blood 1991 78: 89-93

Blunted erythropoietin production and decreased erythropoiesis in early pregnancy [see comments]

Y Beguin, G Lipscei, H Thoumsin and G Fillet

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published semimonthly by the American Society of Hematology, 1900 M St, NW, Suite 200, Washington DC 20036.

Blunted Erythropoietin Production and Decreased Erythropoiesis in Early Pregnancy

By Yves Beguin, Gyorgyi Lipscei, Henri Thoumsin, and Georges Fillet

After decreasing in the first trimester of pregnancy, the total red blood cell mass increases in the second and third trimesters to peak at term at about 120% to 125% of nonpregnant values, but how this is brought about by changes in the rate of erythropoiesis is not known. We evaluated erythropoiesis by measuring serum transferrin receptor (TfR) levels in 406 women during normal pregnancy (N = 317), at delivery (N = 63), or in the early postpartum (N = 27). Despite the presence of the placenta and the frequent occurrence of iron deficiency, TfR levels remained low in the first two trimesters and increased in the third trimester and at delivery. To explain why erythropoiesic activity was relatively low in early pregnancy, we also measured serum immunoreactive erythropoietin (Epo) in relation to the degree of anemia. There was a very strong correlation between serum TfR and Epo levels in the entire group ($r = .59$, $P < .0001$) as well as in each period of pregnancy. Epo levels remained low for the degree of anemia and did not correlate with hematocrit in the first two trimesters, but recovered afterwards. In the early postpartum, Epo production and erythropoiesis were normal. We conclude that: (1) erythropoiesis is decreased in the first part of pregnancy but increases afterwards; and (2) blunted Epo production in early pregnancy could be responsible for that observation.

PREGNANCY CAUSES an increase in plasma volume and red blood cell (RBC) mass, which reach, respectively, 150% and 120% to 125% of nonpregnant values near term.[1-3] However, the total RBC mass first decreases in early pregnancy, before gradually returning to nonpregnant values by week 30 and further increasing in late pregnancy.[4] Because the RBC lifespan remains unchanged during pregnancy,[5] modifications in the RBC mass must be preceded by changes in the rate of erythropoiesis. Because until recently only ferrokinetics could provide a quantitative assessment of erythropoiesis, no measurement of erythropoiesis has been performed during pregnancy, except by reticulocyte counts,[6] which are only of semiquantitative value. The measurement of serum transferrin receptor (TfR) levels has recently been proposed as a convenient method to monitor erythropoiesis in animal and in humans.[7-9] In the present study, we measured TfR levels in pregnant women and found them to be significantly decreased in the first part of pregnancy as compared with controls. Production of erythroid cells depends on stimulation by erythropoietin (Epo) produced by the kidney in response to hypoxia.[10] Previous studies in small groups of women, including one by us,[11] have shown increased Epo levels in pregnant as compared with nonpregnant women,[6,12-15] but the relationship of Epo to the hematocrit (Hct) often was not assessed. We therefore evaluated serum Epo levels in relation to the degree of anemia and found relatively low levels as compared with control women. This finding suggests that blunted Epo production could be responsible for the low erythropoietic activity observed in early pregnancy.

SUBJECTS AND METHODS

Subjects. We studied 406 women who gave their consent to having blood drawn for hematologic tests while undergoing routine antenatal and obstetrical care. Mean age was 27 years (range 15 to 45 years). Gestational age, as established by one or more ultrasound scans, ranged from 5 to 42 weeks. Erythropoiesis was evaluated during pregnancy (N = 317), during labor leading to vaginal delivery (N = 63), as well as on day 7 postpartum (N = 27).

Control Epo samples were obtained from 74 women with Hct within the 25% to 44% range. This control group included 33 normal adult subjects who had not donated blood in the last 3 months, and 41 women with hypoplastic/aplastic (N = 9), hemolytic (N = 7), dyserythropoietic (N = 9), or iron-deficient anemia (N = 16), who had not received RBC transfusions in the preceding week. Control TfR samples were also obtained from 43 healthy women with normal Hct and serum ferritin (12 to 120 ng/mL).

TfR assay. Human placental receptor-transferrin complex was purified as described elsewhere[16] and injected repeatedly into rabbits. Serum IgG were isolated from rabbit serum[17] and transferrin antibodies were removed by passing through a column of human diferric transferrin coupled to Affigel 15 (Bio-Rad, Richmond, CA). Characterization of the plasma TfR and receptor antibody has been described elsewhere.[8,9]

An enzyme-linked immunosorbent assay (ELISA)[9] was used with minor modifications to measure serum levels of TfR. Immunoplates I with certificate (Nunc Intermed, Roskilde, Denmark) were used. The aliquots of blanks, standards, and unknown samples were added using a Digiflex automatic pipetor (Micromedic System, Philadelphia, PA). Standards were diluted to between 5 and 100 ng/mL and unknown sera were diluted 1:50 to 1:2,000 with 0.15 mol/L phosphate-buffered saline (PBS) (pH 7.4) containing 0.5% bovine serum albumin and 0.05% Tween 20. After color development, differential absorbance was read in dual wave length mode at 492 and 690 in a Titertek Multiskan MCC/340 plate reader (Flow Laboratories, Herts, England). Each sample was run in triplicate. The between-assay variability (coefficient of variation) was 7.2% when the same control sample was measured in each plate. Because the standard consisted of a complex of receptor and transferrin molecules, all TfR values given are actually receptor-complex values.

Epo assay. Circulating Epo levels were measured by a commercially available radioimmunoassay (Incstar Corp, Stillwater, MN)

From the Department of Hematology and the Department of Gynecology and Obstetrics, University of Liège, Liège, Belgium.

Submitted November 28, 1990; accepted March 1, 1991.

Supported in part by Grant No. 3.4513.88 from the Fund for Medical Scientific Research (FRSM, Belgium) and by a grant from the University of Liège School of Medicine.

Address reprint requests to Yves Beguin, MD, University of Liège, Department of Hematology, SI-3, CHU Sart-Tilman, 4000 Liège, Belgium.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/91/7801-0020$3.00/0

that uses recombinant human Epo (rHuEpo) for tracer and standards. Samples are incubated with rabbit anti-Epo serum for 2 hours at room temperature before Epo tracer is added. After overnight incubation, goat antirabbit serum is added. After centrifugation, the unbound tracer is removed by decantation and the pellet is counted. Several samples had to be diluted 1:10. Twelve control samples were run in each assay, with a between-assay coefficient of variation ranging from 10.3% to 14.1%.

Miscellaneous. Serum iron and total iron-binding capacity (TIBC) were measured by standard procedures.[18,19] Serum ferritin was measured by a radioimmunoassay.[20]

Statistical methods. Log transformed Epo and TfR values were used in statistical analyses. Student's *t*-tests, with pooled or separated variances as appropriate, were used to compare two groups. Analysis of variance (ANOVA), with Snedecor's F-test or Welch's test as appropriate, was used to compare more than two groups. Two-way analysis of variance was used to assess the effect of iron stores on TfR values within each period studied. Pearson and likelihood-ratio χ^2 tests were used to measure associations in two-way or multiway frequency tables. The *r* correlation coefficient between two variables was computed in least squares regression equations. The slopes and y-intercepts of the regression lines between log(Epo) and Hct in controls and study subjects were tested for equality among groups by Student's *t*-tests in an analysis of covariance, using the BMDP 1V program of the BMDP Statistical Software (University of California). A multivariate stepwise regression analysis was performed with serum TfR as the dependent variable.

RESULTS

Controls. Mean serum TfR level in 43 normal women was 6,940 ± 1,480 ng/mL (range, 4,300 to 11,450). Thirty-three normal control women had a mean Hct of 39.5% ± 2.1% (M ± SD) and a mean Epo level of 16.4 ± 4.1 mU/mL, while Epo levels in 41 nonpregnant anemic women ranged from 10 to 237 mU/mL. The following regression (N = 74, $r = -.88$, $P < .0001$) was obtained between Epo (mU/mL) and Hct (%): log(Epo) = 3.8279 − (0.0662 Hct). Based on this formula, a predicted log(Epo) value was derived for each sample and the O/P ratio of observed/predicted log(Epo) ranged from 0.80 to 1.20. Consequently, O/P ratios in study subjects were considered abnormal if lower than 0.80.

Study subjects. Table 1 displays Hct, iron status, serum TfR and Epo levels, as well as O/P ratios in groups of pregnant women. As compared with controls, Hct decreased in the first and further so in the second and third trimesters, before reincreasing at delivery and on day 7 postpartum ($P < .0001$ for comparison between periods of pregnancy). Transferrin saturation as well as serum ferritin levels decreased throughout pregnancy. In the first trimester, only 8% of women were iron deficient (serum ferritin <12 μg/L). This figure rapidly increased to 27% in the second and 59% in the third trimester ($P < .0001$).

TfR levels were decreased in early pregnancy, normalized in the first part of the third trimester, and increased slightly beyond normal values in late pregnancy, at delivery, and in the early postpartum ($P < .001$). The evolution of Hct, TfR, Epo, and O/P ratio throughout pregnancy is illustrated in Fig 1. The fraction of cases with reduced erythropoiesis (TfR < 4,300 μg/L) was 34%, 48%, 16%, 10%, and only 1%, respectively, during weeks 5 through 12, 13 through 20, 21 through 28, 29 through 32, 33 through 36, and after week 37 ($P < .0001$). The effect of iron status on serum TfR levels was analyzed in two-way analysis of variance (Table 2). Within each trimester of pregnancy, women with deficient iron status (serum ferritin < 12 μg/L) had higher TfR levels than women with marginal status (ferritin 12 to 19 μg/mL) and even more so than those with normal iron stores (ferritin ≥ 20 μg/mL). Both time of pregnancy and iron status were significant determinants of TfR levels ($P < .0001$) and there was no interaction between the two.

Epo levels were higher during pregnancy (31.4 ± 20.5 mU/mL), at delivery (33.9 ± 22.3 mU/mL), and on day 7 postpartum (35.1 ± 34.7 mU/mL) than in normal women ($P < .001$). Epo levels increased steadily (Fig 1) throughout pregnancy and into third trimester values ($P < .001$), but remained relatively stable among third trimester, delivery, and early postpartum values (not significant [NS]). The mean O/P ratio was significantly reduced in the first two trimesters ($P < .001$) but returned to normal values in the third trimester, at delivery, and on day 7 postpartum. Epo levels were relatively low for the degree of anemia (O/P ratio < 0.80) in 10% of the cases in the third trimester, and 25% of the cases in the first two trimesters ($P < 0.01$). In the 9 to 16 week period (Fig 1), the percentage of cases with abnormal O/P ratios peaked to 34%.

Figure 2 displays regression lines between Epo levels and Hct in groups of pregnant women as compared with control women. The inverse linear relationship between log(Epo) and Hct was not significant in the first ($r = -.01$) and second ($r = -.16$) trimesters, was present but with a reduced slope ($P < .001$ for difference in slopes) during the third trimester ($r = -.41$, $P < .0001$), and was normal

Table 1. Hct, Iron Status, Erythropoiesis, and Serum Epo Values in Controls and Pregnant Women

		Pregnant Women					
	Controls	1st Trimester	2nd Trimester	3rd Trimester	Delivery	Postpartum	*P* Value
N	43	53	108	156	110	26	
Hct (%)	39.5 ± 2.1	36.3 ± 3.4	34.4 ± 2.4	34.2 ± 3.0	36.8 ± 3.2	36.1 ± 4.0	<.0001
Tf saturation (%)		29 ± 12	23 ± 11	19 ± 10	17 ± 8	14 ± 8	<.0001
Ferritin (μg/L)	33 ± 20	44 ± 30	26 ± 24	14 ± 14	20 ± 16	16 ± 13	<.0001
TfR (μg/L)	6,940 ± 1,480	5,350 ± 2,360	5,130 ± 1,480	7,170 ± 2,990	9,200 ± 3,420	8,380 ± 3,220	<.0001
Epo (mU/mL)	16.4 ± 4.1	19.1 ± 6.2	28.4 ± 15.5	37.7 ± 24.2	33.9 ± 22.3	35.1 ± 34.7	<.0001
O/P ratio	1.00 ± 0.10	0.91 ± 0.17	0.92 ± 0.14	0.98 ± 0.14	1.04 ± 0.21	1.00 ± 0.16	<.0001

P values are given for comparison between groups of pregnant women.

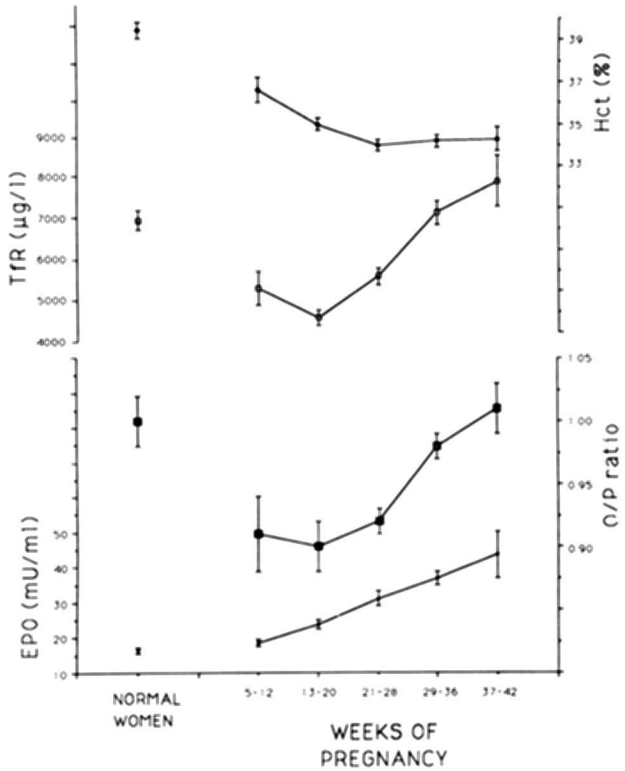

Fig 1. Evolution of Hct, TfR levels, Epo levels, and Epo O/P ratios (Mean ± SEM) throughout pregnancy, as compared with normal control values.

(slopes and y-intercepts not significantly different from controls) in the early postpartum ($r = -.74$, $P < .0001$). The relationship was also normal in the 28 women sampled beyond week 37 ($r = -.70$, $P < .0001$).

There was a very strong correlation between serum Epo and TfR levels in the entire group of pregnant women ($r = .59$, $P < .0001$), as well as in the first ($r = .48$, $P < .001$), second ($r = .56$, $P < .0001$), and third ($r = .54$, $P < .0001$) trimesters, at time of delivery ($r = .56$, $P < .0001$), or on day 7 postpartum ($r = .48$, $P < .001$). There was a striking parallelism between Epo O/P ratio and TfR levels throughout pregnancy (Fig 1). TfR also correlated negatively with parameters of iron status such as SeFe, transferrin saturation, and serum ferritin ($P < .001$), but this was mostly observed in the third trimester. The relationship between Hct and TfR levels was not significant in the first ($r = -.04$) and second ($r = -.14$) trimesters, and was weak in the third trimester ($r = -.26$, $P < .01$). In multivariate stepwise regression analysis ($r = .68$), serum Epo contributed the most to the prediction of serum TfR (change in r^2: .35), transferrin saturation (change in r^2: .08), and ferritin (change in r^2: .02) somehow enhanced precision, while Hct and week of pregnancy did not add significance.

Table 2. Effect of Iron Status on Serum TfR Levels According to Time of Pregnancy

	Time of Pregnancy			
	1st Trimester	2nd Trimester	3rd Trimester	*P* Value
Iron status				
Deficient	7,590 ± 4,810*	5,680 ± 1,400	8,260 ± 3,260	
Marginal		5,490 ± 2,000	5,870 ± 1,640	<.0001
Normal	5,040 ± 1,490	4,570 ± 1,040	5,460 ± 1,710	
P value		<.0001		

*Deficient and marginal iron status combined (N = 7).

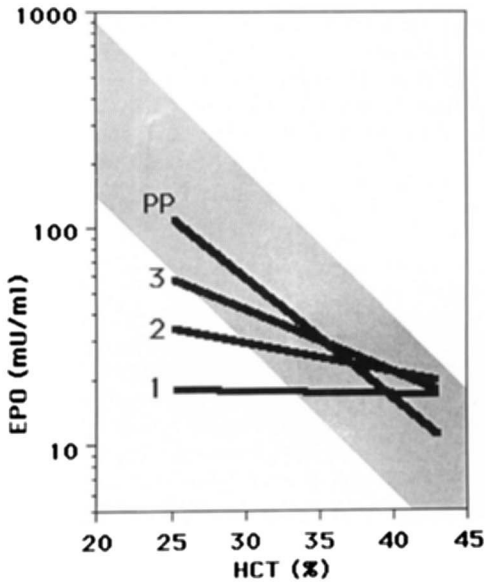

Fig 2. Relationship between Epo levels and Hct in the first trimester (I), second trimester (II), third trimester (III), and on day 7 postpartum (PP). Control subjects are represented by their 95% confidence limits (shaded area).

DISCUSSION

Pregnancy causes considerable alterations in plasma volume and RBC mass. There is little change in plasma volume before week 16, but then a steady increase takes place to 120% of nonpregnant values by week 20, to 140% by week 30, and to 150% at term.[1,2] Although the RBC mass peaks at term to about 120% to 125% of nonpregnant control values,[3] little is known about its evolution throughout pregnancy. Most studies have derived calculations of the RBC mass from measurements of plasma volume using Evans blue dye.[3,21,22] Such indirect measurements are not recommended[23] because whole-body Hct may vary widely, particularly in situations such as splenomegaly and pregnancy[24-26] where considerable redistribution of blood volume and blood flow takes place. In the one study using appropriate methodology for the serial measurement of total RBC mass,[4] there was a decrease in the first trimester of pregnancy, followed by a gradual return to nonpregnant values only by week 30, and a further increase in late pregnancy.

It has been shown that the RBC lifespan remains unchanged during pregnancy.[5] Modifications in the total RBC mass should then be accounted for by variations in the rate of erythropoiesis. No quantitative measurement of erythropoiesis is available from the literature, except near term,[5] because of the prohibitive need to use radioiron.

Methodology has been recently introduced for the measurement of serum TfR levels as a valuable alternative to ferrokinetics for the quantitative assessment of erythropoiesis.[7-9] Using this assay in the present study, we found that erythropoiesis was decreased in early pregnancy, normalized in the first part of the third trimester, and was moderately stimulated in late pregnancy, at delivery, and in the early postpartum. These data are consistent with the changes of RBC mass observed throughout pregnancy.[4] Others[27] have measured TfR levels without other parameters of erythropoiesis in a small group of women, and observed increased levels in late pregnancy, but no change in the first part of pregnancy. The nature and standardization of the material measured in that assay is not well established[28] and direct comparison is difficult.

There are theoretical interferences potentially precluding the use of serum TfR levels as a measurement of erythropoiesis during pregnancy. First, the placenta is very rich in receptors quite similar to the erythroid TfR, allowing its use as immunogen and standard in the present assay.[8,9,16] If placental TfR contributed significantly to circulating TfR levels, they would have caused an apparent overestimation of erythropoiesis in pregnancy, but this was obviously not the case. Second, functional iron deficiency beyond depletion of iron stores has been shown to produce an increase in serum TfR levels.[8,9,29] The effect of iron stores on TfR levels was also observed in pregnancy. Women with deficient iron status had higher levels than women with marginal status and even more so than those with normal iron stores. When only women with normal stores were considered, mean TfR levels in each trimester were even more decreased, while iron deficient women had normal levels. Therefore, iron deficiency does not appear to interfere significantly with the conclusion that the rate of erythropoiesis is low in pregnancy.

Plasma expansion could cause an apparent reduction in the serum concentration of TfR by diluting the total number of soluble receptors in a larger volume. Correcting for plasma volume would give mean receptor levels of about 5,290, 5,710, and 7,930 μg/L for women with normal iron stores, respectively, in the first, second, and third trimester. These levels are still well below expected values, particularly in view of levels of 23,258 ± 5,640 and 32,900 ± 8,352 μg/L in patients with immune hemolytic anemia or hemoglobin H disease, where a similar degree of anemia (Hct 35% ± 5%) induced considerably higher rates of erythropoiesis.[9]

We attempted to identify factors responsible for decreased erythropoiesis in pregnancy. In multivariate stepwise regression analysis, serum Epo contributed the most to the prediction of serum TfR. The correlation between serum TfR and Epo was strong in each period of pregnancy. Epo levels are best expressed in relation to the Hct.[30] We found an excellent correlation between Epo levels and Hct in 74 women with various degrees of anemia. Therefore, Epo levels measured in pregnant subjects could be related to predicted values calculated from the Hct. Absolute Epo levels increased throughout pregnancy and were in agreement with the Hct in the third trimester, at delivery, and in the early postpartum, but in the first two trimesters they were below predicted levels in the vast majority and low relatively to the Hct in 25% of the cases. The inverse linear relationship between log(Epo) and Hct, absent in the first trimester, progressively returned to normal as pregnancy advanced (Fig 2).

Several physiologic adaptations to pregnancy may augment oxygen supply to the kidney sensor, thus depressing Epo release in the first trimester. Erythrocyte 2,3-diphosphoglycerate increases early in pregnancy, producing a shift in the oxygen dissociation curve to the right.[31] In the first trimester, the renal blood flow is considerably increased,[25] but in the second half of pregnancy, a larger part of the expanded cardiac output is directed to the utero-placental and cutaneous circulations.[26] These alterations in oxygen supply to the kidney could in part explain variations in Epo production rate throughout pregnancy and the early postpartum. Modifications in the endocrine status, such as the production of human placental lactogen, have also been suggested to possibly influence changes in Epo production and release during pregnancy.[12,13]

We conclude that serum Epo levels, though increased over nonpregnant values, remain relatively low for the degree of anemia in the first part of pregnancy but return progressively to predicted levels thereafter. These changes in Epo production could explain the slowdown in erythropoietic activity observed in the first two trimesters and its normalization later in pregnancy. It should be emphasized that these alterations in the rate of erythropoiesis are adaptive changes that would certainly not require therapeutic intervention, such as the administration of rHuEpo.

REFERENCES

1. Hytten FE, Paintin DB: Increase in plasma volume during normal pregnancy. J Obstet Gynaecol Br Commonwealth 70:402, 1963

2. Pirani BBK, Campbell DM, MacGillivray I: Plasma volume in normal first pregnancy. J Obstet Gynaecol Br Commonwealth 80:884, 1973

3. Hytten F: Blood volume changes in normal pregnancy. Clin Haematol 14:601, 1985

4. Berlin NI, Goetsch C, Hyde GM, Parsons RJ: The blood volume in pregnancy as determined by P32 labeled red blood cells. Surg Gynecol Obstet 97:173, 1953

5. Pritchard JA, Adams RH: Erythrocyte production and destruction during pregnancy. Am J Obstet Gynecol 79:750, 1960

6. Howells MR, Jones SE, Napier JAF, Saunders K, Cavill I: Erythropoiesis in pregnancy. Br J Haematol 64:595, 1986

7. Kohgo Y, Niitsu Y, Kondo H, Kato J, Tsushima N, Sasaki K, Hirayama M, Numata T, Nishisato T, Urushizaki I: Serum transferrin receptor as a new index of erythropoiesis. Blood 70:1955, 1987

8. Beguin Y, Huebers HA, Josephson B, Finch CA: Transferrin receptors in rat plasma. Proc Natl Acad Sci USA 85:637, 1988

9. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA: Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 75:102, 1990

10. Schuster SJ, Wilson JH, Erslev AJ, Caro J: Physiologic regulation and tissue localization of renal erythropoietin messenger RNA. Blood 70:316, 1987

11. Beguin Y, Lipscei G, Oris R, Thoumsin H, Fillet G: Serum immunoreactive erythropoietin during pregnancy and in the early postpartum. Br J Haematol 76:545, 1990

12. Widness JA, Clemons GK, Garcia JF, Schwartz R: Plasma immunoreactive erythropoietin in normal women studied sequentially during and after pregnancy. Am J Obstet Gynecol 149:646, 1984

13. Cotes PM, Canning CE: Changes in serum immunoreactive erythropoietin during the menstrual cycle and normal pregnancy. Br J Obstet Gynaecol 90:304, 1983

14. Manasc B, Jepson J: Erythropoietin in plasma and urine during human pregnancy. Can Med Assoc J 100:687, 1969

15. Zivny J, Kobilkova J, Neuwirt J, Andrasova V: Regulation of erythropoiesis in fetus and mother during normal pregnancy. Obstet Gynecol 60:77, 1982

16. Huebers HA, Huebers E, Josephson B, Csiba E: A highly efficient chemical isolation procedure for the rat placental transferrin receptor. Biochim Biophys Acta 991:30, 1989

17. Axelsen NH, Kroll J, Weeke B: A manual of quantitative immunoelectrophoresis. Methods and applications. Scand J Immunol Suppl 2:1, 1973

18. Bothwell TH, Conrad ME, Cook JD, Fielding J, Hallberg L, Izak G, Layrisse M, Ramsay WNM: Recommendations for measurement of serum iron in human blood. Br J Haematol 38:291, 1978

19. Bothwell TH, Conrad ME, Cook JD, Fielding J, Hallberg L, Izak G, Layrisse M, Ramsay WNM: The measurement of total and unsaturated iron-binding capacity in serum. Br J Haematol 38:281, 1978

20. Lipschitz DA, Cook JD, Finch CA: A clinical evaluation of serum ferritin as an index of iron stores. N Engl J Med 290:1213, 1974

21. Taylor DJ, Lind T: Red cell mass during and after normal pregnancy. Br J Obstet Gynaecol 86:364, 1979

22. Chesley LC: Plasma and red cell volume during pregnancy. Am J Obstet Gynecol 112:440, 1972

23. ICSH: Recommended methods for measurement of red-cell and plasma volume. J Nucl Med 21:793, 1980

24. Zhang B, Lewis SM: Splenic hematocrit and splenic plasma pool. Br J Haematol 66:97, 1987

25. Davison JM: The urinary system, in Hytten FE, Chamberlain GVP (eds): Clinical Physiology in Obstetrics. New York, NY, Blackwell Scientific, 1980, p 289

26. de Swiet M: The respiratory system, in Hytten FE, Chamberlain GVP (eds): Clinical Physiology in Obstetrics. New York, NY, Blackwell Scientific, 1980, p 79

27. Kohgo Y, Niitsu Y, Nishisato T, Kondo H, Kato J, Tsushima N, Hirayama M, Sasaki K, Urushizaki I: Immunoreactive transferrin receptor in sera of pregnant women. Placenta 9:523, 1988

28. Flowers CH, Skikne BS, Covell AM, Cook JD: The clinical measurement of serum transferrin receptor. J Lab Clin Med 114:368, 1989

29. Skikne BS, Flowers CH, Cook JD: Serum transferrin receptor: A quantitative measure of tissue iron deficiency. Blood 75:1870, 1990

30. Garcia JF, Ebbe SN, Hollander L, Cutting HO, Miller MO, Cronkite EP: Radioimmunoassay of erythropoietin: Circulating levels in normal and polycythemic human beings. J Lab Clin Med 99:624, 1982

31. McDonald RG, McDonald HN: Erythrocyte 2,3-diphosphoglycerate and associated haematological parameters during the menstrual cycle and pregnancy. Br J Obstet Gynaecol 84:427, 1977