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Iron Metabolism in Pregnancy

Volume XX | Friday, April 22, 1949 | Number 24

BULLETIN OF THE
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I.

UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

April 24 - 30, 1949

No. 245

Sunday, April 24

9:00 - 10:30 Surgery Grand Rounds; Station 22, U. H.

10:30 - 11:00 Arterial Transfusion; Claude Hitchcock
Report on the Memphis Cancer Meeting; Geo. Moore & Claude Hitchcock; Rm. M-109, U. H.

Monday, April 25

8:00 - Fracture Rounds; A. A. Zierold and Staff; Ward A, Minneapolis General Hospital.

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; M-109, U. H.

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:00 - 11:50 Physical Medicine Seminar; E-101, U. H.

11:00 - 11:50 Roentgenology-Medicine Conference; Veterans Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Eustis Amphitheater, U. H.

12:00 - 1:00 Physiology Seminar; Altered Physiology of Pulmonary and Hemato-Respiratory Exchange in Pulmonary Emphysema; Craig Borden; 214 M. H.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

12:30 - 1:20 Pathology Seminar; Letterer Siwe Disease; Edward Bauer; 104 I. A.

12:30 - 1:30 Surgery Problem Case Conference; A. A. Zierold, C. Dennis and Staff; Small Class Room, Minneapolis General Hospital.

1:30 - 2:30 Surgery Grand Rounds; A. A. Zierold, C. Dennis and Staff; Minneapolis General Hospital.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - Pediatric Seminar; Report of the Meeting of the Federation of the American Society for Experimental Biology; Vincent Kelly and Robert Good; 6th Floor, Child Psychiatry, U. H.

4:00 - Public Health Seminar; 113 Medical Sciences.

5:00 - 5:50 Clinical Medical Pathologic Conference; Todd Amphitheater, U. H.

5:00 - 6:00 Urology-Roentgenology Conference; D. Creevy and H. M. Stauffer and Staffs; M-109, U. H.

Tuesday, April 26

8:30 - 10:20 Surgery Reading Conference; Small Conference Room, Bldg. I, Veterans Hospital.

9:00 - 9:50 Roentgenology Pediatric Conference; L. G. Rigler, I. McQuarrie and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Surgical Pathological Conference; Lyle Hay and Robert Hebbel; Veterans Hospital.

12:30 - Pediatric-Surgery Rounds; Sta. I, Minneapolis General Hospital; Drs. Bosma, Wyatt, Chisholm, McNelson and Dennis.

12:30 - 1:20 Pathology Conference; Autopsies; Pathology Staff; 102 I. A.

1:00 - 2:30 X-ray Surgery Conference; Auditorium, Ancker Hospital.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III, Veterans Hospital.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U. H.

3:30 - 4:20 Clinical Pathological Conference; Staff; Veterans Hospital.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

4:00 - 5:30 Physiology-Surgery Conference; Tissue Pressure and Necrosis; Drs. A. Lehman, H. S. Wells, L. Tongen; Eustis Amphitheater, U. H.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Todd Amphitheater, U. H.

5:00 - 6:00 X-ray Conference; Dr. Aurelius and Staff, Ancker Hospital; Todd Amphitheater, U. H.

Wednesday, April 27

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-515, U. H.

8:30 - 9:30 Clinico-Pathological Conference; Auditorium, Ancker Hospital.

8:30 - 10:00 Orthopedic-Roentgenologic Conference; Edward T. Evans, Room 1AW, Veterans Hospital.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker and Joe R. Brown; Veterans Hospital.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; O. H. Wangensteen, C. J. Watson and Staff; Todd Amphitheater, U. H.

12:00 - 12:50 Radio-Isotope Seminar; Use of Isotopes for External Radiation; George E. Moore; Rm. 212, Hospital Court, Temp. Bldg.

3:30 - 4:30 Journal Club; Surgery Office, Ancker Hospital.

4:00 - 5:00 Infectious Disease Rounds; Maine Lecture Room, Minneapolis General Hospital.

Thursday, April 28

8:15 - 9:00 Roentgenology-Surgical-Pathology Conference; Craig Freeman and H. M. Stauffer; M-109, U. H.

8:30 - 10:20 Surgery Grand Rounds; Lyle Hay and Staff; Veterans Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-109, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Surgery-Radiology Conference; Daniel Fink and Lyle Hay; Veterans Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

11:30 - 12:30 Clinical Pathology Conference; Steven Barron, C. Dennis, George Fahr, A. V. Stoesser and Staffs; Large Class Room, Minneapolis General Hospital.

12:00 - 1:00 Physiological Chemistry Seminar; Interactions of Atabrine, Thiamine, and Cocarboxylase; Clay E. Pardo, Jr.; 214 M. H.

1:00 - 1:50 Fracture Conference; A. A. Zierold and Staff; Minneapolis General Hospital.

2:00 - 3:00 Errors Conference; A. A. Zierold, C. Dennis and Staff; Large Class Room, Minneapolis General Hospital.

4:00 - 5:00 Bacteriology and Immunology Seminar; Immunochemical Studies of Factors Modifying the Interaction of Egg Albumin -- Anti-egg Albumin; W. J. Kleinschmidt; 214 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 6:00 Urology Seminar; Preparation of Large Intestine for Operation; Michael Feeney; E-101, U. H.

5:00 - 6:00 X-ray Seminar; Current Concepts of Pathogenesis of Tuberculosis; Charles Nice; Todd Amphitheater, U. H.

Friday, April 29

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:20 Medicine Grand Rounds; Staff; Veterans Hospital.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:00 - 12:00 Surgery-Pediatric Conference; C. Dennis, O. S. Wyatt, A. V. Stoesser and Staffs; Minneapolis General Hospital.

11:30 - 12:50 University of Minnesota Hospitals General Staff Meeting; Factors in Patient's Adjustment to Rest Home Care, Helen Kretchmer; The Family as a Factor in the Epileptic's Social Adjustment, Jean Cummins; Study of Referrals to Social Service, Rose Baldwin; Powell Hall Amphitheater.

12:00 - 1:00 Surgery Clinical Pathological Conference; Clarence Dennis and Staff; Large Classroom, Minneapolis General Hospital.

1:00 - 1:50 Dermatology and Syphilology; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

1:00 - 3:00 Pathology-Surgery Conference; Auditorium, Ancker Hospital.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

4:00 - 5:00 Electrocardiographic Conference; George N. Aagaard; 106 Temp. Bldg., Hospital Court, U. H.

Saturday, April 30

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; Station 20, U. H.

8:30 - 9:30 Surgery Conference; Auditorium, Ancker Hospital.

8:00 - 9:00 Pediatric Psychiatric Rounds; Reynold Jensen; 6th Floor, West Wing, U. H.

8:00 - 9:00 Surgery Literature Conference; Clarence Dennis and Staff; Minneapolis General Hospital, Small Classroom.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; E-101, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amph., U. H.

9:00 - 11:30 Surgery-Roentgenology Conference; Todd Amphitheater, U. H.

9:00 - 12:00 Child Psychiatry Conference; Powell Hall Amphitheater.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - 12:00 Anatomy Seminar; Further Studies on the Allergic Etiology of Encephalitis, Berry Campbell; Chromotolysis, Harold Haft; 226 I. A.

II. IRON METABOLISM IN PREGNANCY

Roy G. Holly

1. Introduction

In the past decade a great many advances have been made in our knowledge of the general problem of iron metabolism. The use of bone marrow examination, radio-active iron studies, determination of serum iron, iron binding capacity and erythrocyte protoporphyrin, animal experimentation on the properties of ferritin and a study of their relationship in health and disease permits more accurate description of iron absorption, excretion, transport, storage and utilization. It is reasonable to assume on the basis of the known physiologic variations in normal pregnancy that iron metabolism will be altered from that in the male or non-pregnant female. It is the purpose of this paper to review briefly the general background of iron metabolism and to present the results of investigations on iron metabolism in pregnancy, particularly as they relate to iron deficiency anemia.

Pregnancy anemia is of major interest to the obstetrician. From our experience and from investigations of others we know that the iron deficiency anemia is the common anemia of pregnancy. The role of diet, iron loss to the fetus and blood loss at parturition in the production of an iron deficiency state is well recognized. It is probably better to think in terms of an iron deficiency state rather than an iron deficiency anemia, since the anemia is only one manifestation of the general deficiency. Associated with the anemia there must be depletion of iron stores and available iron for tissue metabolism will be reduced. It appears probable that the infant does not suffer from the iron deficiency state in the mother, in a sense being a perfect parasite and taking that which it needs at the expense of the maternal organism.

By the use of techniques to be described later, it is possible to accurately diagnose the iron deficiency state and associated iron deficiency anemia. Obviously this is possible only as a research problem. From a practical point of view the presence of an anemia should suggest iron deficiency and iron medication should be begun. It is probably commendable practice to routinely administer iron with all pregnancies, particularly in the last trimester when the fetal demand is so great. As will be indicated iron medication should be carried beyond mere correction of the anemia in order to thoroughly replenish the iron stores. An occasional anemia will be refractory to iron therapy or will develop in spite of iron treatment. These cases have been of special interest. On the basis of our knowledge at the present time they will fall into one of our groups:

a. anemias not peculiar to pregnancy, e.g., familial hemolytic anemia
b. nutritional macrocytic anemias including the megaloblastic anemia of pregnancy
c. the "refractory anemia of pregnancy" associated with bone marrow hypoplasia
d. iron deficiency anemia with subnormal iron absorption.

In this latter group intravenous iron therapy is effective.

2. Method of Study

Only brief mention of techniques employed in this study will be made except for the iron studies. Standardization of techniques has been attempted as much as possible. Blood for the routine determination of hemoglobin, erythrocyte count and hematocrit was drawn without stasis from the antecubital vein. Earlier in the study heparin was used as the anticoagulant but more recently double oxylate has been used. Standardized pipettes have been used throughout. The hemoglobin was determined by the Evelyn colorimeter oxyhemoglobin method. Hematocrit readings were obtained after a full 30 minutes of centrifugation at 3000 r.p.m. in Wintrobe tubes. Reticulocyte smears were made from finger-tip blood

and stained supravitally with brilliant cresyl-blue. Cell diameter was obtained in a Halometer standardized against a Price-Jones curve.

The erythrocyte protoporphyria (E.P.) was determined by the method of Grinstein and Watson.[1] Readings were made in the Evelyn colorimeter with a 400 filter against a blank prepared with 5% hydrochloric acid.

The serum iron was determined by a procedure modified from Barkan.[2] 4 cc. of serum was used instead of 2 cc. as described by Barkan in that greater accuracy could be obtained in recovery experiments using the larger quantity. The blood for the serum iron determination was drawn without a syringe. Centrifuge tubes, pipettes and colorimeter tubes were made "iron free" by rinsing with iron free water after the usual acid cleaning. Reagents were all prepared as iron free. 2 cc. of 1.2% HCl was added to the serum and incubated at 37° for one hour. After cooling to room temperature, 2 cc. of 20% trichloracetic acid was added, mixed, and allowed to stand for an hour. This mixture was then centrifuged at 3000 r.p.m. for 15 minutes. 4 cc. of the supernatant fluid was placed in a colorimeter tube along with 1 cc. of saturated sodium acetate solution, 1 cc. of buffered 1% hydrazine sulfate solution and 1 cc. of 0.1% O-phenanthroline monohydrate. Full color was allowed to develop by standing over night, though in his paper Barkan suggested that readings could be made after 1 hour. Results were obtained in the Evelyn colorimeter against a blank prepared with all reagents. The result is expressed as gamma per cent.

The method for determining the iron binding capacity (IBC) of the serum is that described by Finch.[3] 2 cc. of serum and 4 cc. of saline were added to a colorimeter tube. The serum was obtained by the same method as that employed for the serum iron. The blood was drawn from a fasting patient to eliminate cloudiness of the serum. Standard iron solution was made so as to contain 10 gamma of iron per cc. of solution. 0.1 cc. additions of the standard iron solution were made and allowed to stand 4 minutes before reading in the Evelyn colorimeter. These 0.1 cc. increments were added until a "break" in the downward trend of the colorimeter readings was noted. Results were plotted on the graph paper and the point of intersection of two lines drawn as illustrated in the accompanying Figure 1 was used as the final determination. The 520 filter was used throughout the present experiments though in duplicate experiments it was found that the 490 filter gave similar results. Calculation of iron binding capacity was then made by the formula:

cc. standard solution x 10 x 50
= IBC in gamma per cent.

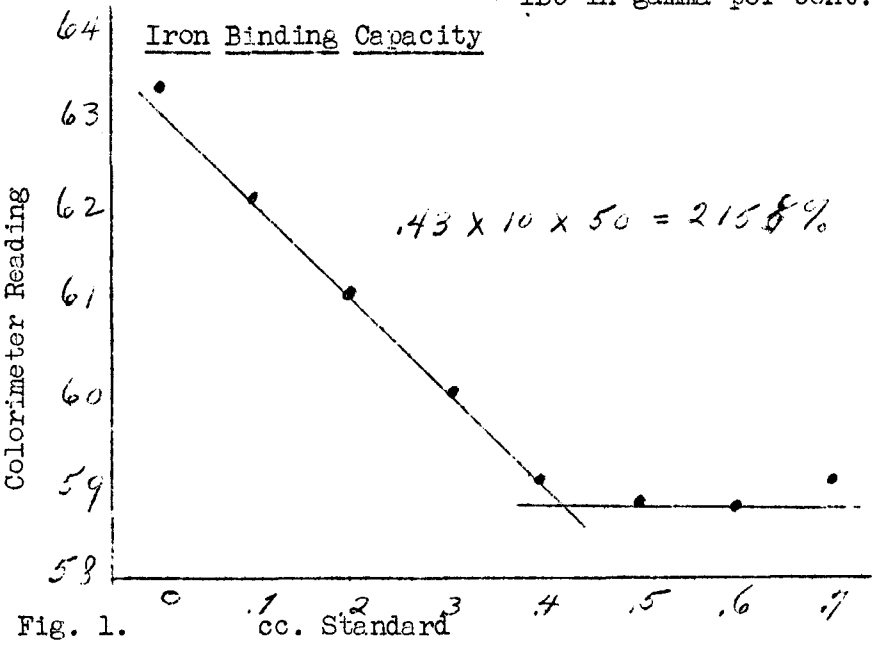


Fig. 1.

Patients studied for iron tolerance were fasted for 2-3 hours after the test dose of iron was given. After withdrawal of the fasting specimen 1 gram of ferrous gluconate was given by mouth. Repeat blood samples were drawn at 1 hour, 3 hours, 5 hours, 8 hours, and 12 hours.

3. The Bone Marrow in Iron Deficiency Anemia of Pregnancy

Logically the bone marrow in iron deficiency anemia of pregnancy should be influenced by two factors, first the pregnancy itself and secondly, the iron deficiency. Distinct changes are produced in the marrow by a normal pregnancy. Daniachij[4], Hansen[5], Markoff[6], Pitts and Packham[7], and Wolff and Limarzi[8] have reported findings in the bone marrow of the normal pregnancy. There is an increased cellularity beginning as early as the 3rd month of pregnancy involving both the erythroid and myeloid lines of development. A slight shift to the left as manifest by increased numbers of younger developing cells has been reported. Megakaryocytes are increased in all cases. Wolff and Limarzi[8], using a technique similar to our own, report an average myeloid-erythroid volume of 14% for the normal pregnancy as contrasted with 6.8% for the non-pregnant individual. While giving no actual statistics these same authors report a normal differential count for the normal pregnancy marrow.

There is not an abundance of material reported on the bone marrow alteration in iron deficiency. Scott[9] in 1939 reported on 23 cases of his own and reviewed the literature up to that time. None of his cases were pregnancy anemias. Normally the marrow contains about 20% normoblasts with only 1-2% being pronormoblasts and basophilic normoblasts. In iron deficiency he described the marrow as being highly cellular with a relative increase in normoblasts proportionate to the degree of the anemia. The typical cell was a small polychromatic normoblast with a scant jagged rim of cytoplasm around a pyknotic nucleus.

Wolff and Limarzi[8] described 10 cases of pregnancy iron deficiency anemia. They observed a normoblastic hyperplasia and a definite shift to the left. As one would expect there was also a myeloid and megakaryocytic hyperplasia. Markoff[6] and Segerdahl[10] describe macronormoblasts in the marrow of pregnancy complicated by iron deficiency anemia.

Correlation of the bone marrow pattern with the peripheral blood findings has been an interesting phase of the current investigation. The accompanying Figure 2 gives the results of 22 marrows obtained from proven cases of iron deficiency anemia of pregnancy. With one exception they have all been obtained during pregnancy. F.T.#19 was obtained on the third post-partum day.

The method of study is similar to that described by Sundberg[11] in a previous Staff Meeting Bulletin. Approximately 1 cc. of marrow was obtained from the sternum by use of a Klima-Rosseger needle. More recently we have used the Illinois modification of this needle. The skin, subcutaneous tissue and periosteum overlying the site of puncture were infiltrated with 1% procaine. The needle was then placed against the outer bone plate and the guard adjusted to $2\frac{1}{2}$ turns. By gentle pressure the needle was then inserted into the marrow cavity. After removal of the stylet quick suction with a dry 20 cc. syringe was used to aspirate marrow content. The liquid was placed in a small vial containing powdered heparin, shaken to avoid clotting and then immediately prepared for study. 1 cc. or the total aspirated material was first placed in a Wintrobe hematocrit tube and centrifuged at 2000 r.p.m. for 8 minutes. The values for the fat, plasma, myeloid-erythroid, and erythrocyte layers were obtained. Smears were made from the concentrated myeloid-erythroid layer after diluting with a small quantity of plasma and stained with Wright stain.

The pregnancy effect could be observed in all cases of iron deficiency with an increase in megakaryocytes and myeloid cells. As indicated the average volume of the M-E layer was 21.5%. In the few nor-

Figure 2

Bone Marrow

Iron Deficiency Anemia of Pregnancy

	% M-E Volume	% Normoblast	% Neutrophil	% Eosinophil	% Basophil	% Lymphoid
1.	19.0	33.6	51.6	2.0	0.0	12.8
2.	14.5	-	-	-	-	-
3.	17.0	36.6	53.6	1.2	0.0	8.4
4.	29.0	37.6	53.0	1.6	0.0	7.8
5.	35.0	34.0	52.6	0.8	0.2	12.4
6.	26.0	31.4	56.8	3.2	0.6	8.0
7.	13.0	38.6	53.4	1.8	0.0	6.2
8.	20.0	23.0	64.5	1.7	0.1	10.7
9.	-	27.0	57.4	1.6	0.0	14.0
10.	15.0	36.8	51.0	4.2	0.0	8.0
11.	28.0	33.4	58.2	1.4	0.4	6.6
12.	26.0	35.4	57.0	1.2	0.4	6.0
13.	36.0	31.0	55.0	3.2	0.0	10.8
14.	17.0	32.5	61.5	3.0	0.0	3.0
15.	24.0	44.6	45.8	1.2	0.4	8.0
16.	15.0	52.6	37.2	4.4	0.2	5.6
17.	8.0	39.2	44.9	3.9	0.3	11.7
18.	42.0	20.2	67.8	2.2	0.4	9.4
19.	24.0	31.4	55.8	2.6	0.0	10.2
20.	22.0	21.0	72.4	0.4	0.4	5.8
21.	12.0	23.6	50.4	6.4	0.8	18.8
22.	20.0	31.8	61.8	1.4	0.0	5.0
Average	21.5%	33.1%	55.3%	2.4%	0.2%	9.0%

mal pregnancy marrows studied an M-E volume over 20% has not been observed. While the range of values is quite great, in a majority of cases the increase in M-E volume is greater than expected from the pregnancy alone. It undoubtedly reflects the added erythropoiesis associated with iron deficiency.

The differential count reveals the outstanding feature of these marrows, the significant relative increase in developing normoblasts. The average for the 22 marrows was 33.1%.

Two fairly distinct patterns were observed on detailed scrutiny of these marrows. In the majority of smears examined the normoblasts were small polychromatophilic cells which occurred in clusters or nests. Similar to those described by Scott[9] the cytoplasm was scant with a jagged edge and the nucleus was pyknotic and small. There was usually an apparent shift to the left with a moderate increase in the number of pronormoblasts and basophilic normoblasts. In other cases and often in association with the above changes, macronormoblasts were observed.

In nearly all the marrows examined there was an increase in the number of histiocytes, plasma cells and pigment containing macrophages. In many instances the neutrophils showed toxic changes, however all of these findings have been seen in the few normal pregnancy marrows examined and are probably then associated with pregnancy effect and not the result of the iron deficiency.

The changes associated with iron de-

ficiency anemia of pregnancy can be briefly summarized as follows. There is an increase in the M-E volume, greater than anticipated from the pregnancy alone. The percentage of normoblasts is relatively increased and there is a moderate shift to the left. Other changes such as the megakaryocytic and myeloid hyperplasia and increase in lymphoid cells are seen but are normal pregnancy alterations in the marrow.

The findings have been of great interest in another connection and only brief mention will be made of this at this time. A few pregnancy anemias of moerate degree have been observed which are refractory to all treatment. No iron deficiency could be demonstrated. In these cases the M-E volume is normal, only slightly increased to 8-11% or actually decreased. On differential count the percentage of normoblasts has varied from 5-18%. These have been interpreted as representing "toxic depression" of the bone marrow and have been classified as "refractory anemias of pregnancy". The etiology is not known but they could represent sensitivity to estrogen which is known to increase markedly in pregnancy. Reference is made to the previous Staff Meeting Bulletin where details of 2 such cases were given.[12]

It is very probable that study of the bone marrow will indicate the possibility of response to iron medication. The two cases of the 22 described with 20 and 21% normoblasts did not respond adequately to iron medication in spite of clearcut demonstration of the presence of iron deficiency. A third case of refractory anemia with M-E volume of 5% and only 18% of the marrow consisting of normoblasts has been studied. Only by constant iron medication over a 3 month period was her overlying iron deficiency corrected. Details of this case will be presented later under discussion of treatment. It seems reasonably clear that the status of the marrow in iron deficiency anemia is one feature that must be studied in order to clearly evaluate the presence or absence of response to iron medication.

4. The Erythrocyte Protoporphyrin in Pregnancy

The determination of erythrocyte protoporphyrin has been extremely valuable as an aid in diagnosis and for judging response to therapy. Considerable interest has centered around the relation of the erythrocyte protoporphyrin to various disease states. No investigation of pregnancy anemia has reported porphyrin studies. In a previous Staff Meeting Bulletin[12] our own preliminary experiences on a few normal pregnancies and anemias were reported. The finding of a normal E.P. value in the presence of anemia led to further study and description of the "refractory pregnancy anemia" which will be discussed more in detail later. To conserve space the initials E.P. will henceforth refer to erythrocyte protoporphyrin.

No discussion of many of the fundamental problems of porphyrin metabolism currently being investigated by others will be attempted, such as how and where the protoporphyrin is synthesized, whether it represents an "excess" in the erythrocyte after hemoglobin synthesis or whether it is a degradation product of hemoglobin breakdown. A protoporphyrin in the erythrocyte free from the hemoglobin molecule was first demonstrated by van den Bergh and Hymans in 1928.[13] After identification as a Type III isomer Grinstein and Watson[1] in 1943 reported a quantitative method for determining this protoporphyrin with the Evelyn colorimeter. Numerous clinical applications in disease states have followed. Uniformly elevated E.P. values have been demonstrated in iron deficiency anemia, heavy metal poisoning, uremia and the anemia of infection. Low to normal values are the rule in pernicious anemia. Variable values from normal to high have been found in hemolytic anemia, aplastic anemia, polycythemia vera and the leukemias.

A major consideration is the normal E.P. value for the adult male and female before supposedly abnormal values can be taken as significant. Watson, Grenstein and Hawkinson[14] in 1944 reported values

on 12 healthy normal subjects. No value exceeded 45 gamma %. The E.P. values for the female subjects were slightly higher, were associated with lower hemoglobin and hematocrit percentages and thus were thought to represent minimal iron deficiency on the basis of menstrual blood loss. Recently Wintrobe[15] and his group have reported results on a larger series of normals but unfortunately did not correlate their values with the hemoglobin or hematocrit. 33 males had a mean value of 32 gamma % with a range from 13-79 gamma %. 33 females had a mean value of 39 gamma % with a range from 16-140 gamma %.

In Figure 3 are shown the results of 24 E.P. determinations done on normal female subjects with corresponding hemoglobin values. The mean value for this group is 48 gamma % but is unquestionably influenced by the one high value. In a larger series of 113 normal females to be published by Holly and Watson[16] the mean value was 40 gamma %. For 28 normal males the mean value was 27 gamma %. Even though the "average" female may have a determined E.P. of 23-67 gamma % it is more than likely that values in excess of 45-50 gamma % represent minimal iron deficiency and could be lowered by appropriate iron therapy, with a corresponding increase in hemoglobin. For our diagnostic purposes E.P. values in excess of 60 gamma % have been taken to represent iron deficiency.

Figure 3

Erythrocyte Protoporphyrin

The Normal Female Value

Subject	Age	Gram % Hemoglobin	Gamma % Erythrocyte Protoporphyrin
1.	21	13.2	40
2.	23	13.1	28
3.	25	12.5	57
4.	23	13.6	44
5.	24	13.2	52
6.	23	13.4	55
7.	31	13.6	52
8.	23	13.2	34
9.	24	13.4	56
10.	24	13.3	40
11.	23	12.0	54
12.	23	13.0	45
13.	25	13.9	34
14.	23	13.3	55
15.	23	12.2	50
16.	22	11.6	40
17.	24	13.3	23
18.	23	14.5	105 106
19.	28	13.8	29
20.	24	13.1	67
21.	26	12.6	37
22.	24	13.1	63
23.	26	12.7	39
24.	24	12.8	48

The question arises as to whether pregnancy in itself, aside from iron deficiency, will alter the determinable E.P. Figure 4 shows values on 21 normal pregnancies, assumed normal on the basis of hemoglobin values of over 10 gram %. In the first 7 months of pregnancy all values are within the normal range though the number of observations is not great. Figure 5 reveals values on normal pregnancies but represents continuous determinations on individual subjects. Two values (#11,17 Fig.4) do not fall within expected variation. Variable results are seen where the subject has a hemoglobin between 10-11 Gm. %. Three such cases (#25,26,27, Figure 5)

Figure 4

Erythrocyte Protoporphyrin

Normal Pregnancy

Subject	Age	Para	Mo.Preg.	Gram % Hemoglobin	Gamma % Erythrocyte Protoporphyrin
1.	23	1	3	13.2	39
2.	26	0	3	12.9	35
3.	26	;	4	11.3	33
4.	15	0	5	10.5	29
5.	21	1	5	11.4	39
6.	20	0	6	11.1	50
7.	16	0	7	11.7	53
8.	18	0	8	11.8	30
9.	24	0	8	12.3	48
10.	17	0	8	11.1	48
11.	24	1	8	12.1	137
12.	19	0	8	11.7	27
13.	30	0	8	10.9	48
14.	34	8	9	10.4	53
15.	21	1	9	13.3	19
16.	16	0	9	12.2	60
17.	19	0	9	11.7	101
18.	25	0	9	11.8	31
19.	18	0	9	11.4	53
20.	17	0	9	10.1	70
21.	18	0	9	10.4	72

with E.P. in excess of 50 gamma % were assumed to be iron deficien and were treated with ferrous gluconate. In each instance the hemoglobin promptly rose and there was a tendency for the E.P. to decrease. In Case #25 a severe post partum hemorrhage resulted in iron deficiency and was reflected in the lowered hemoglobin and elevated E.P. value.

By continuation of similar observations it should be possible to determine how low the hemoglobin can fall in connection with known plasma dilution before it represents iron deficiency. On the basis of experience to the present, a hemoglobin below 10 gram % is associated with demonstrable iron deficiency. The critical point is probably between 10-11 gram % for the last trimester of pregnancy.

Figures 6 and 7 show E.P. values in chronic blood loss anemia, in refractory anemia of pregnancy and in one case of megaloblastic anemia of pregnancy. The initial high E.P. value in

Figure 5

Erythrocyte Protoporphyrin

Normal Pregnancy

Subject	Age	Para	Mo. Preg.	Gram % Hemoglobin	Gamma % Erythrocyte Protoporphyrin
22.	20	0	6	12.5	35
			7	13.0	31
			8	13.4	28
			8	13.4	31
			9	12.5	26
			Del.	13.6	28
23.	19	0	7	10.8	32
			8	11.4	57
			9	11.0	49
			Del.	12.3	43
			P.P.	12.2	37
24.	17	0	6	13.7	22
			7	12.9	60
			8	12.5	30
			9	14.1	39
			Del.	13.8	47
			P.P.	11.2	42
25.	26	0	7	10.4	51
	Ex-Iron		8	10.7	38
			9	12.6	-
			P.P.	9.3	76 **
26.	33	3	7	10.8	86
	Ex-Iron		8	12.3	56
27.	16	0	6	10.7	65
	Ex-Iron		8	12.9	83
			9	13.0	62
			Del.	14.8	62

** Post Partum Hemorrhage - 1000 cc.

Figure 6

Erythrocyte Protoporphyrin
Chronic Blood Loss Anemia

Subject	Age		Hgb. Gm%	E.P. γ %
1.	44	Endometrial Hyperplasia	8.9	173
			8.9	142
2.	21	Menorrhagia	8.5	274
			11.3	110
			13.2	52
3.	53	Bleeding Myoma	5.3	489
			12.7	175
4.	22	Incomplete Abortion	7.7	141

chronic blood loss anemia decreases as iron becomes available and the continued blood loss is checked. Case #2, Figure 6 represents observations over a time interval of six weeks. Treatment was with oral ferrous sulfate. Reference has previously been made to the refractory anemias of pregnancy. The syndrome is essentially that of lowered hemoglobin, erythrocyte count and hemato-

Figure 7

Erythrocyte Protoporphyrin

Refractory Anemia of Pregnancy

Subject	Age	Para	Mo. Preg.	Hgb. Gm.%	E.P.γ %
1.	26	1	8	9.0	37
			p.p. 1 mo.	12.8	28
	28	2	4	9.4	49
2.	21	0	7	7.8	39
			8	8.7	62
			9	9.1	68
			9	9.0	57
			Del.	9.0	54
			p.p. 1 wk.	9.6	53
			p.p. 1 mo.	9.6	119
			p.p. 2 mo.	11.0	130
3.	19	0	8	9.4	53
			9	9.2	52
			p.p. 1 wk.	10.7	83
4.	22	0	7	8.8	47
			8	8.0	58
			8	8.7	62
			8	8.3	62
			8	8.5	60
			9	10.0	80
			Del.	11.4	52
5.	21	0	5	9.0	49
			6	10.3	26
			9	10.0	20
			p.p. 2 wk.	12.1	36
6.	23	0	7	9.0	32
			8	9.5	28
			p.p. 2 wk.	10.7	30
			Megaloblastic Anemia of Pregnancy		
1.	40	7	p.p. 1 day	8.4	37
			p.p. 3 wk.	10.0	96
			p.p. 6 wk.	11.5	50

crit percentage. The anemia is normocytic and normochronic. Blood volume studies have shown plasma dilution but not of a degree sufficient to lower the hemoglobin to the level noted. The E.P. is normal indicating lack of associated iron deficiency although it will be shown that iron deficiency may overlie a refractory anemia. The bone marrow, in contradictinction to normal changes in pregnancy, is relatively hypoplastic. Case #1, Figure 7 is of particular inter-

est in that the same type of anemia has reappeared in a second pregnancy. Case #2, Figure 7 was valuable in that prolonged antepartum and post partum observations were made. No amount or type of therapy was effective during pregnancy. Termination of the pregnancy allowed active regeneration to occur with development of a relative iron deficiency with corresponding rise in E.P. Case #3, Figure 7 is similar. Case #4, Figure 7, reveals similar findings for which no therapy was effective.

The megaloblastic anemia of pregnancy was proven by bone marrow study. Similar to true pernicious anemia the E.P. was normal. Without therapy spontaneous remission followed delivery. As others have observed, in the remission phase the E.P. rises, probably indicating iron deficiency which later disappears as sufficient iron is mobilized for normoblastic development.

The E.P. in proven iron deficiency anemia of pregnancy are given in Figure 8. Many of these individuals are currently under treatment and observations are incomplete. In each instance an elevated E.P. has given a clue to the diagnosis. It is quite apparent from examination of these cases that response to iron therapy given orally was extremely variable. Cases 1, 4, 13, 18, and 20 reflect fairly adequate response to oral iron medication. Cases 2, 3, 5, 6, 8, 15 and 21, 24 show slow or lack of response to oral medication. Further discussion of this problem will be given in a later section. Suffice it to say that for practical clinical handling iron medication should be carried beyond mere correction of the hemoglobin decrease. Often up to 5 months elapsed after delivery before the E.P. value had returned to normal.

Figure 9 shows E.P. values determined on cord blood with corresponding maternal values. The maternal E.P. determinations were done within 24 hours of delivery, usually the blood was drawn during the course of labor. Cord hemoglobin values are routinely higher than the corresponding maternal value. In 7 of the 10 cases the cord reticulocyte percentage was elevated slightly. Cord blood erythrocyte protoporphyrin was elevated in the majority of cases but bore no correlation with maternal values as one would expect. In view of the relative iron saturation of the fetus elevated E.P. values can not be explained on this basis. There is no apparent transmission of erythrocyte protoporphyrin across the placenta.

5. Serum Iron in Pregnancy: Fetal Demand for Iron

The iron present in serum free from the erythrocyte is generally regarded as transport iron. It remains in equilibrium with the iron storage depots (liver, spleen and bone marrow), with absorbed iron, with the iron released by hemoglobin breakdown, and with iron being diverted to the bone marrow for hemoglobin synthesis. The quantity of iron in the total plasma volume is approximately 3.5-4.0 mgm. and represents only a very small fraction of the total body iron. For a 70 kg. adult, total body iron averages 4.3 grams, of which about 30-35% is storage iron, approximately 55% is hemoglobin iron and the remainder is in body tissue. Serum iron is non-dialyzable at the pH of blood and forms a complex compound with a specific B_1 fraction of plasma. With improvement of methods for determining the serum iron, its variations in disease have been widely investigated. A review of these investigations is not the purpose of this presentation but attention should be called to the monographs of Heilmeyer and Plötner[17], Waldenstrom[18], Vahlquist[19] and Laurell[20] and the excellent articles of Moore[21,22,23], Powell[24], and Cartwright and Wintrobe[15].

The normal adult will have a serum iron value between 70-200 gamma %. There is an apparent sex difference which is not present before puberty or after the climacteric. Waldenstrom[18]

Figure 8

Erythrocyte Protoporphyrin

Iron Deficiency Anemia

Subject	Age	Para	Mo.Preg.	Hgb.Gm.%	E.P. γ %
1.	36	0	5	9.9	99
			7	12.2	37
2.	17	0	8	8.6	150
			9	10.3	113
			Del.	11.6	97
			p,p. 3 wks.	13.1	120
3.	24	0	7	7.7	160
			8	7.0	105
			9	7.9	119
			p.p. 2 wks.	8.5	131
			p.p. 2 mo.	12.6	83
			p.p. 5 mo.	13.4	66
4.	34	4	8	8.8	226
			Del.	11.0	103
			p.p. 2 wks.	13.9	135
5.	20	1	8	8.3	275
			Del.	11.9	108
			p.p. 2 wks.	12.5	145
6.	19	0	7	11.0	30
			8	10.3	65
			9	9.2	63
			Del.	9.9	43
			p.p. 2 wks.	10.4	68
			p.p. 1 mo.	10.8	68
7.	24	1	9	8.0	152
			Del.	8.0	92
8.	20	0	8	9.4	271
			9	9.6	297
			p.p. 2 wks.	10.4	147
9.	28	5	p.p. 1 wk.	7.1	129
			p.p. 6 wk.	11.3	70
10.	33	3	8	9.5	92
11.	24	1	7	9.3	96
12.	41	5	p.p. 1 wk.	8.3	96
13.	26	2	8	8.6	72
			9	9.2	88
			Del.	11.2	48

Figure 8 (Cont.)

Erythrocyte Protoporphyrin

Iron Deficiency Anemia

Subject	Age	Para	Mo. Preg.	Hgb.Gm.%	E.P.γ %
14.	28	1	8	9.4	112
15.	27	0	9	9.4	80
			p.p. 3 wk.	9.9	105
			p.p. 6 wk.	12.9	73
			p.p. 5 mo.	12.6	43
16.	42	16	p.p. 1 day	6.8	170
		17	8	8.4	182
17.	19	0	9	8.5	101
			Del.	12.0	123
			p.p. 1 wk.	12.5	127
18.	36	10	7	8.4	71
			8	10.5	71
			9	10.4	40
			Del.	12.5	35
			p.p. 1 wk.	11.3	44
19.	24	4	9	7.8	297
			Del.	9.7	282
20.	20	0	8	9.5	109
			9	10.7	86
			Del.	13.5	68
			p.p. 1 wk.	13.9	97
			p.p. 6 wk.	13.0	38
21.	19	0	6	9.3	113
			7	10.4	133
			8	11.0	95
			9	11.3	109
			Del.	12.5	84
			p.p. 6 wk.	11.7	64
22.	21	0	5	10.0	86
23.	36	0	9	9.9	93
			Del.	10.2	68
24.	23	2	7	7.9	122
			8	8.0	92
			9	8.6	86

Figure 9

Cord Blood Erythrocyte Protoporphyrin

	Hgb.	Patient Retic.	E.P.	Hgb.	Cord Retic.	E.P.
1.	12.0	1.0	123	16.3	1.8	74
2.	11.6	1.7	97	17.0	-	104
3.	12.6	1.3	24	17.1	3.8	40
4.	9.9	1.3	43	15.6	1.6	51
5.	9.7	4.2	282	14.8	4.4	64
6.	10.5	0.4	35	13.4	3.0	91
7.	7.2	1.2	212	17.5	5.2	83
8.	11.0	0.6	103	17.4	-	44
9.	11.9	0.5	108	-	1.2	68
10.	10.7	1.2	75	14.3	-	52
11.	12.3	0.6	43	17.2	3.3	83
12.	12.5	0.5	84	14.9	-	187
13.	7.0	0.8	131	14.4	-	97
14.	11.2	-	48	15.6	2.6	95
15.	13.5	0.8	68	19.1	3.0	105

Figure 10

Normal Serum Iron Values

	No.	Sex	Serum Iron Range	% Mean
Heilmeyer and Plötner	25	M	81-162	126
	25	F	64-128	89
Vahlquist	50	M	68-263	142
	50	F	53-210	123
Skouge	50	M	79-158	118
	50	F	66-164	104
Brochner and Mortensen	50	M	78-194	128
	50	F	79-191	118
Hemmeler	100	M		132
	100	F		103
Laurell	124	M	70-214	124
	39	F	57-196	108
Harjola	8	F	67-127	95
		M		121.5
		F		98
Powell		M		143
		F		117
Cartwright and Wintrobe	49	M		105
	43	F		104
Holly	23	F	54-142	93

and Strom[25] believe this difference to be an effect of hormone differences rather than menstrual blood loss. Powell[24] in 28 subjects noted a definite cycle in the female corresponding to the menstrual cycle. Lowest values (mean 100 gamma %) were observed during the bleeding phase while the highest values (mean 131 gamma %) were observed in the week just prior to menstruation. Vahlquist[19] has observed a diurnal variation with higher values being found in the evening. In Figure 10 are shown the mean values of reported series including those of the present investigation. For our purposes, values from 60-150 gamma % have been assumed to be normal.

Figure 11

Serum Iron

The Normal Female

Subject	Age	Gram % Hemoglobin	Gamma % Serum Iron
1.	21	13.2	82
2.	25	12.5	87
3.	23	13.4	102
4.	23	12.2	92
5.	24	13.1	126
6.	31	13.6	65
7.	26	12.6	92
8.	23	14.5	142
9.	28	13.8	78
10.	22	11.6	80
11.	24	13.3	72
12.	24	13.3	86
13.	23	13.0	92
14.	24	13.2	97
15.	22	13.1	80
16.	23	13.3	54
17.	25	13.9	117
18.	24	13.4	83
19.	26	12.7	93
20.	23	13.2	123
21.	23	13.6	96
22.	23	12.0	107
23.	24	13.1	93
Average			93 gamma %

Serum iron has been studied in a variety of clinical states. Low values have been reported in the anemia of infection, chronic blood loss anemias, following acute blood loss, ideopathic hypochromic anemia and during lactation. Normal to high values are found in aplastic anemia, pernicious anemia, hemolytic anemia, hemachromatosis and during iron therapy. In the few studies done in normal pregnancy the results are conflicting though the majority report lower values in the last trimester of pregnancy. Albers[26] reported increased values during pregnancy but his methods have been subject to considerable criticism. Sundelin[27] in a large series found the average serum iron of 118 gamma % in the first 5 months of pregnancy with a decrease to a mean

value of 48 gamma % in the 8th and 9th month. Heilmeyer[17] refers to a "latent iron deficiency" after noting low values in several of 17 subjects investigated. Renaer[28], likewise, has noted the general tendency for a decrease in serum iron late in pregnancy. Dahl[29] studied 43 pregnant subjects in the last month of pregnancy of which 25 had values below 70 gamma %. Laurell[20] in a study of 51 women with multiple observations at various stages of pregnancy noted a beginning decrease in serum iron in the 7th month with a rather consistent lowering in the 9th month.

Sixteen normal subjects have been studied here. The results are reported in Figure 12. These findings are in agreement with investigations of others. It seems clear that the majority of women have a latent iron deficiency

Figure 12

Serum Iron

Normal Pregnancy

Subject	Age	Mo.Preg.	Gram % Hemoglobin	Gamma % Serum Iron
1.	26	4	11.3	92
2.	17	6	11.1	68
3.	28	6	12.5	118
4.	29	7	11.4	88
5.	14	7	14.1	128
6.	13	7	13.3	119
7.	17	7	12.1	156
8.	16	7	11.7	89
9.	19	7	11.1	65
10.	20	7	13.0	142
11.	19	7	10.7	70
12.	17	8	13.2	88
13.	18	8	11.8	75
14.	19	8	11.4	70
15.	20	8	13.4	110
16.	25	9	13.2	60
17.	17	9	10.1	40
18.	18	9	10.4	50
19.	16	9	12.2	55
20.	19	9	11.7	31
21.	19	9	11.0	58
22.	20	9	12.5	160

in the last month or two of pregnancy. The studies on iron binding capacity of serum to be discussed in the next section likewise bear this out. The iron demand of the fetus and of increased erythropoiesis in the presence of relatively inefficiency iron absorption undoubtedly creates this iron deficiency state. Adequate iron store alone prevent the appearance of an anemia. Where these stores are depleted, as with frequent pregnancies, inadequate diet or from blood loss, anemia will appear. Iron deficiency anemia would undoubtedly

occur more frequently were it not common practice to routinely administer iron in pregnancy.

The fetus obtains its iron from the mother. 350-400 mgm. of iron is transferred to the fetus in the course of a pregnancy.[30] Most of this transfer occurs in the last month of pregnancy. This amount of iron represents approximately a tenth of the total maternal iron. How this transfer occurs is not known. Marshall[31] and Stander[32] believed that the transfer is effected by phagocytosis and hemolysis of the erythrocyte in the chorionic villi with liberation of iron which is carried to the fetus. It seems improbable that this series of events is the only mechanism involved. Hahn[33] and associates reported radio-active iron in the cord plasma 40 minutes after oral administration. Similarly more than simple diffusion is necessary in view of the high serum iron concentration of the cord as cpposed to that of the mother. The findings in Figure 13 are those of eight cord blood values contrasted with maternal values. The serum

Figure 13

Serum Iron

Maternal and Cord Serum

	Mother Serum Iron	Cord Gamma %
1.	40	253
2.	118	235
3.	28	125
4.	20	145
5.	112	265
6.	73	237
7.	65	191
8.	44	258

iron of the mother was determined from blood drawn during labor. Laurell[20] reports a mean maternal serum iron value of 80 gamma % and cord serum iron value of 154 gamma %. Dahl[29] found the average cord serum iron to be 234 gamma %. Further study is necessary to learn more of the mechanism by which iron is transmitted to the fetus by the placenta against a concentration gradient. Recent investigations[20] reporting low iron binding capacity values for cord serum suggest the possibility that cord serum has a greater affinity than maternal serum for iron.

Serum iron in iron deficiency anemia is low. The value is usually below 35 gamma %. The serum iron values in 12 cases of iron deficiency anemia of pregnancy are given in Figure 14. Some confusion might exist in the last month of pregnancy, since as previously discussed, values tend to be lower even with supposedly adequate hemoglobin concentration. Those subjects studied in the ninth month of pregnancy with anemia had distinctly lower values than those present in normal subjects. With treatment there is a slow return toward normal. Case #4 is of interest in that the anemia and corresponding marked decrease in serum iron developed while under observation. Figure 15 shows values in the four cases of chronic blood loss anemia. Higher values were noted in Case #2 under iron therapy than those seen for the pregnancy anemias under treatment. This at least suggests a more efficient response in the non-pregnant subject. Further observations of this sort are needed.

6. Iron Binding Capacity of Serum in Pregnancy

In studies on serum iron it was noted that the value for serum iron never exceeded a certain "saturation limit" [17,18,19,34]. Intravenously injected iron raised the serum iron level sharply to a critical level but a "braking" effect was noted even though amounts injected were in excess of a calculated amount assumed to be necessary to raise the serum iron to the observed level. Recent studies on iron binding capacity of serum have explained the mentioned observations. The studies of Schade and Caroline[35], Holmberg and Laurell[34],

Figure 14

Serum Iron

Iron Deficiency Anemia

Subject		Age	No. Preg.	Gram % Hemoglobin	Gamma % Serum Iron
1.		19	9	8.9	
	Rx-Iron		Del.	11.8	40
			P.P. 2 wk.	12.5	58
2.		20	8	9.5	35
	Rx-Iron		Del.	13.5	112
			P.P. 2 mo.	13.0	87
3.		36	5	9.9	28
			6	10.9	150
			7	12.2	113
4.		19	6	10.7	122
			7	11.0	95
			8	10.3	33
	Rx-Iron		9	9.2	17
			Del.	10.1	28
			P.P. 1 mo.	10.8	100
5.		24	9	7.9	37
6.		21	5	10.0	62
7.		19	9	9.2	31
	Rx-Iron		9	10.3	59
			Del.	12.0	44
8.		19	6	9.3	27
	Rx-Iron		9	11.3	52
			Del.	12.5	73
			P.P. 6 wk.	11.7	32
9.		23	9	7.9	32
	Rx-Iron		P.P. 2 wk.	8.5	20
			P.P. 3 mo.	12.6	40
			P.P. 5 mo.	13.4	40
10.		17	8	8.6	19
	Rx-Iron		Del.	11.6	25
			P.P. 2 wk.	13.1	39
11.		33	7	10.8	59
	Rx-Iron		8		103
			8		88
			9	12.3	130
12.		16	6	10.1	26
	Rx-Iron		7	9.0	25
			7	11.9	49
			8	12.9	33
			9	13.0	78

Figure 15

Serum Iron

Chronic Blood Loss Anemia

Subject	Age	Gram % Hemoglobin	Gamma % Serum Iron
1.	44	8.9	23
2.	21	8.5	12
		9.4	152
		13.2	160
3.	53	5.3	27
		12.7	48
4.	22	7.7	20

Laurell[20], and Rath and Finch[3] show that a B_1 globulin fraction is a specific metal combining component of human plasma. This fraction has been separated and crystallized by Cohn and associates.[37] The globulin has a molecular weight of 90,000, is colorless by itself but combined with iron develops a salmon red color. It is this color formation which is used in the current method of determination. It has been determined that one molecule of protein binds two molecules of iron. This fraction similarly binds the copper and zinc present in the serum.

The studies of Laurell[20], Finch[3], and Cartwright and Wintrobe[36] indicate that the iron binding capacity (IBC) in the healthy subject is approximately 200 gamma %. For normal females Rath and Finch found a mean value of 194 gamma % (15 cases), Laurell 207 Gamma % (39 cases) and Cartwright and Wintrobe 248 gamma % (15 cases). Our own data on 23 normal females is summarized in Figure 16. The mean value of 175 gamma % is somewhat lower than those reported by other investigators.

The technique employed measures only the unbound B_1 globulin. Since the serum iron concentration on these same subjects was 93 gamma %, it follows that 35% of the total iron binding protein is bound in the normal subject.

Figure 16

Normal Females

Iron Binding Capacity of the Serum

Name	Age	Hgb.	IBC gamma %
1.	21	13.2	145
2.	25	12.5	210
3.	23	13.4	180
4.	23	12.2	285
5.	24	13.1	140
6.	31	13.6	190
7.	26	12.6	230
8.	23	14.5	130
9.	28	13.8	180
10.	22	11.6	200
11.	24	13.3	150
12.	24	13.3	185
13.	23	13.0	220
14.	24	13.2	200
15.	22	13.1	100
16.	23	13.3	210
17.	25	13.9	100
18.	24	13.4	185
19.	26	12.7	185
20.	23	13.2	150
21.	24		140
22.	20		210
23.	23	13.6	100
Average			175

The relation of the bound protein fraction (serum iron) to the unbound fraction (IBC) in disease states is now being investigated. In iron deficiency anemia it has been found that coincident with the serum iron decrease the IBC increases. It is an interesting observation that the total iron binding capacity or "saturation limit" exceed that of the normal subject in iron deficiency anemia.

The IBC and "saturation limit" in the normal pregnancy in the investigation of Laurell[20] was found to be increased in the last months of pregnancy. Fourteen normal subjects in various months of pregnancy have been studied here. The results are given in Figure 17. Similar to the serum iron picture by beginning in the eighth month, the IBC is elevated late in pregnancy. The "saturation limit" or total iron binding capacity is likewise greater than normal in this period but has not been computed in the present material. Figure 18 is a chart of the IBC before and after treatment in thirteen anemias, nine of which were pregnancy iron deficiency anemias. Several of these subjects are under treatment at present and post treatment values have not been determined.

Figure 17

Iron Binding Capacity of the Serum

Normal Pregnancy

Name	Mo.Preg.	Hemoglobin	IBC
1.	4	11.3	200
2.	6	12.5	150
3.	6	11.0	245
4.	7	11.7	270
5.	7	11.3	220
6.	8	13.0	250
7.	8	12.3	320
8.	8	11.8	470
9.	8	12.1	370
10.	8	11.7	250
11.	8	11.1	385
12.	9	11.4	450
13.	9	11.8	395
14.	9	10.1	470

Figure 18

Iron Binding Capacity of Serum in Anemia

Name	Age	Diagnosis	Before Rx Hemoglobin	IBC	After Rx Hemoglobin	IBC
1.	21	Chronic Blood Loss	8.5	420	13.2	165
2.	44	" " "	8.9	340	8.9	310
3.	53	" " "	5.3	350	12.7	--
4.	17	Pregnancy Iron Deficiency	8.6	380	9.1	425
5.	16	" " "	10.7	450		
6.	19	" " "	9.3	400	11.7	245
7.	33	" " "	10.8	360	12.3	250
8.	19	" " "	9.2	290	10.4	200
9.	20	" " "	8.3	455		
10.	20	" " "	9.5	400	13.0	100
11.	19	" " "	8.5	410		
12.	21	" " "	10.0	430		
13.	24	" " "	7.8	470		

The significance of the increase in IBC and total iron binding capacity in the latter part of the pregnancy is not known. The one observation #7 E.W. suggests that the elevation of IBC during pregnancy represents latent iron deficiency. This individual, as yet undelivered, has a normal IBC following iron treatment.

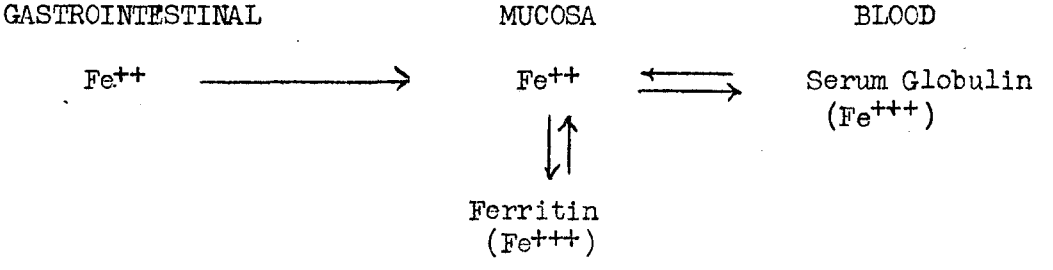
VII. Iron Absorption and Excretion. The Iron Tolerance Test.

Iron equilibrium in the body is maintained apparently by the gastrointestinal mucosa. It is not freely absorbed as is the case for sodium, potassium and other ions where excess amounts are excreted by the kidney. The excretion of iron from the intestine and from the kidney is negligible. It has been measured as less than 1 mgm. per day by the kidney.[30] Intravenously injected radio-active iron does not appear in the feces or in the urine. An insignificant and transient increase in urinary iron appears following the intravenous administration of iron. There is no evidence that pregnancy increases the excretion of this ion.

Because iron excretion is constant and negligible, to avoid over supply to the body the absorption mechanism must play the major role by regulating the intake. Absorption of iron takes place in the upper gastrointestinal tract, principally in the duodenum. Granick's[38] explanation of the mechanism by which absorption takes place and is regulated is the best of current theories. He suggested on the basis of animal experiments that the protein, apoferritin, mediates the transfer of iron from the interior of the intestine to the serum. The iron absorbed as the ferrous ion is combined with apoferritin to form the protein-iron complex called ferritin. The significance of ferritin to storage iron is well known. The mucosal cell ferritin then exists in equilibrium with werum iron. Where body stores of iron are normal, dietary iron is absorbed only in small quantities, and a relative mucosal block exists. Depletion of body stores permits greater absorption of iron. It is known that absorption of iron in iron deficiency states is many times greater than normal. The scheme of Granick's theory is shown in Figure 19.

Iron absorption in pregnancy was

Figure 19



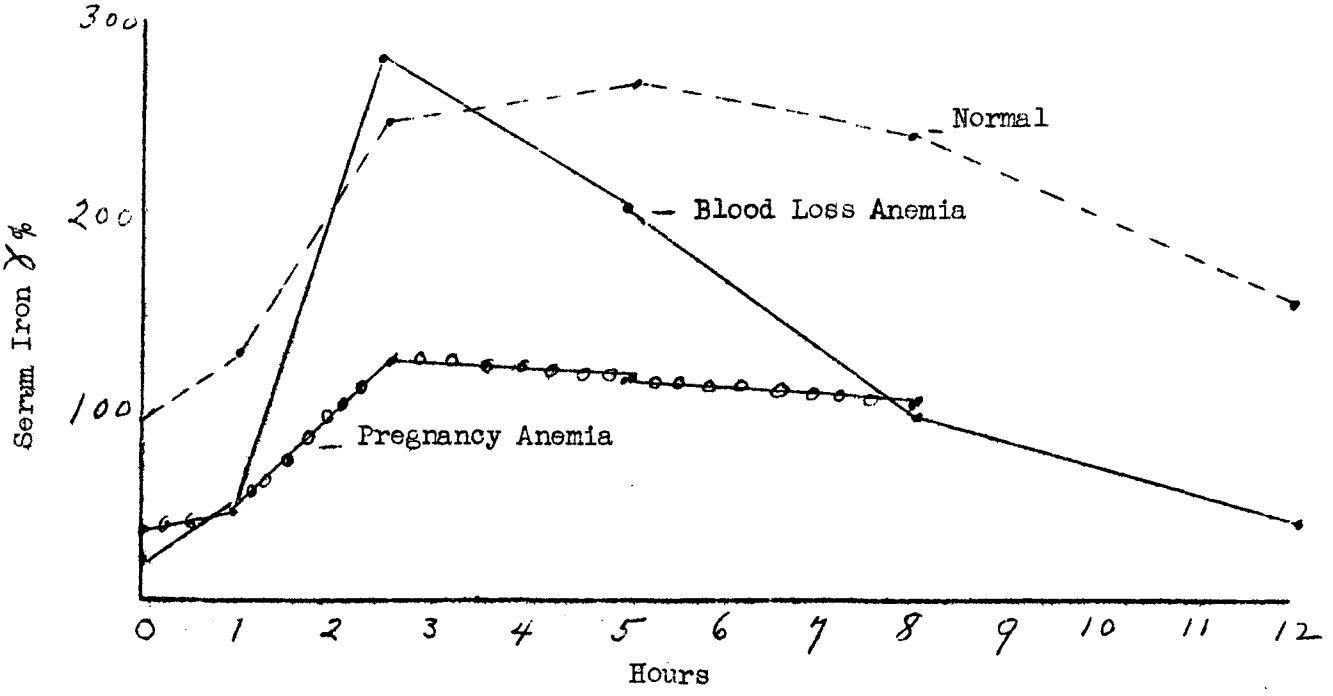
studied by Hahn[39] using radio-active techniques. The uptake of iron was 17% of administered dose in the first quarter and 36% in the last quarter of pregnancy. Renaer[28], using iron tolerance tests similar to those to be described, noted an increase in iron uptake in pregnancy. Our own experiences have not confirmed these reports but only patients with proven iron deficiency anemia have been studied in detail.

Heilmeyer[17], Waldenstrom[18], Vahlquist[19], and Moore[23] noted that following the ingestion of a test dose of iron the serum iron level rose. Maximum values were noted 2-5 hours following the administration of the iron. The level to which the serum iron was elevated was roughly proportionate to the amount of the test dose used.

Figure 20 is a graph of iron tolerance

Figure 20

Iron Tolerance Curve



tests performed here. One curve represents the average for eight normal subjects tested. The serum iron rose to a peak value of 271 gamma % followed by a gradual fall to normal in approximately 12 hours. Another curve is the average for 3 chronic blood loss anemias tested prior to treatment. The serum iron rose to a peak of 282 gamma % but the fall in serum iron values was more rapid than that seen in the normal individual. Ten cases of iron deficiency anemia of pregnancy were similarly tested. The serum iron rose on the average to only 126 gamma %. In this latter group the individual variation was great. Those individuals with normal or only slightly subnormal curves responded well to iron therapy. Two individuals with only minimal rise in serum iron were refractory to iron therapy.

The significance of these findings in iron deficiency anemia is open to some question. It may well be that elimination of iron from the serum in the iron deficient individual is rapid enough to lower the observed rise. In addition iron tolerance curves cannot be interpreted to represent quantitative uptake of iron. However, corresponding clinical observation suggests that a relative "mucosal block" may operate in certain individuals during pregnancy. This factor may in part be responsible for the anemia and for the lack of response to therapy.

Details of Hahn's extensive study are not available. The increased absorption was noted on normal subjects as was that observed by Renaer. Our own material is selected in that only iron deficient subjects have thus far been tested in detail. Moore[40] has emphasized that in radio-active iron studies the status of the bone marrow influences the measured uptake. Thus in bone marrow aplasia the measured values were low and where bone marrow hyperplasia existed the measured values were high. It is possible that the increase in measured uptake of radio-active iron in part reflects the bone marrow hyperplasia of pregnancy.

8. Diagnosis and Treatment of Iron Deficiency

Blood morphology in pregnancy may be misleading and has proven of little value in many cases. In severe long standing iron deficiency states, hypochromic microcytes are seen but in milder cases the cells are normocytic and normochromic. Greater accuracy is possible by determination of the erythrocyte protoporphyrin, serum iron and iron binding capacity. The bone marrow study yields confirmatory evidence of the existence of iron deficiency. In every instance of iron deficiency anemia studied the following was observed: (1) Elevation of erythrocyte protoporphyrin over 60 gamma %: (2) Decrease of serum iron below 60 gamma %: (3) Elevation of iron binding capacity above 300 gamma %: (4) And decrease in hemoglobin below 11 grams %. The hemoglobin value should be evaluated with respect to time of determination. A hemoglobin of 10.5 grams % early in pregnancy is more suggestive of iron deficiency than a similar value in the last two months of pregnancy.

Treatment is most effectively handled by the sulfate or gluconate of iron. Ferrous gluconate is tolerated better by the pregnant patient. Liver and folic acid are not effective in treatment of iron deficiency anemia.

Not all iron deficiency anemias respond adequately to oral iron. This failure of response has led Dreckmann[41] to investigate the properties of molybdenized iron. Goran and Scott[42] have recently reported the use of intravenous iron as a means of treatment. Two of their cases did not respond to prolonged treatment with oral iron. The interesting problem is why response to iron is poor in many anemias. Two possible factors are best shown by study of the four cases. (Figures 21-24)

Figure 21

Iron Deficiency Anemia of Pregnancy

P.M. Age 20

Hgb.	H'crit	E.P.	S.I.	IBC	Retic.	Date	Therapy
9.5	30	109	35	400	1.4	12/15/48	Oral Iron
10.7	34	86			2.9	12/31	
12.8	40				1.8	1/21/49	
13.5	39	68	112		0.8	2/9	(Delivery)
13.0	40	38	87	100	0.8	4/5	

Iron Tolerance Test:	12/15/48	4/5/49
Fasting	35	87
2½ hour	122	174
5 hour	133	160

Serum Iron in gamma %

Figure 21 gives the data on a typical case of iron deficiency anemia of pregnancy. Oral iron in the form of ferrous gluconate gram 1 per day produced a normal response. At term the anemia had been corrected. The E.P. drop and serum iron increase indicate adequate filling of the iron stores. Adequate absorption of iron could be predicted by a fairly normal iron tolerance test.

The response in a second case was very different. (Figure 22) Two months of oral iron did not in any way correct the anemia. The bone marrow was hyper-

Figure 22

Iron Deficiency Anemia of Pregnancy

A.H. Age 24

Hgb.	H'crit	E.P.	S.I.	Retic.	Date	Therapy
7.7	28	160		1.3	7/6/48	Oral Iron
7.0	30			1.8	7/21	
7.9	28	119	32	0.8	8/4	
7.0	26			1.0	8/16	
7.0	28		20		9/4	(Delivery)
8.5	34	131		0.6	9/20	
12.6	42	83	40	1.0	12/17	
13.4	41	66	40	0.8	2/8/49	

Iron Tolerance Test	8/4/48	2/8/49
Fasting	32	40
$2\frac{1}{2}$ Hour	64	172
5 Hour	44	120

Serum Iron Values in gamma %

plastic. Inadequate amounts of iron were being absorbed as indicated by the serum iron curve. Following delivery slow recovery took place. The serum iron curve five months after delivery revealed better absorption of iron. Three months after delivery an E.P. of 86 gamma % and a serum iron of 40 gamma % indicated incomplete saturation with iron even though the hemoglobin was 12.6 gram %. Two months later at her last check iron saturation was not complete.

Recognizing that the validity of the iron tolerance test as a measure of iron absorption is questioned, the next case refractory to oral iron therapy was

Figure 23

Iron Deficiency Anemia of Pregnancy

P.W. Age 17

Hgb.	H'crit	E.P.	S.I.	IBC	Retic.	Date	Therapy
8.6	31	150	19	380	1.2	11/12/48	Oral Iron
8.6					2.4	11/24	
9.1	32			425	1.4	12/1	I.V. Iron
9.2					4.4	12/7	
10.3	38	113			2.5	12/9	
11.6	42	97				12/9	

Iron Tolerance Test:	11/12/48	1/7/49
Fasting	19	39
$2\frac{1}{2}$ hour	40	94
5 hour	46	53

Values in serum iron gamma %.

treated with intravenous iron. (Figure 23) Three weeks of oral iron did not produce an appreciable response. The maximum reticulocyte count observed was 2.4%. 140 mgm. of ferrous ascorbate given intravenously on 12-1-48 raised the serum iron to 418 gamma %. This single dose of I.V. iron produced a second and more pronounced reticulocyte response of 4.4%. The hemoglobin and hematocrit promptly rose. No oral iron was given after the intravenous injection. The pre-treatment iron tolerance curve again suggested that iron absorption was suppressed. Re-testing shortly after delivery indicated a return toward normal of absorption.

A second factor responsible for inadequate response to treatment is suggested by case 4, Figure 24. Iron absorption was normal in this individual. Oral iron over a three month period produced only a slow and gradual recovery. The bone

Figure 24

Iron Deficiency Anemia of Pregnancy

B.B. Age 19

Hgb.	H'crit	E.P.	S.I.	IBC	Retic.	Date	Therapy
9.3	32	113	27	330	0.6	11/12/48	Oral Iron
9.0					2.0	11/22	
9.5	33				1.4	11/29	
10.1	33	133			0.8	12/28	
11.3	36	109	52		0.8	1/19/49	
12.5	39	84	73	285	0.5	2/15	(Delivery)
11/7	36	64	32	245	1.3	4/5	

Iron Tolerance Test: 11/15/48

Fasting	23
2½ hour	163
5 hour	160

Serum Iron in gamma %

Bone Marrow 11/15/48 M.E. Volume- 5% Normoblasts 19%

marrow from this patient was distinctly hypoplastic. It is probable that only blood transfusion could have produced a more rapid recovery.

Circumstances may be produced during pregnancy which enable the individual to inadequately absorb and utilize iron. Fortunately these circumstances do not appear in the majority of pregnant women. Iron absorption may be impaired which may be one factor responsible for the presence of iron deficiency and for the failure of response to oral iron. Intravenous iron will be effective in these individuals. A second factor of bone marrow hypoplasia may be responsible for incomplete utilization of iron.

Conclusions

1. Bone marrow study of 22 iron deficiency anemias is reported. The basic feature of these marrows is the normoblastic hyperplasia.

2. The erythrocyte protoporphyrin is not elevated in the normal pregnancy. Elevated values are consistently found in association with iron deficiency anemia.

3. There is a tendency for decrease in serum iron in the last two months of pregnancy. A corresponding increase in iron binding capacity is noted. This evidence suggests a latent iron

deficiency state in nearly all pregnant women.

4. Poor response to orally administered iron is found in some iron deficiency anemias of pregnancy. Inadequate iron absorption and bone marrow hypoplasia are suggested as factors responsible for this poor response.

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III. MEDICAL SCHOOL NEWS

Faculty News

Members of the Department of Physiology who presented papers at the Detroit meetings of the American Physiology Society, April 18-22, included Doctors Maurice B. Visscher, Joseph T. King, E. Gellhorn, Nathan Lifson, Roger M. Reinecke, Gilbert S. Campbell, Francis J. Haddy, Carmen B. Casas, Claire J. Carr, J. Hyde, J. Gay, Francis L. Stutzman, Akira Omachi, and Shirley L. Michel. Dr. Berry Campbell and Mr. Charles Good of the Department of Anatomy also presented papers before the meetings of the American Physiological Society.

Doctors Marthella Frantz and Arthur Kirschbaum presented papers at the meetings of the American Association for Cancer Research in Detroit on April 16-22. Dr. Lane Williams, also of the Department of Anatomy, reported on "Clearance of Rose Bengal by Livers of Normal and Carbon Tetrachloride-Treated Mice" at the meetings of the American Society for Experimental Pathology.

Dr. Wallace, Dr. Armstrong and Dr. David Glick of the Department of Physiological Chemistry presented papers at the meetings of the American Society of Biological Chemists which also met in Detroit, April 18-22.

Seniors to Visit Mayo Clinic

A large group of senior medical students will journey to Rochester on Saturday, April 30, to visit the Mayo Clinic. Plans for the trip have been made by Dr. Victor Johnson of the Mayo Foundation and Assistant Dean Myron Weaver of the Medical School. The tour will include the clinic building, Foundation office, museum, library, Medical Sciences Building, and perhaps the Research Institute. There will also be a brief visit at St. Mary's Hospital. Any junior or senior member of the Medical School faculty who would be interested in a tour to the Mayo Clinic is welcome to join with the students on this trip.

Biographical Briefs -- Chief of Psychiatry

Dr. Donald W. Hastings was born in Madison, Wisconsin, and had his undergraduate work in the premedical and medical spheres at the University of Wisconsin. He served his internship at Philadelphia General Hospital from 1934 to 1936. Following this, he was awarded a Rockefeller fellowship in Psychiatry and spent the years 1936 to 1938 as a resident at the Pennsylvania Hospital, also in Philadelphia. The following year was spent in Boston in psychiatric research on an appointment as psychiatrist to Harvard University.

In 1939, Dr. Hastings returned to Philadelphia as Director of Psychiatry at Pennsylvania Hospital. He remained in Philadelphia until 1942 when he entered military service. During his second stay in the Quaker City, Dr. Hastings held simultaneously several medical school appointments. These posts included Associate in Psychiatry in both the University of Pennsylvania Medical School and Jefferson Medical College, Associate Professor of Psychiatry in Woman's Medical School, and Assistant Chief of Psychiatry at Philadelphia General Hospital.

Dr. Hastings' military service began in 1942 when he was assigned to the Army Air Force. He later served in the European theater of operations as Chief Psychiatrist to the Eighth Air Force. At the war's end, he was stationed in Washington as Chief Psychiatrist to the Army Air Force. He is still called upon as civilian psychiatric consultant to the Air Surgeon. He also serves the State Department as psychiatric consultant.

In April, 1946, Dr. Hastings came to this Medical School as Professor of Psychiatry and Head of the Department of Psychiatry and Neurology.