

# Megakaryocytopoiesis and Platelet Production Are Stimulated During Late Pregnancy and Early Postpartum in the Rat

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Platelet count during uncomplicated pregnancy shows considerable patient variation. To gain a better understanding of thrombocytopoiesis during pregnancy, megakaryocytes and platelets were examined during gestation and the early postpartum period, using as a model the rat. Platelet counts and megakaryocyte concentrations and DNA content distributions of timed-pregnant rats were examined at intervals from day 10 of gestation through parturition on day 22 and days 1 through 7 postpartum. Platelet survival was studied in late gestation and the early postpartum. Platelet volume was measured on gestation day 21. Platelet counts were moderately increased on gestation days 17 and 19 through 22, and on days 2 to 3 postpartum. However, the actual rate of platelet production was much higher than the platelet count suggests because the blood volume increased in late gestation to 1.5 times the nonpregnant level. Mean platelet volume and platelet volume distribution width of day 21 gestation rats were not significantly altered. Platelet survival in pregnant rats was not significantly different from that in nonpregnant females. In contrast, megakaryocyte concentration was significantly increased on gestation days 12, 17, and

19 through 21, and 2 to 3 days postpartum. In addition, in late gestation, megakaryocyte DNA content distributions displayed a marked increase in the proportion of high ploidy cells, which peaked 1 day before parturition. At that time, the proportions of 32N (43%) and 64N cells (3%) were, respectively, three and four times nonpregnant values. In contrast to megakaryocyte concentration, megakaryocyte DNA content distributions had returned to the nonpregnant pattern by day 1 postpartum. The changes in megakaryocyte DNA content distribution were accompanied by changes in megakaryocyte size. These data indicate that thrombopoiesis is substantially increased during late pregnancy, and that this increase is accomplished through an increase in megakaryocyte DNA content and size, as well as megakaryocyte number. The more rapid return of megakaryocyte DNA content than of megakaryocyte concentration to nonpregnant levels postpartum suggests that pregnancy-associated hormonal changes which produce an increase in megakaryocyte DNA content and size differ from those which cause an increase in megakaryocyte number.

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**A** RECENT REPORT suggests that a qualitative platelet alteration may precede and predict the development of increased vascular resistance and preeclampsia in pregnant women.<sup>1</sup> Several observations suggest that thrombocytopoiesis also may be altered in uncomplicated pregnancy; however, comprehensive studies have not been performed. Most large cross-sectional studies of platelet counts of pregnant women imply that platelet concentration decreases slightly during uncomplicated pregnancy.<sup>2,3</sup> A longitudinal study of platelet counts during pregnancy in 26 healthy women by Sill et al<sup>4</sup> also showed a small decrease in average platelet counts, but an analysis of platelet counts in individual patients showed that the decrease in average platelet count of the group was due to a substantial decrease in a few individuals. However, a platelet size index, namely platelet volume distribution width, showed a

positive correlation with gestation time in 19 of 20 women.<sup>4</sup> A positive correlation between platelet volume distribution width and gestation also was reported by Fay et al<sup>2</sup> from an analysis of 2,881 random blood samples from 2,114 women with uncomplicated pregnancy. Mean platelet volume was significantly increased after gestation week 35 in that study.<sup>2</sup> Another pertinent pregnancy-related change is a 35% increase in  $\alpha_2$ -adrenoceptor binding capacity of intact platelets of women studied late in pregnancy compared with that of nonpregnant females.<sup>5</sup> In addition, a recent report described a 20% increase in platelet lactic dehydrogenase activity during the last trimester in healthy pregnant women.<sup>6</sup> Because platelet size and cellular contents are primarily determined during thrombopoiesis, the increases in these platelet indices suggest that megakaryocytopoiesis and platelet production are altered during normal pregnancy in humans. Of the few experimental data available, Marien and McFadden<sup>7</sup> reported that splenic megakaryocytes of pregnant rats were maximally increased (threefold) 1 day before parturition, while marrow megakaryocytes were increased about 20% at this time. A similar increase in splenic megakaryocytes has been noted during late gestation in mice.<sup>8</sup> However, these studies left many questions unanswered because platelet kinetics and other megakaryocyte parameters such as size and DNA content were not examined, and splenic megakaryocytopoiesis does not appear to be a major contributor to platelet production in rodents with functioning marrow.<sup>9,10</sup> We then conducted a detailed examination of platelets and marrow megakaryocytes during gestation and in the early postpartum period in the rat to define pregnancy-associated changes in megakaryocytopoiesis and platelet kinetics. The preliminary results of megakaryocyte concentration and DNA content distribution 1 day before parturition and 2 to 3 days postpartum were reported in a workshop proceedings.<sup>11</sup>

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## MATERIALS AND METHODS

Timed-pregnant Long-Evans hooded rats were purchased from Harlan Industries, Inc (Indianapolis, IN) or Blue Spruce Farms, Inc (Altamont, NY). The day after mating was designated as day 1 of gestation. Pregnant rats at a particular day of gestation were usually studied in groups of 3 or 4. Nonpregnant females of the same strain and age were killed in parallel as controls (usually three or four at each sampling time). The timed-pregnancies were scheduled so that rats at 2 or more gestation days were usually studied at the same time to minimize day-to-day methodological variations. To obtain data on larger numbers of pregnant rats at the peak of the megakaryocytopoietic response, two or more groups of pregnant rats were studied at each gestation day, from days 19 to 22, and data for particular gestation days were pooled. Parturition usually occurred on day 22 of gestation. The females studied postpartum were housed with their litters and, therefore, were lactating. Platelet counts were determined by phase-contrast microscopy after blood collection in platelet Unopettes (Becton-Dickinson, Rutherford, NJ). Hematocrits were determined using a microtechnique. Blood for platelet counts and hematocrits was obtained by puncturing a vaseline-coated tail vein with a 25-gauge needle after vasodilation induced by briefly immersing the tails in warm water.

### Platelet Volume Measurements

Mean platelet volumes (MPVs) were determined with a microcomputerized Elzone electronic particle counter (Particle Data Laboratories, Elmhurst, IL) equipped with a 48- $\mu\text{m}$  orifice and a 128-channel analyzer.<sup>12</sup> Data were accumulated on each platelet sample until a count of 4,000 was collected in the peak channel. The samples were diluted so that the coincidence level was less than 1%. The volume distributions were analyzed as log-normal distributions. MPVs and platelet distribution widths<sup>2</sup> were calculated. For platelet size analysis, blood ( $\approx 5$  mL) was collected via cardiac puncture under general anesthesia into platelet syringes containing 1 mL of 3.8% sodium citrate with 8  $\mu\text{mol/L}$  prostaglandin E<sub>1</sub> (PGE<sub>1</sub>). Platelet-rich plasma (PRP) was prepared by centrifugation of the blood in 12  $\times$  75 mm plastic tubes at 160g for 4.5 minutes at 22°C. The PRP samples were diluted 1:500 in Isoton (Coulter, Hialeah, FL) yielding a platelet concentration of 10,000 to 12,000 platelets/100  $\mu\text{L}$  for size analysis.

### Blood Volume Estimation

Blood volume was estimated with a <sup>59</sup>Fe-labeled red blood cell (RBC) dilution method.<sup>13</sup> A female Long-Evans rat was administered 138  $\mu\text{Ci}$  <sup>59</sup>Fe-ferrous citrate intravenously. Six days later, the rat was exsanguinated under ether anesthesia with acid citrate as anticoagulant. The RBCs were sedimented by centrifugation and the plasma discarded. The RBCs were resuspended to their original volume in Hanks' Balanced Salt Solution (HBSS) without Ca<sup>2+</sup> and Mg<sup>2+</sup>, and 0.6 mL containing 1,792,200 cpm was transfused into each of four pregnant rats on day 15 of gestation and four nonpregnant rats of the same age. A 20- $\mu\text{L}$  blood sample was collected from puncture of a vaseline-coated tail of each recipient rat on gestation days 15 and 17 through 22, and at the same times from the nonpregnant females. The blood was diluted with 1.98 mL of 1% ammonium oxalate, radioactivity counted in a gamma scintillation spectrometer, and total blood volume in milliliters calculated. Total blood volume of each pregnant rat was divided by the average total blood volume of the four nonpregnant rats on each sample day to calculate the percentage increase in blood volume during gestation. Blood volume also was expressed as milliliters per 100 g of body weight.

### Measurement of Platelet Survival

Platelet survival was estimated using <sup>51</sup>Cr as the label.<sup>14</sup> Platelets were separated by differential centrifugation from whole blood collected into acid citrate from donor rats under ether anesthesia. Donors were pregnant rats at the same stage (day) of gestation as the pregnant recipients on which platelet survival studies were to be performed. The separated platelets were resuspended in HBSS without calcium and with one half the magnesium ion concentration and incubated with 1 mCi of Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub> for 45 minutes at room temperature. After incubation, the platelets were pelleted, washed twice with rat plasma diluted 1:1 with the HBSS described above, and resuspended in the plasma-HBSS for transfusion. The <sup>51</sup>Cr-platelet suspensions were transfused via a tail vein. In one study, five gestation day 19 rats and five age-matched nonpregnant females each received 0.55 mL of <sup>51</sup>Cr-platelet suspension containing 4,090,075 cpm. In a second study, seven gestation day 22 rats and six nonpregnant rats of the same age each received 0.6 mL of <sup>51</sup>Cr-platelet suspension containing 3,313,500 cpm. <sup>51</sup>Chromium bound to contaminating erythrocytes constituted 1.3 and 1.1%, respectively, of the radioactivity in the labeled platelet suspensions in the two studies. Blood samples (20  $\mu\text{L}$ ) were obtained by puncture of a vaseline-coated tail vein at 1 or 2 hours and daily through day 4. The blood was immediately diluted with 1.98 mL of 1% ammonium oxalate and the samples counted for radioactivity in a gamma scintillation spectrometer. To construct platelet survival curves, the radioactivity of the later time periods was expressed as a percent of the 1- or 2-hour cpm. The proportion of the platelet radioactivity injected that was circulating at 1 or 2 hours was also calculated.

*Measurement of megakaryocyte DNA distribution.* The DNA distribution of megakaryocytes in unfractionated marrow was determined as previously described.<sup>15</sup> Marrow from one femur of each rat was washed out with 3 mL of Hanks' BSS without Ca<sup>2+</sup> and Mg<sup>2+</sup>, but containing 0.38% sodium citrate, 10<sup>-3</sup> mol/L adenosine, and 2  $\times$  10<sup>-3</sup> mol/L theophylline (CATCH) medium<sup>16</sup> with 20 U/mL of DNase I.<sup>17</sup> A monoclonal antibody (MoAb) (BF3) with high specificity for rat platelets and megakaryocytes (kindly provided by Dr Kathryn Kellar, Centers for Disease Control, Atlanta, GA) was used as the primary antibody for labeling megakaryocytes for the flow cytometric analysis. Fluorescein isothiocyanate (FITC)-goat-antimouse IgG F(ab')<sub>2</sub> (TAGO, Inc, Burlingame, CA) was used as the secondary antibody. DNA was stained with propidium iodide in hypotonic citrate.<sup>15</sup> Using an Epics 753 flow cytometer (Coulter Electronics, Inc, Hialeah, FL) and two-color flow cytometry,<sup>15</sup> DNA contents of all BF3-positive cells were measured (DNA contents from 2N to 64N); however, for the purposes of this study, only those cells with DNA contents  $\geq 8N$  were considered because the proportions of 2N and 4N platelet-antibody-positive cells detected in unfractionated marrow is very dependent on the selection of the green fluorescence gate. The coefficient of variation for the modal polyploid DNA peak routinely averaged less than 2%. The proportion of cells in each ploidy class was determined by integrating the number of cells under each DNA peak.

*Bone marrow megakaryocyte concentration and size.* The average number of megakaryocytes per high power field (HPF, 500  $\times$  magnification, field area of 0.07 mm<sup>2</sup>) was determined in B-5-fixed, hematoxylin-eosin stained, sternal marrow sections. One entire longitudinal section was evaluated for each rat. Megakaryocytes were recognized by their large size, large lobulated nuclei with thick, clumped, deeply basophilic staining, and usually abundant cytoplasm. Megakaryocyte concentrations of animals with increased average megakaryocyte diameter were corrected for multiple counting error<sup>18,19</sup> by dividing the number of megakaryocytes

per HPF by the ratio of the average megakaryocyte diameter of the individual pregnant rats to the average megakaryocyte diameter of nonpregnant rats.

The cross-sectional area of megakaryocytes (50 per rat) was determined in the same sternal marrow sections with a computer-assisted image analysis system. All measurements were made by the same observer.

#### Platelet Hypertransfusion of Pregnant Rats

Timed-pregnant rats were transfused via a tail vein with platelet concentrates or plasma prepared from retired breeder male Long-Evans rat donors for 4 days beginning on gestation day 16 or 17. Donor rats were anesthetized with metofane and blood was collected from the abdominal aorta into 0.1 mol/L acid citrate. Platelets were separated and concentrated by differential centrifugation. Recipient rats were briefly restrained in plastic rat restrainers for daily blood collection (10 or 20  $\mu$ L for platelet count from a tail vein) and infusion of platelets or plasma. The recipient rats were killed after four daily platelet transfusions (1 day after the fourth transfusion) on days 20 or 21 of gestation, and megakaryocyte analyses performed.

#### Statistical Analysis

Unless stated otherwise, the Mann-Whitney rank sum test was the usual test for statistical differences between groups and between megakaryocyte DNA distributions. In this report, significance indicates a  $P < .05$ . A one-way analysis of variance showed that platelet counts, hematocrits, and megakaryocyte concentration for nonpregnant female rats killed at different times were not significantly different, so data on these parameters for all nonpregnant rats were pooled for statistical analyses. However, the analysis of variance showed a significant difference in megakaryocyte size

for nonpregnant females collected at different times; therefore, statistical comparisons for this megakaryocyte parameter were made with values of nonpregnant females killed at the same time as the pregnant rats to which they were compared.

## RESULTS

Platelet counts were significantly increased ( $P < .04$ ) on gestation days 17 (27%) and 19 through 22 (15% to 24%), and days 1 (13%) and 2 to 3 (30%) postpartum compared with nonpregnant females (Table 1). Platelet counts had returned to normal by day 7 postpartum.

MPV studied on gestation day 21 averaged  $3.60 \pm 0.26$  fL in five pregnant rats, and  $3.83 \pm 0.15$  fL in seven nonpregnant females; this difference was not significant. Platelet volume distribution width (10.21) of day 21 gestation rats was not significantly different from that of nonpregnant females (10.0).

Hematocrits were significantly decreased ( $P < .007$ ) on gestation days 12 and 16 through 22, and days 1 and 2 to 3 postpartum (Table 1). The nadir occurred on gestation day 22 (day of parturition) when average hematocrits were 26% lower than those of nonpregnant females. Hematocrits had returned to nonpregnant levels by day 7 postpartum.

Marrow megakaryocyte concentration was significantly increased ( $P < .015$ ) on gestation days 12 (26%), 17 (26%), and 19 through 21 (22% to 32%) and days 2 to 3 postpartum (18%) (Table 1).

Megakaryocyte size measured as cross-sectional area in marrow sections was significantly increased on gestation

**Table 1. Changes in Platelet Counts, Hematocrits, and Megakaryocyte Number and Size During Gestation and the Early Postpartum**

Day of Gestation	No. of Rats	Platelets $\times 10^3/\text{mm}^3$	Hematocrit (%)	Corrected MK/HPF*	MK Size† $\mu\text{m}^2$
Nonpregnant controls	56	929 $\pm$ 124 (48)	41.7 $\pm$ 2.1 (53)	7.2 $\pm$ 0.9	251 $\pm$ 27
10	3	867 $\pm$ 81	39.6 $\pm$ 2.3	7.9 $\pm$ 1.3	221 $\pm$ 24
12	6	959 $\pm$ 77	36.5 $\pm$ 1.4§	9.1 $\pm$ 1.2	268 $\pm$ 24
14	3	960 $\pm$ 26	40.6 $\pm$ 1.5	7.4 $\pm$ 0.8	307 $\pm$ 24
16	4	1,001 $\pm$ 74	37.2 $\pm$ 2.2§	7.0 $\pm$ 1.0	297 $\pm$ 45†
17	4	1,179 $\pm$ 49‡	37.8 $\pm$ 1.3§	9.1 $\pm$ 1.1	288 $\pm$ 18
18	3	1,035 $\pm$ 122	37.7 $\pm$ 1.1§	8.1 $\pm$ 0.7	268 $\pm$ 5
19	8	1,134 $\pm$ 51‡	35.8 $\pm$ 1.8§	9.5 $\pm$ 2.3	301 $\pm$ 34
20	7	1,098 $\pm$ 108‡	33.4 $\pm$ 2.0§	9.3 $\pm$ 1.8	318 $\pm$ 56
21	7	1,070 $\pm$ 157‡	33.7 $\pm$ 3.8§	8.8 $\pm$ 1.5	311 $\pm$ 40#
22	8	1,149 $\pm$ 128‡	30.9 $\pm$ 2.7§	8.2 $\pm$ 1.9	293 $\pm$ 14#
1 Postpartum	5	1,051 $\pm$ 66‡	34.0 $\pm$ 4.9§	7.8 $\pm$ 1.2	267 $\pm$ 33
2-3 Postpartum	7	1,205 $\pm$ 145‡	33.2 $\pm$ 2.6§	8.5 $\pm$ 0.7	248 $\pm$ 33
7 Postpartum	5	892 $\pm$ 186	41.8 $\pm$ 1.5	6.4 $\pm$ 1.1	281 $\pm$ 26

Values are means  $\pm$  1 SD. Numbers in parentheses indicate no. of rats where different from no. in second column.

\*The average number of megakaryocytes per high power field (MK/HPF) for each rat was determined by counting megakaryocytes in all HPF (30-110) available in one entire longitudinal section of sternal marrow. MK/HPF was corrected for changes in megakaryocyte size. The MK/HPF values represent the means of the individual rat average MK/HPF.

†MK size is the average of the median cross-sectional area in  $\mu\text{m}^2$  of 50 megakaryocytes in marrow sections of each rat.

‡ $P < .04$ .

§ $P < .007$ .

|| $P < .015$ .

† $P < .05$ .

# $P < .0015$ .

‡§ (for MK/HPF) Compared with all nonpregnant females.

|| (for MK size) †# Compared with nonpregnant females killed at that time.

days 12 (7%) ( $P < .015$ ), 16 (18%) ( $P < .05$ ), and 21 to 22 (17% to 24%) ( $P < .0015$ ) (Table 1).

The polyploid megakaryocyte DNA content distribution also was shifted toward larger values late in pregnancy (Fig 1). The average polyploid megakaryocyte DNA distribution of 60 nonpregnant female rats was: 8N, 21.1%  $\pm$  5.8%; 16N, 64.3%  $\pm$  3.3%; 32N, 13.8%  $\pm$  4.9%; and 64N, 0.8%  $\pm$  0.4%. The polyploid megakaryocyte DNA content distribution of pregnant rats on day 10 of gestation was not different from that of nonpregnant females studied at the same time. However, at all later gestation time points, megakaryocyte DNA distributions showed increases in the proportion of 32N cells with concomitant decreases in 8N and 16N megakaryocytes. On gestation day 12, the percentage of 32N cells was increased to 24% and, except for an isolated increase to 35% on day 14, remained in the 22% to 24% range through day 18. Gestation day 19 marked the onset of a further substantial increase in the percentage of 32N cells with peak values of 42% and 43%, or three times the nonpregnant percentage on gestation days 20 and 21. One day later (parturition day), 32N cells showed a significant decline to 35% ( $P < .024$ ), and by day 1 postpartum had decreased to 21%.

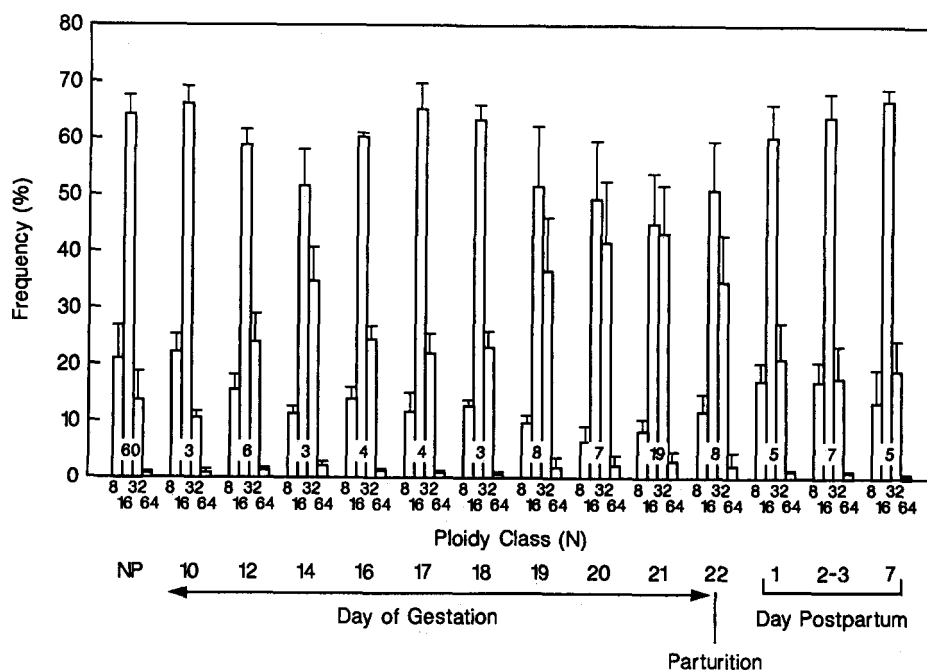
The megakaryocyte DNA content data presented above include values of all pregnant rats studied with one exception; the exception was one gestation day 18 rat that had only one fetus and whose megakaryocyte DNA distribution (23% 8N; 65.9% 16N; 10.2% 32N; and 0.9% 64N) was like that of nonpregnant females. The number of fetuses in all the other pregnant rats studied ranged from 3 to 17 (median of 12).

Total blood volume was 1.35 times the nonpregnant level on day 15 of gestation, and increased to 1.5 times normal by gestation day 21 (Fig 2). The increase in blood volume closely paralleled that of body weight, which was increased

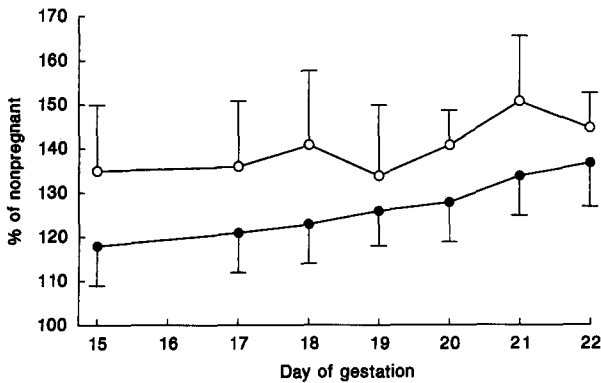
an average of 18% on gestation day 15 compared with age-matched nonpregnant females, and continued to increase, reaching 1.4 times the nonpregnant level before parturition (Fig 2). Thus, although total blood volume was substantially increased, blood volume expressed as a percentage of body weight (data not shown) was only modestly increased (high of 17% on gestation day 18) in the pregnant rats; this increase was significant on gestation days 15, 18, and 20 through 22 ( $P < .03$ ).

The disappearance rate of  $^{51}\text{Cr}$ -platelets during late gestation (Fig 3) and during parturition and the early postpartum (Fig 4) was not significantly different from that in age-matched nonpregnant females.

To determine if stimulated megakaryocytopoiesis in pregnant rats was subject to feedback regulation by platelet count elevation, pregnant rats were transfused with rat platelet concentrates for 4 days beginning on day 16 or 17 of gestation. Another group of pregnant rats was transfused in parallel with an equal volume of rat plasma. Both groups were killed on day 20 or 21 of gestation, and megakaryocytopoiesis examined (Table 2). Platelet counts and megakaryocyte indices also were obtained on a group of untreated, nonpregnant rats killed in parallel. The initial platelet transfusion raised platelet counts to about three times baseline. Subsequent transfusions raised and maintained platelet counts at four to five times normal. Megakaryocyte concentration and size were decreased in the platelet-transfused pregnant rats, compared with the plasma-transfused pregnant group or with nonpregnant controls (Table 2). Polyploid megakaryocyte DNA content distributions of platelet-transfused pregnant rats were shifted toward lower values compared with those of plasma-transfused pregnant rats or nonpregnant controls; the percentage of 32N cells was reduced from 42% in the plasma-transfused pregnant group to 4% in the platelet-



**Fig 1. Changes in polyploid megakaryocyte DNA content distributions of rats during pregnancy and the early postpartum. Bars represent means  $\pm$  1 SD. Numbers on bars indicate number of rats. NP, nonpregnant. From 559 to 2,426 platelet antibody-positive cells with DNA contents  $\geq$  8N were analyzed per rat.**

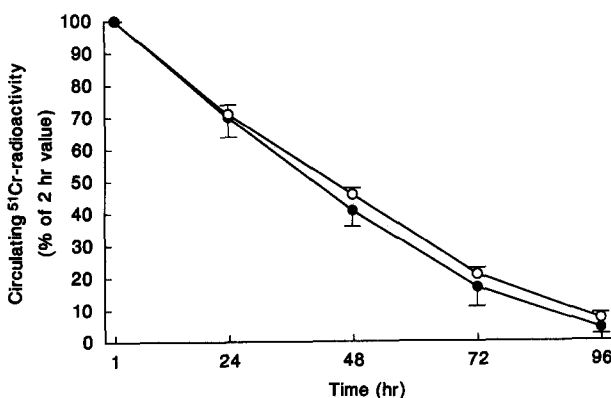


**Fig 2.** Increases in blood volume and body weight during gestation. (●), Blood volume; (○), body weight. Each point represents the average of four pregnant rats. The percentages were obtained by dividing the values for the individual pregnant rats by the average value for four age-matched nonpregnant female rats. Bars represent 1 SD.

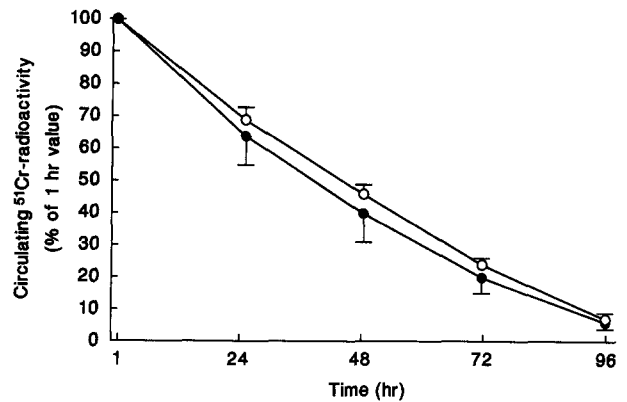
transfused pregnant rats, while the percentage of 8N megakaryocytes (45%) nearly equaled that of 16N cells (50%) in the platelet-transfused group (Table 2).

#### DISCUSSION

Few studies of megakaryocytopoiesis during pregnancy have been conducted. The literature indicates that during uncomplicated pregnancy, platelet counts show considerable individual variation<sup>4</sup>; some women maintain normal platelet counts,<sup>4</sup> some show a slight platelet decrease,<sup>4</sup> and in a small subset of women, platelets decrease into the thrombocytopenic range.<sup>3,20</sup> However, platelet size indices appear to increase slightly in healthy pregnant women regardless of platelet count,<sup>2,4</sup> suggesting that megakaryocytopoiesis is altered by this physiological stress. Here, we have examined the effects of pregnancy on megakaryocytopoiesis and platelet kinetics in detail, using the rat as an



**Fig 3.** <sup>51</sup>Chromium platelet survival during late gestation. <sup>51</sup>Cr-platelets were infused on gestation day 19. (●), Pregnant rats; (○), age-matched nonpregnant females. Five rats in each group. The platelet recovery (circulating radioactivity at 2 hours expressed as a percent of injected) was 59.6% ± 6.2% and 47.4% ± 10.9%, respectively (not significantly different), in the pregnant and nonpregnant recipients. Bars represent 1 SD.



**Fig 4.** <sup>51</sup>Chromium platelet survival during parturition and the early postpartum. <sup>51</sup>Cr-platelets were infused on gestation day 22. Parturition occurred before collection of day 1 samples. (●), Pregnant rats (n = 7); (○), age-matched nonpregnant females (n = 6). The platelet recovery (circulating radioactivity at 1 hour expressed as a percent of injected) was 77.3% ± 8.3% and 75.8% ± 6.5%, respectively, in pregnant and nonpregnant recipients. Bars represent 1 SD.

animal model. Platelet count as well as megakaryocyte size, concentration, and polyploid DNA content were increased in late pregnancy in the rat. However, the polyploid megakaryocyte DNA content distribution quickly returned to baseline in the early postpartum, while megakaryocyte concentration and platelet count were still elevated 2 to 3 days postpartum. MPV and platelet volume distribution width of gestation day 21 rats were not significantly different from those of nonpregnant females. Platelet survival in pregnant rats studied during late gestation, parturition, and the early postpartum also was not significantly different from that of nonpregnant females.

The small increase in platelets observed here in pregnant rats contrasts with the slight platelet decrease reported in most large studies of pregnant women<sup>2,3</sup>; however, the longitudinal study of Sill et al<sup>4</sup> suggests that considerable individual variation exists in uncomplicated pregnancy. In that study, platelet counts showed numerical increases during pregnancy in 7 of 20 women (although the slopes were not significant), while platelet levels decreased in the remaining 13 women, but in only two was the platelet change significantly different from the horizontal. These investigators concluded that the decrease in average platelet count of the group was due to a substantial decrease in a few individuals.<sup>4</sup> We did not observe decreased platelet counts in individual pregnant rats; however, our sample size was relatively small. Likewise, the maximum increase in platelet count we observed (1.3-fold) was not as large as that reported by Marien and McFadden<sup>7</sup> in pregnant and postpartum Wistar rats (1.65 times the nonpregnant value on day 2 postpartum). This discrepancy may be related to differences in the rat strains used; however, no indication of the variation in platelet counts of pregnant rats was given in that report, so that the significance of this difference cannot be judged.

Two groups have reported a positive correlation between platelet volume distribution width and time of gestation in

**Table 2. Reversal by Platelet Hypertransfusion of Pregnancy-Associated Megakaryocyte Stimulation**

Treatment Group	No. of Rats	Platelets $\times 10^3/\text{mm}^3$	Hematocrit (%)	Corrected MK/HPF*	MK Size† ( $\mu\text{m}^2$ )	MK Polyploid DNA Distribution‡			
						8N (%)	16N (%)	32N (%)	64N (%)
Platelet transfused	5	4,548 $\pm$ 473§	32.6 $\pm$ 1.9¶	5.1 $\pm$ 0.7§	207 $\pm$ 18#**	45.0 $\pm$ 9.2	49.8 $\pm$ 8.1	4.2 $\pm$ 1.0	0.9 $\pm$ 0.5
Plasma transfused	5	1,201 $\pm$ 165	33.2 $\pm$ 1.3¶	8.5 $\pm$ 1.2	319 $\pm$ 20¶	6.4 $\pm$ 2.1	49.2 $\pm$ 6.3	41.7 $\pm$ 6.9	2.7 $\pm$ 1.0
Nonpregnant control	5	968 $\pm$ 98	41.8 $\pm$ 1.5	7.7 $\pm$ 0.8	250 $\pm$ 20	21.7 $\pm$ 6.2	65.3 $\pm$ 1.7	12.2 $\pm$ 4.7	0.8 $\pm$ 0.5

All values represent means  $\pm$  1 SD and are those at time of sacrifice (gestation day 20 or 21 for the platelet- and plasma-transfused groups).

Abbreviation: MK, megakaryocyte.

\*The average number of megakaryocytes per high power field (MK/HPF) for each rat was determined by counting megakaryocytes in all the high power fields (29-94) available in one entire longitudinal section of sternal marrow. MK/HPF was corrected for changes in megakaryocyte size. The MK/HPF values represent the means of the individual rat average MK/HPF.

†Megakaryocyte size is the average of the median cross-sectional area in  $\mu\text{m}^2$  derived from measurement of 50 megakaryocytes in marrow sections of each rat.

‡The polyploid megakaryocyte DNA content distribution for each rat was derived from analysis of 499-1,958 platelet antibody-positive marrow cells with DNA contents  $\geq$  8N.

§P = .012 compared with plasma-transfused pregnant or nonpregnant controls.

¶P = .037 compared with nonpregnant controls.

¶P = .012 compared with nonpregnant controls.

#P = .012 compared with plasma-transfused pregnant rats.

\*\*P = .037 compared with nonpregnant controls.

healthy pregnant women<sup>24</sup>; however, the actual maximal increase above initial values in the Fay et al<sup>2</sup> study was 6% at gestation times  $\geq$  36 weeks, and in the study by Sill et al<sup>4</sup> at only one timepoint, 239 to 259 days of gestation, was the platelet volume distribution width significantly above the initial value in the same individuals. In both studies, platelet sizing was performed on platelets collected in EDTA. EDTA causes a 30% to 45% increase in platelet volume.<sup>21</sup> In our rat study, platelets were collected in citrate with PGE<sub>1</sub> to avoid the EDTA size artifact, and platelet size was studied only on gestation day 21. Platelet volume distribution width of pregnant rats was not significantly different than that of nonpregnant females.

The platelet survival times in the pregnant rats were like those reported in healthy pregnant women<sup>22-24</sup>; values were not significantly different from nonpregnant females. Although significant differences in platelet lifespan during uncomplicated pregnancy have not been detected, gradual increases in plasma  $\beta$ -thromboglobulin<sup>24,25</sup> and platelet factor 4 levels<sup>25</sup> during uncomplicated pregnancy suggest increased platelet activation.

Although the increase in platelet count during gestation was modest (15% to 27%), the rate of megakaryocytopoiesis and platelet production must have been substantially higher than the platelet count increment reflects, because total blood volume increased to 1.5 times normal by the end of gestation. The sum of the increases in platelet count and blood volume would equate to a platelet production rate of approximately 1.75 times normal near the end of gestation. This increase in platelet production was accomplished by an increase in both megakaryocyte concentration and size (related to higher DNA content). The abrogation of the pregnancy-associated megakaryocyte stimulation by platelet hypertransfusion indicates that this feedback loop can override the megakaryocyte stimuli present during late gestation. (Although nonpregnant rats were not platelet hypertransfused for comparison here, the reductions in

megakaryocyte concentration and size present in the platelet-hypertransfused pregnant rats were the same as those reported by Harker<sup>26</sup> in male rats platelet hypertransfused to the same degree with the same experimental protocol, suggesting that the responsiveness of megakaryocytopoiesis in the pregnant rat to biofeedback regulation was the same as that in nonpregnant rats.) Whether the same changes in megakaryocytopoiesis observed here in the rat occur in women with uncomplicated pregnancy remains to be determined.

The stimulated megakaryocytopoiesis may be related to pregnancy-associated elevations of progesterone, estrogens, or other steroid hormones. Plasma progesterone levels in the pregnant rat peak a few days before parturition and show a substantial decrease toward normal 1 day before parturition,<sup>27-30</sup> while estradiol peaks near parturition with a reduction to normal by day 1 postpartum.<sup>27,30</sup> Like estradiol, serum cortisol and oxytocin levels also increase and peak just before parturition, and decrease rapidly within 1 day postpartum.<sup>30</sup> Hence, the pattern of change in megakaryocyte DNA content most closely parallels that of progesterone, while the change in megakaryocyte concentration follows that of estradiol, cortisol, and oxytocin, suggesting that different hormones may be responsible for these two different megakaryocyte responses. Consistent with this interpretation is a report by Matsumura et al<sup>8</sup> that estradiol injections at 2- to 3-day intervals produced an increase in splenic megakaryocyte concentration in intact or castrated male mice, while progesterone did not. Unfortunately, megakaryocyte DNA content was not examined in that report. Our observation that a gestation day 18 rat with only one fetus did not show a right-shifted megakaryocyte DNA content distribution also fits with the idea that the increase in megakaryocyte DNA content is progesterone related, as rat plasma progesterone levels during the last one third of gestation are related to fetal number.<sup>31-33</sup> Progesterone is a product of the corpus

luteum, and is regulated by placental luteotropins. Therefore, the amount of progesterone produced is related to placental mass, and hence, to the number of fetuses.<sup>31-33</sup>

In summary, although platelet counts are only modestly increased, platelet production rate, and megakaryocyte size, number, and DNA content show substantial increases during the latter part of gestation in the rat, while platelet survival remains normal. Differences in the timing with which megakaryocyte number, and size and DNA content

return to nonpregnant levels postpartum suggest that different pregnancy-associated hormonal stimuli are responsible for the increases in these megakaryocyte indices.

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#### REFERENCES

- Zemel MB, Zemel PC, Berry S, Norman G, Kowalczyk C, Sokol RJ, Standley PR, Walsh MF, Sowers JR: Altered platelet calcium metabolism as an early predictor of increased peripheral vascular resistance and preeclampsia in urban black women. *N Engl J Med* 323:434, 1990
- Fay RA, Hughes AO, Farron NT: Platelets in pregnancy: Hyperdestruction in pregnancy. *Obst Gynecol* 61:238, 1983
- Matthews JH, Benjamin S, Gill DS, Smith NA: Pregnancy-associated thrombocytopenia: Definition, incidence and natural history. *Acta Haematol* 84:24, 1990
- Sill PR, Lind T, Walker W: Platelet values during normal pregnancy. *Br J Obstet Gynaecol* 92:480, 1985
- Metz A, Stump K, Cowen PJ, Elliott JM, Gelder MG, Grahame-Smith DG: Changes in platelet  $\alpha_2$ -adrenoceptor binding postpartum: Possible relation to maternity blues. *Lancet* 1:495, 1983
- Zappacosta B, De Sole P: Lactic dehydrogenase and glycolytic activity of human platelets in uncomplicated pregnancy. *Thromb Res* 61:395, 1991
- Marien GJ, McFadden KD: Splenic megakaryocytes and circulating platelets in pregnant rats. *Am J Anat* 128:225, 1970
- Matsumura G, Sasaki K, Ito T: Quantitative observation of megakaryocytes in the spleen and bone marrow of the mouse: Effects of sex, sex hormones, pregnancy and lactation. *Arch Histol Jpn* 47:251, 1984
- Ebbe S, Yee T, Phalen E: 5-Fluorouracil-induced thrombocytosis in mice is independent of the spleen and can be partially reproduced by repeated doses of cytosine arabinoside. *Exp Hematol* 17:822, 1989
- Chenaille PJ, Steward SA, Ashmun RA, Jackson CW: The prolonged thrombocytosis in mice after 5-fluorouracil results from failure to down-regulate megakaryocyte concentration. An experimental model which dissociates regulation of megakaryocyte size and DNA content from megakaryocyte concentration. *Blood* 76:508, 1990
- Jackson CW, Steward SA, Hutson NK, McDonald TP: Genetic and physiological variations in megakaryocyte DNA content distributions. *Int J Cell Cloning* 8:260, 1990
- McDonald TP: A comparison of platelet size, platelet count, and platelet <sup>35</sup>S incorporation as assays for thrombopoietin. *Br J Haematol* 34:257, 1976
- Paxson CL, Smith LH: Blood volume of the mouse. *Exp Hematol* 17:42, 1968
- Aster RH: Studies of the mechanism of "hypersplenic" thrombocytopenia in rats. *J Lab Clin Med* 70:736, 1967
- Jackson CW, Brown LK, Somerville BC, Lyles SA, Look AT: Two-color flow cytometric measurement of DNA distributions of rat megakaryocytes in unfixed, unfractionated marrow cell suspensions. *Blood* 63:768, 1984
- Levine RF, Fedorko ME: Isolation of intact megakaryocytes from guinea pig femoral marrow; successful harvest made possible with inhibitors of platelet aggregation; enrichment achieved with a two-step separation technique. *J Cell Biol* 69:159, 1976
- Rabellino EM, Nachman RL, Williams N, Winchester RJ, Ross GD: Human megakaryocytes. I. Characterization of the membrane and cytoplasmic components of isolated marrow megakaryocytes. *J Exp Med* 149:1273, 1979
- Harker LA: Megakaryocyte quantitation. *J Clin Invest* 47:452, 1968
- Cullen WC, McDonald TP: Effects of isobaric hypoxia on murine medullary and splenic megakaryocytopoiesis. *Exp Hematol* 17:246, 1989
- Burrows RF, Kelton JG: Incidentally detected thrombocytopenia in healthy mothers and their infants. *N Engl J Med* 319:142, 1988
- Levin J, Bessman JD: The inverse relationship between platelet volume and platelet number. *J Lab Clin Med* 101:295, 1983
- Wallenburg HCS, Van Kessel PH: Platelet lifespan in normal pregnancy as determined by a nonradioisotopic technique. *Br J Obstet Gyn* 85:33, 1978
- Rakoczi I, Tallian F, Bagdany S, Gati I: Platelet life-span in normal pregnancy and pre-eclampsia as determined by a non-radioisotopic technique. *Thromb Res* 15:553, 1979
- Pekonen F, Rasi V, Ammala M, Viinikka L, Ylikorkala O: Platelet function and coagulation in normal and preeclamptic pregnancy. *Thromb Res* 43:553, 1986
- Ayhan A, Akkok E, Urman B, Yarah H, Dunbar S, Kirazh S: Beta-thromboglobulin and platelet factor 4 levels in pregnancy and preeclampsia. *Gynecol Obstet Invest* 30:12, 1990
- Harker LA: Kinetics of thrombopoiesis. *J Clin Invest* 47:458, 1968
- Labhsetwar AP, Watson DJ: Temporal relationship between secretory patterns of gonadotropins, estrogens, progesterins, and prostaglandin-F in periparturient rats. *Biol Reprod* 10:103, 1974
- Sanyal MK: Secretion of progesterone during gestation in the rat. *J Endocrinol* 79:179, 1978
- Weisz J, Ward IL: Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. *Endocrinology* 106:306, 1980
- Yoshida T, Suzuki H, Hattori Y, Noda K: Hormonal changes around the parturition in rats. *Tohoku J Exp Med* 135:87, 1981
- Kato H, Morishige WK, Rothchild I: A quantitative relation between the experimentally determined number of conceptuses and corpus luteum activity in the pregnant rat. *Endocrinology* 105:846, 1979
- Golos TG, Sherwood OD: Control of corpus luteum function during the second half of pregnancy in the rat: A direct relationship between conceptus number and both serum and ovarian relaxin levels. *Endocrinology* 111:872, 1982
- Castracane VD, DeLa Cruz JL: The relationship of conceptus number and fetal sex to maternal serum testosterone concentration in the rat. *Proc Soc Exp Biol Med* 195:109, 1990