and found that the diet of chlorotic girls contained only from 1.3 to 3.4 milligrams of elemental iron as compared to a normal diet of from eight to 10 milligrams.

Von Noorden,[10] in 1912, after extensive study of chlorosis came to the conclusion that iron exhibits a "powerful stimulus on the hematopoietic cells of the bone marrow. But iron, according to our point of view, is only one of many therapeutic and hygienic measures which affect a stimulation of the hematopoietic organ without the slightest importance being laid to its chemical relationship to the hemoglobin molecule."

Not until 1932 did Heath, Strauss and Castle[11] suggest the incorporation of iron into the hemoglobin molecule by injecting iron subcutaneously in persons with iron deficiency anemia and demonstrating a close corrolation between the amount injected and increase in total hemoglobin mass. They were also able to demonstrate that iron given orally gave only a fraction of the response as when it was given subcutaneously, indicating that only a small fraction of oral iron is absorbed. Their results were soon confirmed by complementary studies of Reimann, Fistach and Schick[12] who performed elaborate iron balance studies.

Thus, as man's study of iron has incorporated more refined methods, his knowledge of the element and its functional importance

has grown from that of empirical suggestions to its present highly knowledgeable state made possible by use of enzymological and radio-isotope tracer methods.[13] Nasse has often been credited with the earliest description of the anemia of pregnancy in 1835.

As early as October, 1842, W. Channing,[14] the Professor of Obstretics and Dean of the Harvard Medical School, presented a paper on anemia in the New England Quarterly Review of Medicine and Surgery. Most of his case histories describe women with anemia not associated with pregnancy. However, nine of the cases involved pregnant women, and in each instance the outcome was fatal. He minimized excessive blood loss as a cause since one patient was "seen to live for eighteen days without flowing before dying." He expressed the conviction that there was some less obvious and less appreciable cause of the phenomenon, and even suggested that blood transfusions might be satisfactory treatment. However, in his series there is no record of such treatment.

In 1874,[15] H. N. Bennett defined the anemia of pregnancy as "a morbid state resulting only from the process of reproduction." He described symptoms of a sore mouth and gastric disturbances as signs of a transition from the physiological anemia of pregnancy to a pathologic anemia.

One of the first writen descriptions of the plasma expansion during pregnancy was that of Spiegelberg[16] in 1872 who wrote that "it is an old doctrine that as the quantity of blood increases a plethora sets in during pregnancy---indeed I have shown that such

augmentation takes place in dogs---during pregnancy, and a similar change probably in healthy women."[17]

Wilcocks, in 1881, also seemed to understand the concept of a physiologic anemia of pregnancy when he wrote that the apparent anemia in healthy pregnant women does not constitute a true anemia, but is accounted for "by the large increase of the water of plasma."

Just what part this physiologic expansion of the blood volume plays in iron defeciency anemia of pregnancy is a question that many investigators have studied and will be discussed in more detail in this paper.

Osler[18] wrote a paper on "The Severe Anemias of Pregnancy and the Postpartum State" in the British Medical Journal in 1919 which stressed the importance of such anemias. He also attempted to classify such anemias as:

1. Anemia from postpartum hemorrhage
2. The severe anemia of pregnancy
3. Postpartum anemia
4. The accute anemia of postpartum sepsis

Such a classification is incomplete by modern standards. Groups two and three seem rather arbitrary and may actually be extensions of groups one and four. A more modern classification of the anemias encountered in pregnant women is given in William's Obstetrics,[19] twelveth edition:

A. The Anemias Directly Related to Pregnancy

1. Iron Deficiency Anemia (95% of all anemias of pregnancy)

2. Megaloblastic Anemia

3. Hypoplastic Anemia

B. The Anemias Not Directly Related to Pregnancy

1. Sickle Sell Anemia

2. Hemolytic Anemia

3. Miscellaneous Rare Anemias

Brief Review of Iron Metabolism:

Any discussion of the iron deficiency anemia of pregnancy would be incomplete without some description of iron metabolism. One must attempt to understand the dynamics of iron balance to properly evaluate the studies of various investigators of this condition. Therefore, a brief review of iron absorption and metabolism is included at this point.

1. Role of Iron:

Man, as a living creature, has made use of the chemical properties of iron to function in several enzyme systems, i.e. catalase, cytochromes, and peroxidase, all of which are important to the utilization of oxygen for energy. Since iron undergoes oxidation-reduction reactions it is well suited to function in the enzymes of electron transport. Therefore, cytochromes, cytochrome oxidase, succinic dehydrogenase and xanthine oxidase all utilize iron. This element in conjunction with a porphyrin ring also has the capacity to decompose hydrogen peroxide and is utilized in this fashion by the enzymes catalase and peroxidase. Hemoglobin

reversibly binds oxygen for storage and transport in the form of a porphyrin-iron-protein complex. Not only does this compound take oxygen from the lungs and transport it to the tissues, it also carries carbon dioxide and acts as a buffer. Myoglobin also has a capacity for oxygen storage. In addition, iron appears to be essential for the function of several other enzyme systems including aconitase.

2. Dietary Sources of Iron:

Many tables have been written listing the iron content of various foods and diets.[20, 21] These figures are not completely representative of the relative amounts of available iron, however. Many factors affect availability. Available iron has been defined as that which would react with a,a' dipyridyl on reduction.[22, 23, 24] However, even iron in an organic salt, which would be 100% available by this definitation, might be only five to 40% available in vivo. Thus, many other factors affect the physiologic availability of iron.

3. The Absorption of Iron:

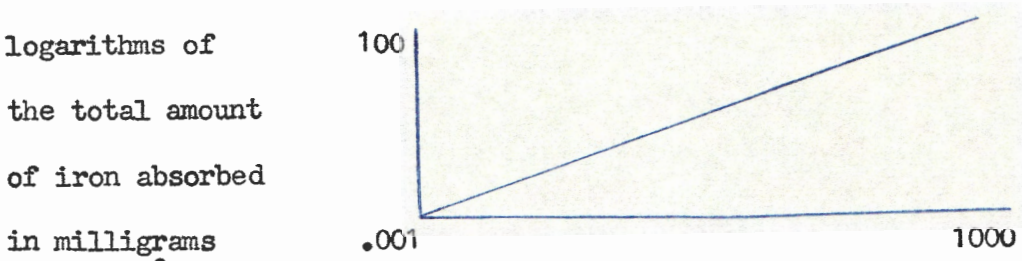
A number of factors exert a favorable or unfavorable influence on the degree of absorption of iron from the gastrointestinal tract.

A. Iron in ferrous form is better absorbed than that in ferric form when presented to the intestinal mucosa.[25, 26] Ferrous complexes with inorganic and organic anions and ferrous hydroxide are relatively more soluble than coresponding ferric complexes. Therefore an environment favorable to the reduction of ferric iron would be conducive to

better absorption. Various reducing elements can be found in the diet in the form of ascorbic acid and sulfhydryl compounds which liberate sulfhydryl groups by protein hydrolysis during digestion.[27]

B. Efficiency of iron absorption seems to be dose dependent. Smaller doses of oral iron are more efficiently absorbed than relatively larger doses. Assuming all other factors influencing iron absorption are equal, a greater percentage of a 10 milligram oral dose would be absorbed than of a 100 milligram dose. This does not mean that the total amount of iron absorbed would be greater. One can readily see that if only five % of a 100 milligram dose is absorbed 50% of a 10 milligram dose must be absorbed to give an equal amount of physiologically available iron. It is unlikely, under normal conditions, that the percentage of absorption would be as high as 50% even at the smaller dose. Therefore the total amount of iron absorbed would be greater with the larger dose even though the percentage of efficiency would be less. When the relationship between dosage of oral intake and the total amount of iron absorbed is plotted a straight line relationship is obtained. There are undoubtedly limits to this straight line relationship, but it apparently holds within physiologic ranges (Fig.1). Therefore, it is good clinical practice to give as large a dose as can be tolerated.

C. It has been said that an acid medium may enhance iron absorption by converting iron in food and in ferric form to the more soluble ferrous state.[29] This medium also inhibits the formation of insoluble



[28]

Figure 1.

iron complexes and facilitates reduction of iron by ascorbic acid and sulfhydryl groups. An acid p.h. must be regarded as only one of many enhancing factors since absorption normally takes place in the duodenum and small bowel where p.h. is alkaline. Also, patients with achlorhydria do not necessarily become iron deficient unless other factors of increased demand for iron or decreased supply are present.[30, 31]

D. Phytates and phosphates appear to inhibit iron absorption by forming insoluble iron complexes. Diets either low or high in phosphate content may significantly enhance or inhibit iron absorption. A corn meal diet which is low in phosphate content may lead to hemosiderosis, or excess iron accumulation in the body.[32, 33, 34] One might also postulate that calcium in moderate amounts would favorably affect iron absorption by combining with and lowering phosphate concentrations. However, an excessive amount of calcium in the diet will inhibit absorption.

E. There have been cases of hypochromic, mycrocytic anemias reported which responded favorably to the administration of pyridoxine. Generally these cases have had normal to increased serum iron

values. Actually it has been shown that the absorption of iron increases in pyridoxine deficiency.[35] Copper in the diet favorably affects absorption as does ascorbic acid.

F. Absorption of iron taken with food may be retarded due to formation of insoluble complexes with organic acids in the food or digestion products.[36]

4. Regulation of Iron Absorption:

With the advent of refined iron balance studies using radioactive isotopes of iron we have learned that, under normal conditions, the amount of iron excreted each day, ½ to 1½ milligrams, is very small compared to the total amount of iron in a normal diet.[37, 38, 39] This fact leads to the conclusion that if iron balance is to be maintained only a fraction of the total iron presented to the gastrointestinal mucosa may be absorbed. If there was no regulation of absorption there would be a toxic accumulation of the element.

It has been shown that patients with iron deficiency absorb iron more efficiently than normal persons. In patients who have been made anemic by bleeding the amount of iron absorbed from a given dose may be increased five to 15 times the amount absorbed before bleeding. This increased efficiency is noted only after a period of time, six to seven days after bleeding, and does not occur immediately with the drop in hemoglobin and red cell masses. Investigators have interpreted this as reflecting the fact that accelerated hematopoesis is supplied for a time by iron from iron

stores. Once such stores are depleted the gastrointestinal tract responds in some manner by increasing the percentage of iron absorption.[40] It is possible that the same factors which stimulate erythropoetic activity, i.e. anemia, anoxia, and colbalt administration, also affect the gastrointestinal mucosa directly to cause increased absorption.[41] Moore and Bothwell[42] have suggested such a theory. Whether the rate of absorption is dependent on the rate of erythropoesis or is directly stimulated by the hormone stimulating marrow activity, erythropoetin, is not known. Moore's experiments have failed to stimulate iron absorption in recipient animals by transferring plasma from iron deficient dogs. He also has observed that there is no significant difference in iron absorption in iron deficient dogs under normal atmospheric conditions or in 70% oxygen atmosphere. Further, he has shown that iron deficient women still have increased iron absorption after they have been transfused to a normal hemoglobin level. Perhaps this reflects the possibility that even though the hemoglobin level has been restored, iron stores have not been replaced. At any rate, these studies indicate that anemia and anoxia may not be the most important regulating factors. There seems to be little doubt that the status of body iron stores influences the rate of iron absorption. Most studies indicate that iron loading inhibits, for a time, the absorption of iron from the gastrointestinal tract. It has been suggested that this phenomenon may be due to the saturation of an

acceptor substance for iron in the intestinal mucosa. The protein, apoferritin, siderophylin, has been proposed as such a substance.[43, 44, 45] Hallberg and Sowel[46] have shown that injection of large amounts of this iron-binding protein will increase the rate of iron absorption in human subjects. However, the total amount of iron absorbed does not increase, and the magnitude of increased rate is below that seen in iron deficiency. There is also a recent report of a child with a congenital absence of iron-binding protein who shows normal iron absorption.[47] This theory of regulation of absorption is diagramed below:

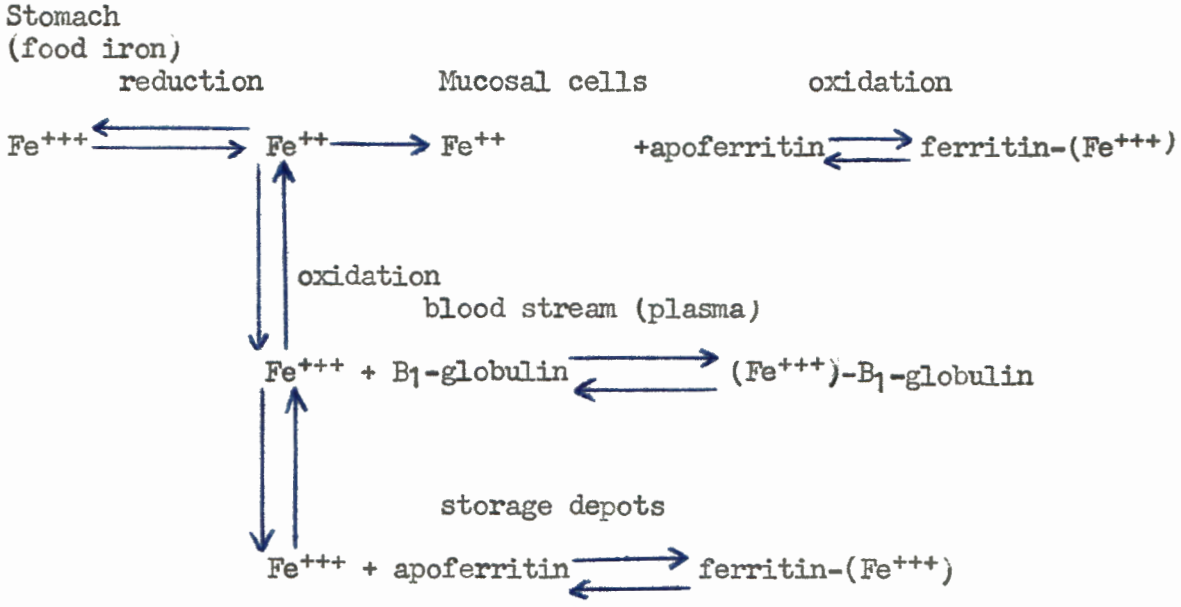


Figure 2.

Iron penetrates the mucosal cell as ferrous irons. Then Fe^{++} is in equilibrium with the Fe^{+++} form which is stored as ferritin. If there is increased demand on iron stores Fe^{++} is drawn out of the mucosal cells. Absorption begins only after the ferritin-(Fe^{+++},

diminishes to the point where the cell is below the point of "physiological saturation". The theory of a mucosal acceptor substance regulating the rate of absorption according to its availability is convenient but is not the only control since a person with an adequate or even excess amount of iron stores may absorb considerable amounts of iron. For instance, people with hemolytic and pernicious anemias, or pyridoxine deficiency may have adequate iron stores but exhibit an increase rate of iron absorption.[48, 49]

While the factors of decreased iron stores, hypoxia, and increased erythropoietic activity have been incriminated as increasing iron absorption the exact mechanism is not completely understood.

5. Iron Transport:

Iron passes from the mucosal cells into the plasma where it is oxidized from the ferrous to the ferric state by dissolved oxygen.[50] The ferric ion is then transported in combination with transferin, a beta-1 globulin, siderophylin. Most of the iron is carried to the bone marrow and liver, but some is distributed throughout all tissues of the body for use in various enzymes. When more iron reaches the plasma than the available transferin can handle, the excess iron is bound in a nonspecific manner with other plasma proteins. Normally transferin carries an amount of iron which represents about 30 to 40% of its capacity to combine

with iron, thus leaving an unsaturated iron binding capacity of 60 to 70% of the total iron binding capacity.[51] Measurement of the unsaturated iron binding capacity is usually interpreted as being elevated in the presence of low serum iron or iron deficiency anemias and decreased in the presence of high serum iron, hemochromatosis, hemosiderosis, and hemolytic disease.[52]

Although the serum iron is only .1% of the total iron circulating in the blood its level reflects all other processes of iron balance and metabolism in the body. A simplified diagram demonstrates this fact:[51] (Fig. 3)

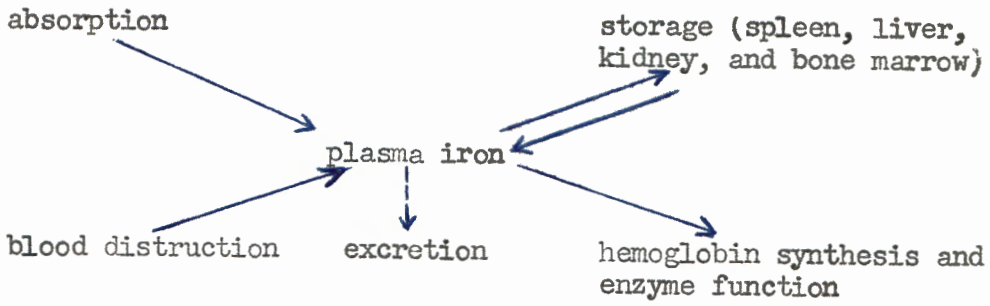


Figure 3.

Thus, the plasma iron level is a resultant value and is subject to varriation if any of the contributory factors change. For example, if iron absorption is sub-normal, the plasma iron level will drop unless there are other compensatory factors such as: decreased excretion, decreased hemoglobin synthesis or mobilization of iron stores. In order to know the particular mode of compensation, in an individual case, one must measure each of these factors, i.e. iron stores, hemoglobin and red cell masses, and iron

excretion.

6. Iron Storage:

The amount of total body iron is usually given as three to five grams. Its distribution in the body is depicted on the following graph: (Fig. 4)

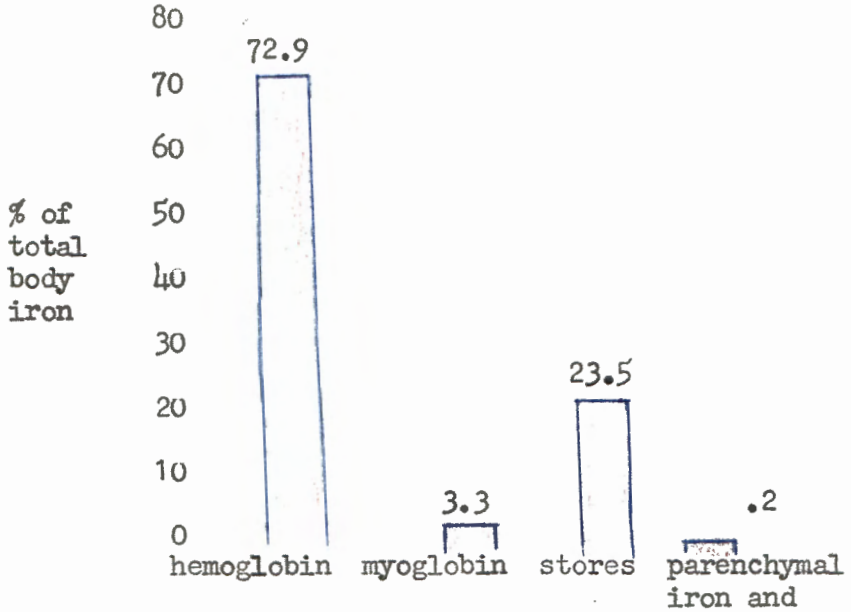


Figure 4.

It should be mentioned that investigators differ somewhat on the precise nature of distribution of iron in the body. The amount of iron in each category undoubtedly varies in individuals, especially in disease, but also in health. The segment of iron contained in the stores is subject to the greatest variation. It decreases when needs for iron exceed absorptive capacities, as in chronic blood loss or pregnancy. Stores are usually somewhat less in women than in men.[53] However, anemias not due to iron deficiency

may have increased iron stores, especially if iron or blood has been given to the patient. The amount of iron in the hemoglobin compartment can be calculated from the hemoglobin concentration and the blood volume. In polycythemia a greater percentage of the total body iron is concentrated in the hemoglobin at the expense of the storage iron. It has frequently been stated that, as a person becomes progressively iron deficient, first the iron stores become depleted then the hemoglobin level decreases while the amount of iron in the enzyme systems remains fairly constant. Haln and Whipple[54] presented this concept in the following diagram: (Fig. 5)

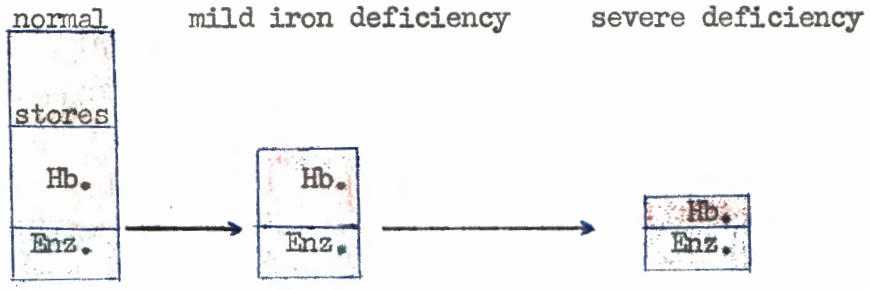


Figure 5.

This theory is not entirely reasonable since there would be no depletion of iron containing enzymes until all hemoglobin in the body was gone. This is in conflict with Gubles[55] who has shown that myoglobin and cytochrome c content in the muscles of severely iron deficient baby pigs is markedly decreased. Perhaps a more accurate picture of the progressive depletion of the various compartments containing iron in an increasingly severe iron deficiency state is depicted by the following graph. (Fig.6) In this case

hemoglobin is shown to decrease slightly, before all stores are gone in mild deficiency. Likewise, enzymes decrease slightly, before the hemoglobin compartment is totally exhausted.[56] In addition, a graph is shown representing iron distribution in iron deficiency as compared to other anemias. (Fig.7)

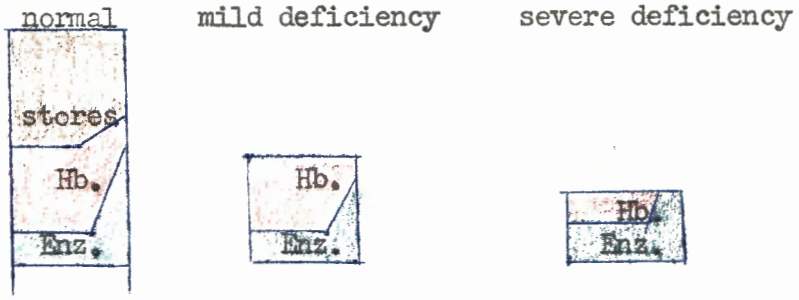


Figure 6.

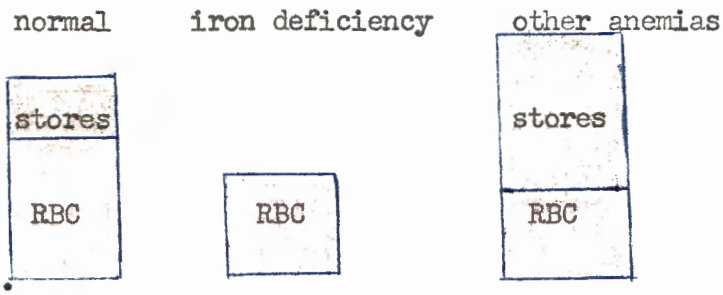


Figure 7.

In the average adult slightly less than thirty milligrams of iron is liberated everyday by the normal breakdown of hemoglobin.[57] The porphyrin fraction of the hemoglobin is degraded further and excreted. Most of the liberated iron is conserved for synthesis of new hemoglobin with only a small fraction being excreted. Newly absorbed iron and iron liberated into the plasma pool from hemoglobin breakdown is used for new hemoglobin synthesis in preference to that in storage.[58]

Ferritin and hemosiderin are storage forms for iron in the liver, kidneys, spleen, and bone marrow.[40] Ferritin is more soluble than hemosiderin and can be extracted from tissues of storage with water or saline. Ferritin is composed of a protein, apoferritin, that combines with iron in the form of micelles of ferric hydroxide. Hemosiderin, the other storage form of iron, is also made up of an iron-ferric hydroxide-protein complex but is relatively insoluble compared with the extraction of ferritin.[59]

While it is known that these two storage forms may exist simultaneously, the exact relationship between these two compounds is not fully understood. Hemosiderin may not be detected by iron storage staining proceedures unless the quantity of iron in stores is normal or increased. If there is only a small quantity of iron present in the stores, ferritin is probably formed in preference to hemosiderin. If iron is administered in excess of the ability of apoferritin to combine with it the capacity of hemosiderin as a storage form is utilized. Roughly then, the presence or absence of hemosiderin in iron stores indicates something of the state of the stores. If hemosiderin is present the stores are normal to increased. If iron stores are present but no hemosiderin can be detected they are probably decreased. This is of practical value since no therapeutic response to iron administration in terms of increased hemoglobin can be expected unless hemosiderin is absent. If iron is administered in the presence of demonstrable hemosiderin

in bone marrow aspirations or liver biopsys, the only probable response will be an increase in iron stores.[51]

7. Physiological Requirements for Iron:

As has already been mentioned, man conserves much of the iron which is liberated from hemoglobin breakdown each day. Most of the iron which is required for synthesis of new hemoglobin is supplied from this source. To maintain iron balance an amount of iron must be absorbed which is equal to the amount excreted. In normal adult males this averages approximately 1.2 milligrams per day as compared with the 26 to 27 milligrams required for daily hemoglobin formation. Blood loss, either from acute or chronic bleeding, increases this requirement.

According to Barker and Fowles[60] the average amount of blood lost during a menstrual period is approximately 50 milliliters. The range in their series was from 6.5 to 187.7 milliliters, but in 50% of their patients blood loss ranged 23.2 and 68.4 milliliters. Assuming a 50 milliliter blood loss and a normal hemoglobin content of 13.5 grams% a woman should lose approximately 6.75 grams during a menstrual period.[61] Applying Butterfield's figure of 3.35 milligrams of iron for each gram of hemoglobin, the amount lost in each period may be calculated as approximately 23 milligrams. When this figure is added to the other excretory routes, excretion in the menstruating woman may average two milligrams per day. It must be remembered also that this figure like all other average values is subject to normal variation. Considering the wide range

in amount of menstrual bleeding, many women probably excrete as much as three milligrams per day.

Since only a small percentage of the iron in an average diet can be absorbed this increased excretion of iron in women, as compared to men and non-menstruating women, contributes to a depletion of iron reserves.[62] In fact, Stevens, Colmen and Finch have shown, by means of evaluating sternal marrow iron content, that nearly 75% of women have some degree of iron storage depletion. Therefore, many women, especially those who are multiparous, probably go into a pregnancy with partilly depleted iron stores and are more susceptible to an iron deficient anemia.

8. The Iron State in Pregnancy:

During pregnancy iron is no longer lost with menstruation. As a working estimate this conserves approximately nine x 23 = 207 milligrams of iron.[63] According to Lob[64] and Widdoinson who studied the iron content of stillborn infants, the content of iron in a full term fetus is six to 10 milligrams per 100 grams of body weight, some 300 milligrams total. A blood loss of 200 to 300 milliliters at the time of delivery means an additional (two to three x 13.5 x 3.35) = 90 to 135 milligrams of iron which is lost. Another 75 milligrams may be added for placental and cord content.[65] These figures account for an approximate deficit of 500 milligrams above normal daily excretion which is about 40% compensated for by decreased menstrual loss during pregnancy. If

this was the only increase in iron loss it would be hard to explain and significant anemia due to iron deficiency. Even if iron stores were 300 milligrams they would be sufficient to maintain normal hemoglobin production.

The other sources of increased iron demand which must be considered are the increase in plasma and red cell volumes which normally expand during the second and third trimesters. The increase in these values is important in two ways. First, the increase in plasma volume has the effect of lowering red cell count and hemoblobin measurements which are recorded by unit volume. Therefore, a significant increase in the plasma volume would have the effect of producing an apparent anemia without actually causing a deficiency of red cell or hemoblobin masses or red cell hemoglobin content. Secondarily, the plasma volume increase is generally paralleled by some degree of increase in red cell mass. This is a highly variable source of increased demand for iron in new hemoglobin synthesis. Because of the importance of these mechanisms they will be reviewed in greater detail.

A schematic outline of iron metabolism is useful in bringing the total picture of iron metabolism into focus: (Fig.8)[66]

Evaluation of Plasma Volume Espansion and Increase in Red Cell Mass

This paper has already eluded to the phenomenon of changing blood volume during pregnancy. In 1934, Dieckmann and Wegner[67]

reviewed the literature concerning this subject as well as conducting experiments of their own. They based their research on three considerations: "1. Is there a sufficient increase in blood volume during pregnancy, as is frequently stated, to permit a loss of from 1000 to 1500 cubic cenitimeters of blood at delivery with no obvious ill effect? 2. The estimations of blood and plasma volumes form the basis for determination of total hemoglobin, erythrocyte mass, serum protein, fibrin, total base, etc. 3. If the blood and plasma volumes are increased, it is a phenomenon well worth study in itself."

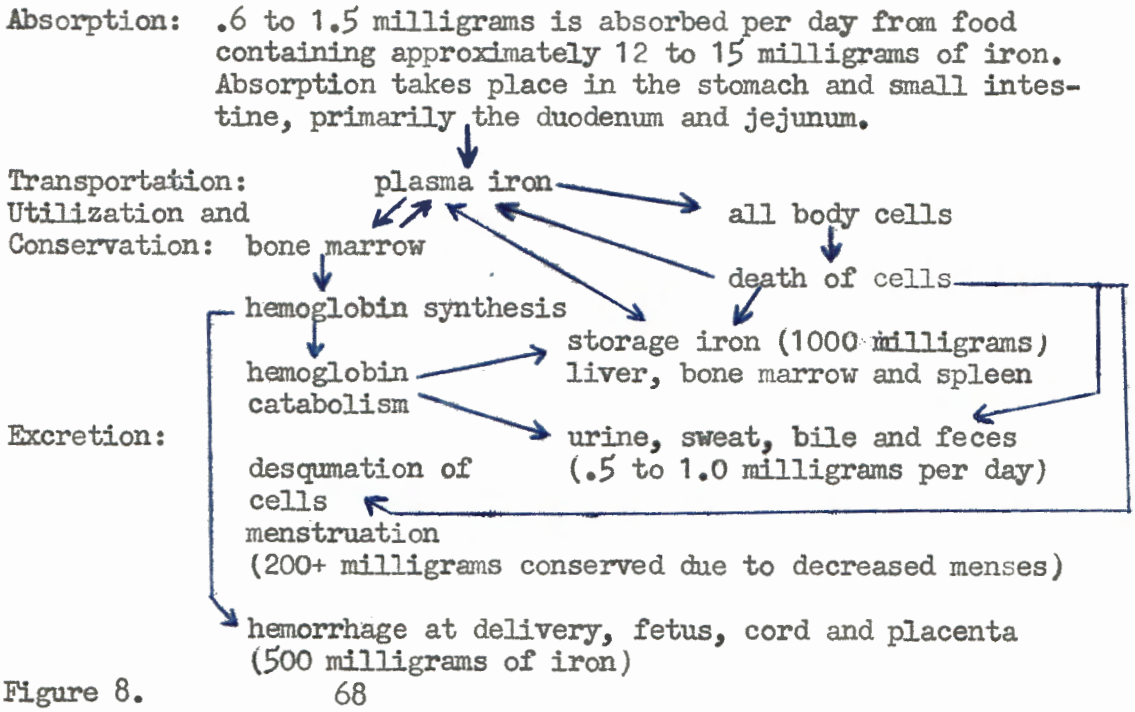


Figure 8. 68

In 1928, "De Lee said," The total quantity of blood is agumented, especially in the last months of pregnancy.---The need for more blood must be admitted; the addition of the fetus and the fetal

circulation, the development of the uterine arteries and veins, the enlargement of the veins of the lower extremities, which are sometimes so great that they appear to be veritable caverns."[69]

This agrees with the works of Williams, in 1930, who went further and said, "Observations made in my clinic in 1915, by Miller, Keith, and Rountree[70] seem to show that during pregnancy there is a definite increase in both the plasma and blood volume, which disappears during the puerperum."

Many authors have published reports on blood and plasma volumes during pregnancy which seem to have conflicting results. One reason for their discrepancies is that many studies are reported in terms of volume per unit weight, rather than in total volume changes. Thus, they not only represent the volume changes in the blood and plasma, but also the increase in the patient's weight during pregnancy. This means that even if there were no change in the blood and plasma volumes during pregnancy, there would be a variable decrease in the volume per unit weight directly related to the individual weight gain during pregnancy. For example, a girl weighing 50 kilograms with a blood volume of 85 cubic centimeters per kilogram has a total volume of 4250 cubic centimeters. If, at term, she weighs 59 kilograms and still has a total blood volume of 4250 cubic centimeters, her volume per kilogram is only 72 cubic centimeters, based on her current weight. With this method a 15.3% decrease would be reported

without any real decrease in total circulating volume.

Kehrer[71] reviewed the German literature on this subject in 1923 and concluded that the blood volume ranged from five to 6.3% of the body weight, but during the last two months of pregnancy it ranged from seven to 8.3%. Assuming an average non pregnant weight of 55 kilograms and a term weight of 64 kilograms, which are reasonable values, Kehrer's figures could represent a total blood volume increase of as much as:

$$\frac{\frac{.07x64+.083x64}{2} - \frac{.05x55+.063x55}{2}}{\frac{.05x55+.063x55}{2}} = 57\%$$

Obviously, to be meaningful, investigators of this phenomenon must either keep records in terms of total blood volumes or include weight changes along with the findings reported in cubic centimeters per kilogram so that a corrective factor may be subtracted for the weight gain of pregnancy. If the blood volume must be recorded in terms of cubic centimeters per kilogram of body weight, for sake of convenience of comparison, an amount of weight should be subtracted equal to the weight of the baby, placenta, cord and extra vascular fluid which is really not a part of the mother's weight but that of a temporary parasite. This is true since some methods used to measure the blood volume, i.e. various dye techniques, do not pass the placental barrier and therefore do not measure the blood volume of the fetus. As a result, expansions in volume which have been shown are strictly

maternal.

Other factors which are important to the validity of studies on blood and plasma volume changes during pregnancy are: 1. All patients in the series should be checked at the same stages if comparisons are to be made. 2. The first blood volume should be determined as early in pregnancy as possible so that a value near the non-pregnant state can be recorded. 3. Equipment which is to be used should be standardized and checked frequently so that the results may be as nearly accurate as possible. 4. Blood volumes should be determined at least three times, i.e. early in pregnancy, near term, and some time after delivery. 5. All determinations should be made using the same technique so that the errors which are inherent to the various methods, i.e. carbon monoxide, trypan red, congo red, vital red and the refractometer, will tend to be constant and definite serial trends can be shown even though none of the values may be 100% accurate.

Since the fetal blood has a higher hemoglobin content than maternal blood it has a greater capacity to combine with carbon monoxide. Consequently, values using the carbon monoxide method will seem higher for the maternal blood than they actually are. Colorometric dye and carbon monoxide methods measure only the circulating volume which is something less than the total volume. Dye methods deal with the plasma, and carbon monoxide methods deal with hemoglobin, so both depend on hematocrit determinations

for calculation of blood volume. Since the hematocrit may vary in different parts of the vascular system it is important that the same site be used in serial testing. This also points up the fact that comparison of the figures of different investigators is of limited value, but serial results of one investigator using a constant technique may be useful in determining trends.

Using vital red with a colorimeter technique Dieckmann and Wegner stated that for changes in volume to be significant they must exceed a plus or minus five %. In their series they injected either eight or 10 cubic centimeters of vital red dye, depending on the patient's weight, using heparin as an anticoagulant for determining cell volume. Their syringes and colorimeter were standardized, and controls were made for the dyes used for injection.

Their most significant study was done on a group of thirteen patients who were followed throughout pregnancy and puerperium. Blood volumes were performed on their patients in four approximate stages: as early as possible after the discovery of pregnancy or an average of thirteen weeks, seven months pregnant, term and 1½ to two weeks after delivery.

The following is a table reproduced from their article in the Archives of Internal Medicine listing their results. Their calculations of percentage changes in total volume are somewhat confusing so I have recalculated their figures in terms of a percentage increase or decrease rather than using 100% as a normal level, less than 100% as a decrease in volume, and greater than

100% as an increase in volume. While not specifically stated in their article the hematocrit and red cell masses have also been calculated and included in the new set of figures. (Table 1).

	27 weeks	39 weeks	11 days post partum
mean blood volume change %	+14	+19.8	+17.7
standard deviation of mean blood volume change %	±19.9	±23.6	±28.4
standard error of the mean blood volume change %	±5.5	±6.6	±7.9
mean plasma volume change %	+18.5	+24	+17.7
standard deviation of mean plasma volume change %	±19.5	±27.6	±30.6
standard error of the mean plasma volume change %	±5.4	±7.7	±8.5
mean red cell mass change	+8.9	+16.1	+19.4
standard deviation of mean red cell mass change %	±20	±27.7	±28.6
standard error of the mean red cell mass change %	±5.6	±7.7	±8.0

Above is a summary of the results I have calculated from values in Table 1.

Table of Data (Table 1)

	gravida race and age ♀	period of gestation or post partum	loss of blood at delivery c.c.	weight in kg.	absolute weight gain	% weight gain
1	2 w 20	13 wk 29 wk 40 wk 12 d p.p.	200	46.3 55.9 59.5 50.4	+13.2	+28.6
2	1 w 23	14 wk 30 wk 40 wk 12 d p.p.	200	77.9 81 80.9 73.6	+3	+3.9
3	1 w 23	15 wk 30 wk 40 wk 12 d p.p.	200	53 61.1 63.5 53	+10.5	+19.8
4	7 w 30	14 wk 27 wk 40 wk 14 d p.p.	200	92.2 91.7 87 82.7	-5.2	-5.6
5	2 w 25	12 wk 28 wk 39 wk 14 d p.p.	200 c. sed.	53.6 59.5 60 52.5	+6.4	+12.5
6	1 w 19	14 wk 27 wk 40 wk 11 d p.p.	200	45.9 54.8 63.5 56	+17.6	+35.6
7	6 w 31	14 wk 30 wk 40 wk 5 d p.p. 13 d p.p.	150	107.2 112.5 114.6 100.3 100.4	+7.4	+6.9
8	3 c 30	14 wk 28 wk 40 wk 11 d p.p.	200	92.9 103.5 108.9 100	+16	+17.3

	gravida race and age	period of gestation or post partum	loss of blood at delivery c.c.	weight in kg.	absolute weight gain	% weight gain
		14 wk		50.6		
	1	20 wk		58.8		
9	c	40 wk	200	60	+5.2	+10.3
	21	3 d p.p.		55.8		
		12 d p.p.		52.5		
		20 wk		71.8		
	1	23 wk		72		
10	w	36 wk	200	75.5	+3.7	+5.2
	26	3 d p.p.		70.3		
		15 wk		67.2		
	1	28 wk		73.8		
11	w	39 wk	200	76.5	+9.3	+13.8
	22	12 d p.p.		68.1		
		9 wk		61.3		
	1	27 wk		80		
12	c	40 wk	200	86.3	+25	+41
	28	11 d p.p.		75.6		
		11 wk		68.1		
	8	29 wk		75.4		
13	c	40 wk	200	71.5	+3.4	+5
	37	11 d p.p.		65.6		

Table of Data

	surface area in sq. m.	total blood volume	% change in blood volume	total plasma volume	% change in plasma volume	% hematocrit
	1.48	3113		1868		40
	1.50	5009	+60	3106	+66	38
1	1.64	5930	+79	3855	+106	35
	1.53	5520	+77	3478	+86	37
	1.82	4585		2935		36
	1.85	4951	+8.0	3367	+14.7	32
2	1.85	4968	+8.5	3478	+18.5	30
	1.77	4643	+1.3	3018	+2.8	35
	1.66	5221		2978		43
	1.70	6415	+23	3720	+25	42
3	1.73	5444	+4.3	3440	+15.5	37
	1.66	5601	+7.3	3137	+5.4	47
	2.07	5369		3007		44
	2.07	6318	+17.6	3669	+22	42
4	1.99	7143	+33	4000	+33	44
	1.95	5750	+7.1	2990	-.6	48
	1.45	3681		2135		42
	1.52	4448	+21	2758	+29.2	38
5	1.52	4822	+31	2990	+40	38
	1.44	5932	+61	3678	+72.5	38
	1.41	3438		2201		36
	1.52	4448	+29.4	2758	+25.3	38
6	1.61	5091	+48	3106	+41	39
	1.53	5015	+46	2909	+32	42
	2.14	5756	+2.7	3454		40
	2.18	5910	+12.2	3783	+9.5	36
7	2.20	6471	+8.6	3883	+12.4	40
	2.08	6250	+36.1	4000	+43	36
	2.08	7838		4938		37
	2.05	6643		4318		35
	2.15	6269	-5.7	3950	-8.5	37
8	2.20	7050	+6.2	4301	-.4	39
	2.11	5395	-18.6	3669	-15	32

	surface area in sq. m.	total blood volume	% change in blood volume	total plasma volume	% change in plasma volume	% hematocrit
	1.45	3968	+21	2500		37
	1.55	4800	+31.4	3168	+27.5	34
9	1.56	5209	+23.8	3232	+29.2	38
	1.51	4914	+28.8	3047	+18.5	38
	1.48	5106		2962		42
		5341		3205		40
		4540	-22.5	2815	-12.2	38
10		6428	+20.4	3600	+12.3	44
		5301	-.8	3181	-2.7	40
	1.70	5830		3440		41
	1.76	5763	-1.2	3516	+2.2	39
11	1.79	5763	-1.2	3516	+2.2	39
	1.71	6107	+4.8	3298	-4.1	46
	1.69	5213		3076		41
	1.90	6783	+30	4102	+33	40
12	1.95	4901	-6	3137	+2.0	36
	1.85	4961	-4.9	3076	0.0	38
	1.78	6168		3578		42
	1.85	6046	-2	3809	+6.5	37
13	1.81	5554	-9.9	3555	-.6	36
	1.74	5686	-7.8	3298	-7.8	42

	red cell mass in c.c.	percentage changes in red cell mass
	1245	+53
	1903	+67
1	2075	+64
	2042	
	1650	-4.0
	1584	-9.7
2	1490	-1.5
	1625	
	2243	+20
	2695	-10.7
3	2004	+14.3
	2564	
	2362	+12.2
	2649	+33
4	3143	+16.9
	2760	
	1546	+9.3
	1690	+18.5
5	1832	+45.8
	2254	
	1237	+36.6
	1690	+60.5
6	1985	+70
	2106	
	2302	-7.6
	2127	+12.4
7	2588	-2.3
	2250	+26
	2900	
	2325	-.30
	2319	+18.2
8	2749	-25.8
	1726	

	red cell mass in c.c.	percentage changes in red cell mass
	1468	+9.8
	1612	+34.6
9	1977	+27
	1864	+46
	2144	
	2136	-19.3
	1725	+32.4
10	2828	-.80
	2120	
	2390	-6.0
	2247	-6.0
11	2247	+17.5
	2809	
	2137	+25.4
	2681	-17.5
12	1764	-11.8
	1885	
	2590	-13.8
	2237	-23
13	1999	-7.8
	2388	

From analysis of these results several conclusions can be drawn. First, by comparing the relative increases in red cell volume with the increase measured in the plasma volume it is apparent that the mean increase in plasma volume is greater than the corresponding increase in red cell mass at 27 and 39 weeks of pregnancy. The figures are approximately equal at 11 days post partum. In other words, the state at 27 and 39 weeks as compared with the base line at 13 weeks shows a mean blood volume expansion with a greater component of plasma increase than red corpuscular increase. This might be termed a slightly oligocythemic hypervolemia as compared to a normocythemic hypervolemia at 11 days post partum.

These figures show a mean total red cell expansion of greater than 16% near term. As a working model a girl weighing 50 kilograms with a total blood volume of 4250 cubic centimeters and a hematocrit of 40% would have a red cell volume of some 1700 cubic centimeters. At term her red cell volume might be expected to increase to (.16x1700+ 1700) = 1972 cubic centimeters. If one now assumes a hematocrit of 38%, which was the mean value at 39 weeks, this means an increase of approximately 716 cubic centimeters in the blood volume or an increase of (7.16x10) = 71.6 grams in the total body hemoglobin. This is a conservative estimate since it assumes a hemoglobin concentration of only 10 grams % at term. If Butterfield's figure of 3.35 milligrams of

iron for each gram of hemoglobin is applied, this accounts for an additional iron requirement of (3.35x71.6) = 240 milligrams of iron.

Perhaps a more direct way of estimating the increased iron demand is to assume that it increases proportionately to the increase in red cell mass. Approximately 72.9% of the total body iron is in hemoglobin.[51] If a figure of 3000 milligrams is used for the total amount of iron in a normal 50 kilogram woman then she would have about (.73x3000) = 2190 milligrams in hemoglobin. If pregnancy causes a 16% increase in red cell mass it is reasonable to assume that it also causes a 16% increase in the hemoglobin mass. This relationship is dependent on a fairly constant mean corpuscular hemoglobin content, but should hold for a rough extimate. Therefore, (.16x2190) = 350 milligrams of additional iron requirement can be accounted for by this method. This is a fairly conservative extimate compared to the estimate of 500 milligrams by Caton[72] who has studied circulating red cell volumes in pregnancy by using radioactive red cells.

We can say then that the mean red cell expansion occuring during pregnancy probably accounts for a significant source of additional iron requirement. When added to the iron deficit of 500 milligrams calculated earlier in this paper we can account for a total deficit of some 800± milligrams above normal daily excretion. Subtracting 200 milligrams for the compensatory con-

servation via decreased menstrual loss there is a net loss of approximately 600± milligrams of iron due to pregnancy and delivery as compared with the non-pregnant woman. Using Caton's figures this estimate could go as high as 1000 milligrams for a total value and 800 milligrams for net increase in iron demand. Considering the wide range in red cell expansion at term as found by Dieckmann and Wegner (-23% to +67%) with a standard deviation of ±28.6%, one must anticipate that some women may have an increased iron demand from red cell expansion up to four or more times that which I have calculated in the working model. Others like patients #two and #13 in the series will have essentially no demand from this source.

It is interesting to consider hypothetical examples of two different types of patients during pregnancy. First, a woman with normal stores of 1000 milligrams should be able to withstand the net iron deficit of 600 to 800 milligrams without production of an iron deficiency anemia provided her red cell expansion does not greatly exceed the mean of Dieckmann and Wegner's figures. This does not mean, however, that values of her hemoglobin, red cell and hematocrit will not drop since they may do so due to a relatively greater plasma volume expansion. Next, a woman with diminished iron stores of 300 milligrams will probably exceed her reserves by 300 to 500 milligrams during pregnancy and may well experience a mild degree of true iron deficiency with totally

depleted stores. When her red cell plasma and volumes return to normal, some time after delivery, the breakdown from surplus hemoglobin may serve to replenish this deficit but will probably leave her with negligible stores. Unfortunately, Dieckmann and Wegner's serial volume determinations were not continued until their patients had returned to normal. This would have been a valuable addition to their study. It is frequently stated that iron therapy should be continued after delivery not only until the hemoglobin level has returned to normal but for an additional time since it is also necessary to replace the missing iron stores. Perhaps this theory is a bit misleading in view of the endogenous source of iron from excess hemoglobin.

When the patients are divided into two groups, those less than 55 kilograms and 1.66 square meters and those greater than 55 kilograms and 1.66 square meters, it is interesting to note that those of the former group, i.e. numbers 1,3,5,6, and 9 showed a mean blood volume increase of 37.2% at term. The remainder showed a mean increase of only 5.9%. The figures at 11 days post partum show a mean increases of 44% and 2.2% for the same respective groups. Total expansion of the red cell volume for the first group averaged 36% at term and only 5% for the second group. From these comparisons one might conclude that a smaller woman apparently has a relatively greater expansion in terms of percentage of her total red cell mass than the stocky or obese woman. There are two notable exceptions to this trend in patients #4

and #10 who, at term, showed red blood cell volume increases of 33% and 32.4% respectively. Certainly a larger series needs to be correlated with this apparent trend before it can be proven significant. While the same absolute volume increase in an overweight as compared to a normal-weight woman shows a greater percentage increase in the smaller blood volume of the normal woman this would not account for such great discrepancies as have been noted. In fact these figures tend to show that women less than 55 kilograms in this series averaged not only relatively larger increases in red cell volume but absolutely greater increases. A valuable study would be to attempt to correlate the incidence of iron deficiency anemia of pregnancy, diagnosed by direct measurement of iron stores, with the non-pregnant weight of the patients.

Interestingly, the mean weight gain of the first group of lighter patients was 10.6 kilograms while it was only 7.8 kilograms for the heavier women. When this is figured as a percentage of their base line weight the lighter women had a mean increase of 21.4% while the remainder of the patients had a mean increase of 10.9% of their original weight.

Diagnosis:

In the final analysis probably the best way to determine if an anemia in pregnancy is due to iron deficiency, physiologic anemia, or an unrelated anemia occuring during pregnancy is to measure the iron stores in some manner. Ernest Beutler[73] has evaluated several

methods designed for this purpose and described their relative efficiency.

1. Serum Iron Concentration:

Most investigators agree that the serum iron level is generally low in an iron deficiency anemia. A low serum iron level, however, is not a specific test for iron deficiency anemia. Low values may also be encountered in chronic infection, uremia and carcinomatosis. One must remember that the plasma iron is a resultant of many factors. Actually the stores may be increased in the afore mentioned conditions. There are some valid conclusions that may be drawn from this test. If the serum iron is found to be high it is not probable that the stores are decreased. If the value is normal or low many other causes must be ruled out before one can make the diagnosis of iron deficiency.

2. Serum Iron and Iron-Binding Capacity:

The iron-binding capacity refers to the ability of siderophilin, a beta-1 protein, to combine with iron in the plasma. Normally there is an excess of siderophilin in the plasma. Hence, only a fraction of the protein binds the iron. A measure of the free, unbound siderophilin is referred to as the unsaturated iron binding capacity. One method of measuring this capacity is to add increments of iron to the plasma until no further change in absorption at 460 millimicrons occurs. The absorption which is measured at this range is due to the bound form of siderophilin. Another method involves over saturating a sample of plasma with iron and then

measuring the quantity of unbound iron. The iron binding capacity is equal to the total amount of iron added minus the quantity of unbound iron.

The measurement of iron-binding capacity is of some use if differentiating several conditions which all show a low serum iron. One would expect the iron binding capacity to vary inversely with the serum iron concentration. The unsaturated iron-binding capacity usually increases in conditions which involve a depletion of body iron stores just as it decreases in conditions with increased serum iron, like hemochromotosis. However, it usually decreases in chronic infection, uremia and cancer in spite of low serum iron concentrations. To complicate the picture there is some overlap in lab determinations reported for iron deficiency, uremia, chronic infection and normal people. Pregnancy seems to be a unique condition since the iron-binding capacity may be increased but does not seem to be completely dependent on deminishing iron stores.

The determination of serum iron in addition to iron-binding capacity may be of some aid in evaluating iron stores, but to a certain extent there is a degree of unreliability especially in the presence of complication factors such as infection or chronic disease.

3. Iron-Tolerance Curve:

Moore[74] and others have shown that an iron-tolerance curve may be of diagnostic aid in demonstrating iron deficiency. Iron-

tolerance curves are plotted by measuring the rise in serum iron concentration after giving a standard dose of iron as iron gluconate. Assuming the principle that iron deficient subjects are able to absorb iron more efficiently, they should show a greater rise in serum iron concentrations than subjects who are not iron deficient. There are several faults with this test. One source of error in persons with an iron deficiency anemia is the fact that they tend to produce new hemoglobin with the exogenous iron. If serum iron determinations are not made soon after the administration of iron gluconate they may not be at peak levels.

Other factors than the size of the iron stores also influence iron absorption, making the results of this test somewhat hard to evaluate. Presumably patients with steatorrhea would have flat iron-tolerance curves in the face of depleted stores. Thedering[75] has even proposed a decompensation of the absorptive mechanism in the duodenal mucosa in iron deficiency anemia which may cause a flattening of the iron-tolerance curve.

From studies using the iron-tolerance curve Jasinski and Diener[76], and Goldeck and Remy[77] concluded that 40 to 50% of pregnant and parous are iron deficient. This estimate varies considerably from that of Pratt and Johnson[78], using studies on bone marrow hemosiderin as an indication of the state of iron stores. The iron-tolerance curve would also suggest that patients with pernicious anemia must have depleted iron stores since Goldeck[77] demonstrated

peak iron-tolerance curves in all seven patients with pernicious anemia who were tested during the active regeneration that followed treatment. This is in direct conflict with the work of Vries and Izak[79] who were able to show histologic evidence of increased iron stores in patients with pernicious anemia.

4. Phlebotomy:

This diagnostic procedure makes use of the principle that an individual will compensate, to a large extent, for the hemoglobin lost through multiple phlebotomies by producing new hemoglobin from mobilized iron stores. Once the iron stores are depleted the phlebotomies will begin to reflect themselves directly by decreasing hemoglobin concentrations. If the patient is given adequate time for hemoglobin synthesis following a phlebotomy of 500 cubic centimeters a person with completely depleted stores prior to the phlebotomy will probably show a decrease of about $1\frac{1}{2}$ grams % in hemoglobin concentration. A person with normal or greater than normal stores will probably show a decrease of something less than this or possibly no decrease at all. These results assume that there are no other factors depressing the regeneration of new hemoglobin.

Pritchard and Ruble[53] conducted serial phlebotomies on groups of normal male and nulliparous female patients. Each week an amount of blood approximately equal to one sixth of the subject's blood volume was drawn off and tested for hemoglobin concentration,

hematocrit, serum iron and total amount of hemoglobin removed. Circulating hemoglobin mass was measured using an aliquot of the subject's red blood cells labeled with radioactive chromium. Marrow samples were also stained with Prussian blue for hemosiderin. This routine was continued until the patient was no longer able to maintain hemoglobin levels above 10 grams %. This was assumed to be the point at which all available stores had been used up. The total amount of iron utilized was then calculated: 1. Initial hemoglobin mass - final hemoglobin mass = hemoglobin deficit in grams. 2. Total grams of hemoglobin removed by phlebotomies - hemoglobin deficit = amount of new hemoglobin synthesized in grams. 3. Hemoglobin synthesized x 3.4 (amount of iron in one gram of hemoglobin) = iron utilized for hemoglobin production. The amount of iron available from stores was then calculated as the total amount needed for new hemoglobin synthesis - that iron which was calculated to have been available from the diet. Using this technique of calculating iron stores they found that the males in their series averaged 819 milligrams while nulliparous, menstruating women averaged only 254 milligrams of storage iron. If their low figure of iron stores in young nulliparous women can be accepted it is not suprising that many women may become iron deficient with pregnancy even though it is their first pregnancy.

For obvious reasons this test is not of practical use in the severely anemic patient. It is doubtful that there is any place

for use of it to evaluate iron stores in pregnancy although it probably could differentitate between the so called physiologic anemia of pregnancy with adequate stores and a truly iron deficient anemia of pregnancy. Its greatest use lies in confirming the diagnosis of hemochromatosis. Such patients should be able to withstand several phlebotomies without a significant drop in hemoglobin concentration.

5. Studies with Radioactive Iron:

This method of evaluating iron stores in pregnancy is mentioned only to be condemed. As with any other radiologic diagnostic tests, pregnancy is usually considered a direct contraindication unless there is no alternative.

One might suspect that all of a tracer dose of iron introduced into the blood stream would be carried in the circulating red cells of an iron deficient subject and that a somewhat smaller percentage would be found in subjects with adequate iron stores due to uptake by the stores. However, 100 % incorporation of the isotope into circulating red cells has also been reported[80] in those with adequate stores. Therefore, even if there were no serious contraindication to this test in pregnancy there is considerable doubt that there would be much useful information derived.

6. Histologic Determination of Storage Iron:

Of the two storage forms of iron, hemosiderin and ferritin, hemosiderin is the predominant form appearing in marrow stained

by the Prussian blue reaction. Since the bone marrow is one of the storage sites for iron a histologic assay of an aspirate is probably the most direct way to evaluate the iron stores. The disadvantages of this procedure are: the discomfort of a sternal marrow aspiration, expense of the procedure, the discrepancies from one investigator to another in estimating the amount of iron present and the fact that iron stores are not necessarily distributed evenly throughout the marrow leaving the possibility of varying aspirates even in the same person. Several criteria have been suggested by various investigators for grading marrow samples. Rath and Finch[81] who first introduced this method of staining fragments of bone marrow for iron graded their results from 0 to 6+. More recently Pratt and others[78] have lister their criteria as follows:

0 = no stainable hemosiderin

trace = essentially no stainable hemosiderin

1+ = decrease (slight amount)

2+ = moderate (normal)

3+ = increased (moderately heavy)

4+ = very heavy

Almost without exception investigators have found markedly decreased or absent stainable iron in specimens from iron deficient subjects. Increased iron stores were found in many other conditions associated with anemia but not iron deficiency: pernicious anemia, uremia, cirrhosis, disseminated lupus erythematosus, hemosiderosis

and hemochromatosis.

Stevens, Coleman and Finch[62] obtained similar results on a larger number of patients, modifying their method, by merely examining the marrow fragment for golden yellow iron granules without specific staining techniques. The results of various investigators in examining bone marrow fragments for iron stores in several hematologic states is given in tabular form below:

1. Rath and Finch[81] examined marrow samples which were stained and graded for hemosiderin on the basis of a 0 to 6+ classification. They came to two basic conclusions. First, that 16 iron deficient subjects showed no appreciable amounts of stainable iron, and secondly, that stores in patients with pernicious anemia, uremia, cirhosis, disseminated lupus erythematosus, hemosiderosis and hemochromatosis were generally increased.

2. Stevens, Coleman and Finch[62] examined marrow fragments for golden-yellow iron granules without staining for iron. Their results were very similar to those of Rath and Finch.

3. Hutchinson[82] examined marrow sections that were fixed and then stained for iron. He found no iron in sections from 25 iron deficient subjects. Iron was present in all 17 marrow samples from normal subjects.

4. Davidson and Jenison[83] found that iron was present in only two of 32 patients with iron deficiency anemia. One of these had two transfusions three months previously, and the other had intra-

venous iron therapy six months previously. Iron was present in all 12 marrow samples of patients with anemia caused by chronic infection or uremia. It was found that occasionally yellowish-brown granules in hematoxylin-eosin-azure sections could be misleading as far as interpretation of iron stores was concerned.

5. Pratt and Johnson,[78] de Vries and Izak,[79] Wallerstein and Morse[84] and Read[85] have in general confirmed the results of others except they found an abnormally small amount of stainable iron in a greater percentage of their iron deficient subjects.

7. Cells noticed by Dacie and Doniach[86] which are normoblasts in the marrow containing iron granules have been called "sideroblasts". Kaplan and others[87] have counted the percentage of such cells in marrow samples from a variety of hematologic diseases attempting to correlate their frequency with specific types of anemia.

A. The majority of smears from 55 iron deficient infants had less than one % of normoblasts with stainable granules.

B. 24 to 82% of normoblasts had granules in a series of normal patients.

C. Sideroblasts were absent from marrow samples in patients with well marked iron deficiency.

D. There was not necessarily a direct correlation between stainable hemosiderin and the quantity of sideroblasts present.

Douglas and Dacie,[88] and Morse and Read[85] found:

A. Markedly decreased numbers of sideroblasts in well developed

iron deficiency.

B. Some sideroblasts were present even in the presence of mild iron deficiency anemia.

C. 50% of the normoblasts were sideroblasts in a case of idiopathic hemochromatosis.

D. Sideroblasts do not appear to show any consistent increase with the degree of iron excess.

The need for further studies of the pathogenesis of iron deficiency during pregnancy is apparent. Therefore, proposals for such a study are included at this point:

Ideal Study on the Pathogenesis of Iron Deficiency Anemia of Pregnancy

Objectives of the study:

1. To evaluate further the importance of plasma and blood volume expansion

2. To correlate the depletion of iron stores in the pregnant state with the development of anemia

3. To measure lab differences between treated and untreated patients

4. To correlate the clinical state of the patient with recorded serial lab determinations

5. To give an estimate of the duration of iron therapy needed to replenish not only the hemoglobin level but to replace, adequately, the iron stores.

6. To determine the relative validity of other lab testing as compared to direct bone marrow measurements in diagnosis of iron dificiency anemia of pregnancy

7. To investigate any changes in the absorption or metabolism during the pregnant state that contribute to the formation of iron deficiency anemia

Procedure:

1. Do preliminary tests on blood and marrow at the first opportunity after the diagnosis of pregnancy is made

a. Bone marrow - Do a histologic exam for hemosiderin and sideroblasts

b. Blood, plasma, and red cell volume determinations

Caton has used red cells tagged with Fe^{55} to determine serial red cell volume change in pregnancy with no apparent harmful effects.[72] He failed to note that such a method probably includes the fetal blood volume giving misleadingly high results. Perhaps some substance like Cr^{51} would be a more suitable material since it does not cross the placental barrier.

c. Red cell count, hematocrit and hemoglobin determinations

d. Reticulocyte count

e. Serum iron and iron-binding capacity

All of the above determinations must be made using the same techniques and standardized equipment. Repeat b, c, d and e at monthly intervals throughout pregnancy until delivery and then at

weekly intervals until values approach normal.

2. Repeat bone marrow examinations at three month intervals during pregnancy. This means that marrow aspirations will be done at approximately $2\frac{1}{2}$ months, $5\frac{1}{2}$ months, $8\frac{1}{2}$ months, and approximately three weeks post partum.

3. Ideally tagged iron studies should be done to study iron absorption and metabolism during pregnancy. Since the use of such studies may be somewhat limited in pregnancy, tests on animals may provide useful information.

4. Although not completely practical, ideally, identical determinations should be made in like sequence on a non-pregnant population for control purposes.

Summary and Conclusions:

Iron deficiency anemia due to pregnancy has long been recognized as a common clinical entity. As a general rule writers attempt to explain the pathogenesis of an iron deficit strictly on the basis of fetal requirements for iron. Since a normal, full-term infant has a total body iron content of about 300 milligrams this source of demand is probably not sufficient to explain development of a significant iron deficiency. Actually, because of decreased menstrual loss during pregnancy, conserving about 200 milligrams, her net deficit would be about 100 milligrams. Even a woman entering pregnancy with markedly reduced stores should be able to maintain hemoglobin production in face of such a small deficit.

In reality, considerable iron demand can be accounted for in addition to the fetal demand during pregnancy. Blood loss at the time of delivery contains approximately 100 milligrams of iron. The cord and placenta contain another 75 milligrams.

A very large source of increased demand for iron in gravid women which is often overlooked is her expanding red cell volume. Investigators of this phenomenon differ considerably as to the degree of expansion which takes place, but in all cases they seem to agree that there is a definite increase in red cell mass. The works of Dieckmann and Wegner and other investigators have been extensively reviewed in this paper. Inherent errors in the various methods of calculating red cell volume have been discussed. It

was felt that calculations of volumes per unit weight are less meaningful than calculations of absolute volume changes since weight is a variable factor during pregnancy.

From calculations which I have made using the serial determinations of Dieckmann and Wegner a red cell expansion which averages greater than 16% at term has been accounted for. Depending on the original size of a woman's red cell mass this probably means an additional demand of some 300 milligrams of iron. Thus, a total iron deficit of approximately 800 milligrams has been proposed which is about 25% compensated for by the absence of menes during pregnancy.

For the most part studies on blood volumes during pregnancy have been poorly controlled. Therefore, I have proposed such a study which would include direct, serial measurements of iron stores in the bone marrow and other laboratory tests on the peripheral blood. With this study a judgement could be made concerning the degree of correlation between the various tests and the clinical state of the patient.

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