The Natural History of Iron Deficiency
Induced by Phlebotomy

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With the technical assistance of N. R. Benjamin

Sydenham's report of the therapeutic effectiveness of iron in chlorotic patients was the basis for the rational use of this agent and its recognition as an essential body constituent and requirement. Subsequently, the demonstration of microcytic and hypochromic red cells led to a method of differentiating iron deficiencies from other anemias. Newer technics such as serum iron determination, radioactive iron studies and quantitation of iron in tissue stores have provided more precise means of distinguishing iron deficiency from other disorders. We have attempted to study the temporal relation of the characteristic changes of iron deficiency and to learn how they relate to severity. Phlebotomy was selected as the method of studying the natural history of iron deficiency in humans.

Methods

Subjects of the experiment were nine healthy volunteers and three patients with untreated polycythemia vera.

Phlebotomy was performed in 500 ml. increments, one to three times weekly, until each person had been bled 2.0 to 7.5 L. Twenty ml. of blood was taken at each phlebotomy and subsequently at weekly intervals for chemical analyses and blood counts. The loss of hemoglobin was computed by multiplying volume of the shed blood by hemoglobin concentration. The loss of iron was calculated on the basis of 3.38 mg. of iron per Gm. Hb.

Red cell indices were computed from counts of at least 2000 cells, duplicate high speed microhematocrits and calibrated hemoglobinometry. Reticulocyte counts were performed by the method of Brecher. Serum iron was measured by the method of Ramsay (normal: 70–170 μg./100 ml.) and the unsaturated iron binding capacity also by the method of Ramsay or, in some of the earlier studies, that of Ventura (normal: 250–400 μg./100 ml.). Bone marrow iron was demonstrated by the Prussian blue reaction. Gastrointestinal absorption of iron was determined by measuring body retention of orally administered radioactive iron (1 μc in a 1 mg. carrier dose) using a whole-body liquid scintillation counter. Normal volunteers absorbed less than 10 per cent of the administered dose.

Results

Iron deficiency was gradually induced in nine normal and three polycythemic adult males by repeated phlebotomy. Bloodletting was performed once weekly in seven of the normal volunteers (table 1: 2-8). The polycythemic patients and one normal volunteer (case 1) were bled 1–1.5 L. weekly. There

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were no untoward reactions to phlebotomy. Each volunteer continued performing normal daily activities without difficulty.

After three to five phlebotomies, the rate of decline in hemoglobin concentration slowed despite continued bleeding. Increased production of hemoglobin persisted until the subject had lost approximately 1.5 Gm. of iron. Then the hemoglobin concentration again fell at an accelerated rate if phlebotomy was continued (fig. 1). Reticulocytosis was maximal during the period of accelerated hemoglobin production but never exceeded 4 per cent. Volunteers with partially depleted stores due to previous phlebotomy had less pronounced reticulocytosis (cases 1b, 2 and 9).

The plasma iron concentration gradually fell but did not fall below the normal range until at least 500 mg. of iron had been removed. Decreased serum iron was the earliest evidence of iron depletion. Subsequent to a decrease in the serum iron, there was a pronounced increase in the total iron binding capacity of the serum in each person. These changes persisted until

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**Table 1. Phlebotomy in Normal Volunteers**

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<tr>
<th>Volunteer Number</th>
<th>Weight (Kg.)</th>
<th>Number of Phlebotomies</th>
<th>Total Hb. Removed (Gm.)</th>
<th>Total Fe Removed (mg.)</th>
<th>Day Following First Phlebotomy</th>
<th>RBC. (10^6 cells/mm.³)</th>
<th>Hct. (per cent)</th>
<th>MCV (μ)</th>
<th>MCH (mcg.)</th>
<th>MCHC (per cent)</th>
<th>Retic. (per cent)</th>
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Fig. 1.—Following the initial three phlebotomies, a rapid fall in hemoglobin concentration was observed. The rate of decline in hemoglobin slowed with continued bleeding. When hemoglobin production kept pace with loss, an average of 20.8 Gm. of hemoglobin was produced daily (3–4 times normal). Maximum reticulocytosis occurred during this period. As available iron stores were depleted, hemoglobin again declined with each blood-letting. (All illustrations in this article are U. S. Army photographs.)

he had recovered from the induced anemia and the hemoglobin concentration had returned to prephlebotomy values.

Ten to twenty days after the initiation of phlebotomy and coinciding with the reticulocyte response, there was an increase in the mean corpuscular volume of the red cells. Then the cells gradually decreased in size with the most marked microcytosis occurring 90 to 120 days following the initial bleeding in all subjects. Recognizable hypochromia was seen in five of the volunteers. Decreases in the mean corpuscular hemoglobin concentration were less pronounced than changes in cell volume, and occurred subsequent to them. Two subjects had no change in mean corpuscular hemoglobin concentration despite significant decrease in the mean corpuscular volume (fig. 4). Despite the loss of 850 to 1960 mg. of iron by these normal volunteers, only moderate changes in cell size and shape were demonstrable.

Gastrointestinal absorption of iron was studied by means of orally administered radioactive iron$^{59}$ and a whole body liquid scintillation counter. Prior to phlebotomy, the iron-replete volunteers absorbed less than 10 per cent of the radionuclide. Increased absorption (over 10 per cent) was observed during recovery from iron loss in all six of the volunteers tested. Three of these subjects (cases 1, 3 and 4) absorbed increased iron after their hemo-
Fig. 2. Subject Ia. Normal volunteer bled 5 L in 45 days.
IRON DEFICIENCY INDUCED BY PHLEBOTOMY

Fig. 3.—Subject 1b. Subject 1a (fig. 1) bled an additional 2.5 L. after initial recovery from abnormalities in the peripheral blood suggestive of iron deficiency.

globin, cellular indices and serum iron had returned to approximate prephlebotomy levels.

One normal subject (fig. 7) absorbed 64 per cent of the test dose of iron 300 days following the first phlebotomy. At that time there was no other demonstrable evidence of iron deficiency except for the absence of stainable iron in bone marrow sections. This subject was subsequently phlebotomized an additional 2.5 L. with an additional loss of 1.09 Gm. of iron. The hemoglobin returned to normal prephlebotomy levels in 190 days (fig. 3). The hemoglobin iron in this case was replenished from an ordinary diet at a rate of 5.7 mg. daily.14

Three patients with polycythemia vera were bled for therapeutic reasons. This permitted scrutiny of the rate of red cell regeneration when hemoglobin synthesis was limited by iron deficiency. Though these bled patients showed the same temporal relation of changes in cellular indices and serum iron as did the normal subjects, the changes in cell size were more profound. The rapid return of the red cell count to supranormal levels while hemoglobin concentration remained low was associated with marked depression of the
mean corpuscular volume and mean corpuscular hemoglobin and the appearance of extremely small erythrocytes. The microcytosis in these polycythemic patients was greater than that observed in our normal volunteers and in nonpolycythemic patients with more profound iron deficiency (figs. 5 and 6).15

**DISCUSSION**

The total iron present in the adult human is not great, amounting to 3–5 Gm. Approximately 55 per cent is present in circulating hemoglobin, a small fraction is incorporated in bone marrow hemoglobin, myoglobin and respiratory enzymes (10–20 per cent), and the remainder is found in iron stores.16

The normal human preserves a constant level of total body iron throughout adult life by efficient conservation of this element, maintaining rigid control over absorption to balance losses.17,18 The adult male loses approximately 1 mg. of iron daily, mostly in desquamated epithelium.15 The balance of absorption and loss may be referred to as iron equilibrium. In case of chronic continued bleeding, the increased loss may be compensated by increased absorp-
Iron deficiency induced by phlebotomy

Table 2.—Phlebotomy in Polycythemia Vera

<table>
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<tr>
<th>Volunteer</th>
<th>Weight (kg.)</th>
<th>Number of Phlebotomies</th>
<th>Total Hb Removed (Gm.)</th>
<th>Total Fe Removed (Gm.)</th>
<th>Day Following First Phlebotomy</th>
<th>Hb. (Gm./100 ml.)</th>
<th>Hct. (per cent.)</th>
<th>MCV (μL)</th>
<th>MCHC (per cent)</th>
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Iron deficiency may be defined as a reduction of total body iron below levels which are normal for the subject in question. To restore iron repletion, the absorption must temporarily exceed loss until the total body iron becomes normal once again. The demonstration of increased absorption implies iron deficiency (or hemochromatosis). Iron deficiency may exist even in the presence of normal hemoglobin and red cell mass and may be confirmed by finding depleted iron stores. Thus demonstration of increased absorption and reduced iron stores permits the diagnosis of iron deficiency in the absence of anemia, hypoferricemia or changes in erythrocyte morphology. Iron deficiency develops when iron equilibrium is upset, either by insufficient intake or by excessive loss. The most common causes are, on the one hand, prolonged dietary deficiency or poor absorption secondary to gastrointestinal pathology, and, on the other hand, loss by bleeding and childbirth.

The term iron-deficiency anemia is properly used when the total body iron is insufficient to provide a normal mass of hemoglobin. It is not accurately used to describe blood-loss anemia when iron stores are still sufficient, as in the early phase of the present studies. It should not be used to describe depletion of iron stores where there is no anemia, as in the final phase of these studies. This is iron deficiency without anemia, characterized only by depletion of the iron stores.

The concentration of iron in circulating hemoglobin (3.38 mg. iron/Gm. of Hb.) makes hemorrhage an ideal method of rapidly depleting body iron. Induction of iron deficiency in normal subjects by bloodletting produces a controlled disorder which permits planned scrutiny of the characteristic abnormalities of this state. The initially normocytic, normochromic anemia is due to loss of hemoglobin. A mild reticulocytosis, maximal 10–20 days after the initial phlebotomy, is probably responsible for the increase in mean corpuscular volume seen at that time. The earliest evidence of iron depletion is diminution of the serum iron. As the serum iron concentration falls, the mean corpuscu-
Fig. 5.—Subject A. Patient with polycythemia vera bled 7.5 L. in 75 days.
IRON DEFICIENCY INDUCED BY PHLEBOTOMY

Fig. 6.—Microcytosis was more pronounced 100 days postphlebotomy in polycythemic patients (above) than in normal volunteers (below) (X784).

...lar volume and mean corpuscular hemoglobin begin to diminish, and the unsaturated iron binding capacity of the plasma increases. Only subsequently is there a decrease in the mean corpuscular hemoglobin concentration. This seems to indicate that cellular hemoglobin concentration is preserved at the expense of erythrocyte size.
IRON ABSORPTION

Fig. 7.—A normal volunteer who absorbed 5 per cent of an oral dose of iron when iron replete, and absorbed 64 per cent 274 days following phlebotomy. Increased absorption was demonstrated after the indications of iron deficiency in the peripheral blood had returned to approximate prephlebotomy levels, but with bone marrow stores still iron depleted. (Subject 1, figs. 1 and 2.)

The cellular indices and morphology of the peripheral smear are useful tools in establishing the diagnosis of iron deficiency. However, the diagnosis will be missed frequently if departure from “normal values” is used as the principal criterion.22 Others have emphasized the relation of the severity of iron depletion to the degree of hypochromia, microcytosis and the intensity of the anemia.23,24 The time of examination of the blood with relation to the loss of iron is equally important. Despite significant loss of iron, our patients had “normal indices” for at least 60 days following phlebotomy. Maximum changes in circulating erythrocytes occurred when the normal cells produced prior to iron loss were completely replaced by newly formed microcytes (90-120 days). As iron deficiency becomes even more severe, the quality of the red cells deteriorates further. At first they are only smaller. When the stores have been completely exhausted, the mean corpuscular hemoglobin concentration begins to diminish. When the iron deficiency becomes extremely severe, the life span of the red cells may be shortened, complicating the situation with a true hemolytic disease.15

Estimation of iron in marrow stores and radioiron technics provided methods
of detecting iron deficiency when there were no diagnostic abnormalities in the peripheral blood. Increased absorption of iron from the gut can be demonstrated early in the course of iron depletion and these changes persist until iron stores are repleted. Depletion of marrow hemosiderin cannot be used as an early criterion of iron deficiency, but this change persists after peripheral blood abnormalities return to prephlebotomy levels. The mechanism by which the depleted depots transmit their requirement to the epithelium of the gastrointestinal tract remains unknown.

**Summary**

1. The sequence of characteristic changes of progressive iron deficiency was demonstrated by serial bleeding of normal volunteers and polycythemic patients.

2. After bloodletting, changes occurred in peripheral blood in the following order: a) fall in hemoglobin concentration; b) decreased plasma iron; c) reticulocytosis, increased MCV and MCH; d) diminution of MCV and MCH, increased total iron-binding protein; and e) decreased MCHC.

3. Characteristic changes of iron deficiency returned to prephlebotomy levels in the following sequence: a) hemoglobin concentration; b) cellular indices; c) serum iron; d) serum iron binding protein; and e) bone marrow hemosiderin, and finally the increased gastrointestinal absorption of iron reverted to normal.

4. Accelerated production of red cells continued in polycythemic patients despite the induction of moderate iron deficiency. Quality was sacrificed for quantity, and thereby a more profound microcytosis occurred than in normal subjects with a similar degree of iron deficiency.

**Summario in Interlingua**

1. Le sequentia del alterationes characteristic de progressive deficientia de ferro esseva demonstrate per le sanguination serial de voluntarios normal e de patientes con polycythemia.

2. Post le sanguination, alteracione in le sanguine peripheric occurreva in le sequente ordine: a) declino del concentration de hemoglobina; b) declino del nivello de ferro in le plasma; c) reticulocytose, augmento del volumine corpuscular medie e del hemoglobina corpuscular medie; d) declino del volumine corpuscular medie e del hemoglobina corpuscular medie, augmento del total proteina ferro-ligatori; e) declino del concentration medie de hemoglobina corpuscular.

3. Le alterationes characteristic de deficientia de ferro retornava al nivellos de ante le phlebotomia in le sequente ordine: a) concentration de hemoglobina; b) indices cellular; c) ferro seral; d) proteina ferro-ligatori del sero; e) hemosiderina del medulla ossee; e, finalmente, f) renormalisation del augmentate absorption gastrointestinal de ferro.

4. Production accelerate de erythrocytos continuava in patientes polycythemic in despecto del induction de moderate grados de deficientia de ferro. In illos qualitate esseva sacrificate pro quantitate, e assi il occurreva un plus profunde microcytose que in subjectos normal con un simile grado de deficientia de ferro.
REFERENCES

27. Krantz, S., Goldwasser, E., and Jacob-


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