The Bone Marrow in Pregnancy and the Puerperium

By Louis Lowenstein and Catherine A. Bramlage

With the technical assistance of Madeleine Lalonde

IN THIS PAPER morphologic studies of the bone marrow in normal pregnancy and the puerperium are compared with the bone marrow findings in normal non-pregnant women of childbearing age. These studies were initiated for the following reasons:

1. Previous studies have shown that there is an increase of the total circulating red cell volume and hemoglobin mass of about 20% during pregnancy1-3 which is masked by the well-known physiologic hydremia of pregnancy.4-6

2. Reports in the literature concerning the morphology of the bone marrow in normal pregnancy and the puerperium are in considerable conflict.

3. In the absence of an exact knowledge of the bone marrow findings in normal pregnancy and the puerperium it is difficult to interpret properly the bone marrow preparations in megaloblastic and other anemias of pregnancy and the puerperium.

METHODS AND MATERIAL

Bone marrow was aspirated from 200 normal pregnant and puerperal women and from 30 non-pregnant normal women of childbearing age. The bone marrow aspirations during pregnancy and the puerperium were divided as follows: Thirty aspirations during each of the three trimesters of pregnancy, ten aspirations on each of the first eight days postpartum and thirty aspirations at approximately six weeks postpartum. In a few women two or more serial aspirations were taken during pregnancy and the puerperium.

All pregnant and puerperal women were normal as judged by medical and obstetrical histories, physical and hematologic examinations, and their subsequent course. The blood examinations included the determination of hemoglobin concentration, red cell count, packed cell volume, reticulocyte count, total and differential white count and morphologic examination of the red cells in the stained smear. The technics used and the normal hematologic values of the blood in pregnancy and the puerperium were described in a previous publication.5

Patients were accepted for this study if their hemoglobin was no lower than 10.1 Gm.% during pregnancy and the first 8 postpartum days and if the hemoglobin was no lower than 12.0 Gm.% at 6 weeks postpartum. The red cell morphology, mean corpuscular volume, the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration were required to be within the accepted range for the non-pregnant normal6 except for a permissible slight anisocytosis.1-9

The normal non-pregnant women of childbearing age selected for these studies were healthy as judged by careful histories and physical and hematologic examinations.

Bone marrow punctures were performed by aspiration of approximately 0.2 ml. of material from the sternum at the level of the second or third interspace. Cover slip preparations were made and marrow particles were selected and smeared on glass slides without anticoagulant. All preparations were stained with Jenner-Giemsa.

From the Haematology Service of the Department of Medicine and from the Bessborough Laboratory of the Department of Obstetrics and Gynaecology of the Royal Victoria Hospital, McGill University Medical School.

Submitted May 31, 1956; accepted for publication Aug. 18, 1956.

* Supported by a Federal-Provincial grant in aid of Research by the Department of Health and National Welfare of Canada.
Total nucleated cell counts were performed in approximately two-thirds of the cases. Bone marrow particles were present in cover slip preparations from each aspiration; at least 500 and often 1000 nucleated cells were counted in these areas. Both slide and cover slip preparations were used for morphologic study and observation. Polymorphonuclear neutrophils, basophils and eosinophils, and stab forms were not counted in order to minimize the error of dilution of bone marrow with blood. All cell groups were expressed as a percentage of the total nucleated cells counted. Mitotic figures were counted separately and were expressed as an absolute figure per 500 cells.

The results were statistically analyzed using the t-test of Fisher. Callender questioned the statistical significance of bone marrow results having a P-value of the order of magnitude of 0.05. In her studies of the bone marrow in pregnancy, she considered P-values of 0.05 to 0.02 to be of doubtful significance. In the present studies of the bone marrow P-values of greater than 0.02 are not regarded as significant, those of 0.02 or less are regarded as probably significant, those of 0.01 or less as definitely significant and P-values of 0.001 or less are regarded as highly significant. It is recognized that these standards are arbitrary and that the results of studies performed by the bone marrow aspiration technique are conditioned by a number of variables.

The percentages of most cell types differentiated followed a normal curve of distribution. The transformation method as used by Mainland and Snedecor was applied in those instances in which the percentage of cells of a given classification was too small to give a normal distribution curve. The results indicated that the original t-test method of analysis may be considered adequate for all cell types differentiated. The P-values reported for the shift to the left in the red and white cell series were verified by an analysis using an angular transformation.

Morphology and Terminology

The wide variation of nomenclature and classification of cells, particularly of the more primitive ones, by various authors is partly responsible for the difference in reported differential counts of normal bone marrow. A discussion of the derivation, classification and nomenclature of cells is outside the scope of this paper. It would seem desirable, however, that each author should define his nomenclature sufficiently to provide a satisfactory basis for comparison with the writings of other authors.

Reticulum cells: These cells vary from 15-30 microns in diameter. Their cytoplasm is relatively abundant, very fragile, slightly basophilic and may contain azurophilic granules. The nucleus is large, round, or oval, central or eccentric and has a fine pale-staining, almost spongy, or at times, a stippled chromatin pattern. Parachromatin is prominent. There are usually one or two nucleoli, occasionally as many as six. In this study these cells were sub-classified into phagocytic and non-phagocytic to determine whether phagocytosis might be observed more frequently during pregnancy and the puerperium than in the non-pregnant state. The hemocytoblast, with its more basophilic and homogeneous and less fragile cytoplasm, was found too infrequently in this study to permit statistical conclusions and consequently was included in the non-phagocytic reticulum cell series.

Plasma cells: Plasmablasts and early plasma cells (proplasmacytes) were observed so infrequently that they were included with the mature plasma cells (plasmacytes) in the differential counts. They occurred often in groups of two or more in the vicinity of reticulum cells.

Nucleated red blood cells: The classification used for the nucleated red cells is essentially that followed by Dacie and White. The term erythroblast is reserved for any nucleated erythrocyte precursor, whether a pathologic or physiologic cell and regardless of its stage of development. The term erythrocyte precursor and corresponds to the erythroblasts of Dameshek and Valentine and of Downey; the proerythroblast and the macronormoblast of other authors. It cannot be determined morphologically whether this cell will mature to megaloblast, to...
macronormoblast or to ordinary normoblast. It was found too infrequently in these studies to permit statistical conclusions and, for convenience, was grouped with the pronormoblasts. The terms, pronormoblast, basophilic normoblast, polychromatic normoblast and orthochromatic normoblast are used for the normal precursors of the mature erythrocytes. We would agree with others that the fully hemoglobinated orthochromatic normoblast is not common and in this study this term is used for a cell with fully orthochromatic cytoplasm and/or a pyknotic nucleus.

Megaloblasts and intermediate megaloblasts have been discussed in a previous paper.17 We agreed with others that all degrees of transitional forms between normoblasts and classical megaloblasts may be found in certain pathologic marrows and preferred the term intermediate megaloblast for these transitional cells. Atypical mitosis and asynchronism are often observed in the megaloblasts and intermediate megaloblasts and all stages of maturation of these cells may be seen. The earliest megaloblastic transition is reflected by a slightly more open chromatin pattern of the nucleus than is seen in normoblasts with comparable cytoplasmic maturation; these early transitional forms, along with a few intermediate macrogranulocytes may be found in marrows which are predominantly normoblastic.

Macropronormoblasts and macronormoblasts are found in some pathologic marrows and except for their large size do not differ morphologically from normoblasts which are found in the marrows of healthy individuals. They were not seen in the marrows of healthy pregnant or non-pregnant women and presented no problem in these studies.

White Cell Series

Monoblasts, promonocytes, lymphoblasts, and prolymphocytes were not encountered in these studies. The granulocytic series of leukocytes include myeloblasts, progranulocytes (promyelocytes) early myelocytes, late myelocytes, metamyelocytes, stab cells and mature neutrophils. The moderately basophilic cytoplasm of the early myelocyte is easily visible and contains a variable number of specific granules. The stab cell may be distinguished from the metamyelocyte and from the mature neutrophil by the presence of parallel sides throughout most of the length of its nucleus and by the absence of segmentation into lobes connected by filament.

Morphologic changes in the granulocytes usually accompany the transition from a normoblastic to a megaloblastic bone marrow. Comparable intermediate forms between normal granulocytes and classical macrogranulocytes occur and in some marrows may precede or dominate changes in the red cell series, or may persist after the red cell abnormalities have disappeared. The terms intermediate macromyelocytes, intermediate macrometa-myelocytes and intermediate macrostabs and intermediate macropolycytes would seem appropriate for these cells. Not only are these cells intermediate in size between the normal granulocytes and the classical macrogranulocytes but they frequently show some asynchronism in maturation of cytoplasm and nucleus with bizarre shapes, chromatin structure and staining properties of the nuclei. The nuclear chromatin pattern often appears more open and less condensed than in comparable stages of maturation in normal granulopoiesis.

The megakaryocytic series were not separated in this study into megakaryoblasts, pro-megakaryocytes and young and mature megakaryocytes. They were grouped together as megakaryocytes.

Results

Blood

The results of the examination of the blood in all 200 pregnant and postpartum women were compared statistically with the findings of the blood examination in the 30 non-pregnant normal women. In the analysis of the blood studies, P-values between 0.05 and 0.01 were considered to be of probable significance; those of 0.01 or less were considered definitely significant and those of less than 0.001 highly significant.
TOTAL WBC (in thousands)

HEMATOCRIT %

HEMOGLOBIN (gm/100 ml)

ERYTHROCYTES (mill.)

RETICULOCYTES %

Fig. 1. —The peripheral blood of pregnant and non-pregnant women.

The mean values of the observations are shown in figure 1. The findings are in agreement with those of previous studies in normal pregnancy and the puerperium reported from this laboratory. The hemoglobin concentration and the red cell values showed a maximum decrease during the second and third trimesters of pregnancy. During the first eight postpartum days the hemoglobin and hematocrit were within the non-pregnant normal limits; the erythrocyte count, however, was significantly lower than in the non-pregnant normal (P = <0.01). At six weeks postpartum the hemoglobin was slightly and probably significantly lower than in the non-pregnant normal (P = 0.05), which would suggest that erythropoiesis may not have returned to the non-pregnant normal at this time.

A significant neutrophilic leukocytosis (P = <0.01) with some shift to the left of neutrophils occurred during pregnancy and the early puerperium. At 6 weeks postpartum the leucocyte count did not differ significantly from the non-pregnant normal.

Bone Marrow Findings

Observations of the macroscopic appearance of all aspirates, particularly the number and size of the marrow particles, and examination of the cover slip and
Slide preparations from each subject were noted and resulted in the following impressions:

1) As compared with the non-pregnant normal the aspirates contained more and larger particles and were more cellular during the latter part of pregnancy and during the first eight days postpartum.

2) There was a definite increase of normoblastic erythropoiesis with a relative increase of early nucleated red cells during pregnancy and the puerperium.

3) There was a slight increase of plasma cells and of phagocytic reticulum cells during pregnancy and the early puerperium.

4) There was no eosinophilia in the bone marrow during pregnancy and the puerperium.

5) Granulopoiesis was increased during pregnancy and the early puerperium.

Total nucleated counts were performed in approximately 75 per cent of all cases. An increased mean value was obtained for all pregnant and puerperal groups studied (ranging from 154,000 to 177,000) as compared with the normal non pregnant group (mean value 130,000). Although this difference cannot be discarded, the individual results showed such great variation that it was decided not to utilize the total counts in the presentation of data.

The results of the 230 differential counts performed on the bone marrows of non pregnant, pregnant and postpartum women are presented in table 1, which shows the means values, their standard errors and standard deviations for each cell type. In table 2 are listed probability values of 0.05 or less. These values were obtained by comparing the means of each cell type in the pregnant and postpartum bone marrow series with the means of the non-pregnant series.

The values for the red cell precursors and mitotic figures are shown in table 1 and in figures 2, 3, 4. The pronormoblasts (including the erythrogones) were not significantly different during pregnancy and the puerperium as compared with the non-pregnant normal. The basophilic normoblasts increased gradually during pregnancy to a maximum mean of 2.34 per cent in the third trimester, as compared with a mean of 1.08 per cent for the non-pregnant normal. Although there was a slight decrease from this peak during the fifth to the eighth days postpartum these cells were still increased at the sixth postpartum week as compared with the non-pregnant normal. This increase throughout pregnancy and the postpartum period was highly significant ($P = <0.001$). Likewise the polychromatic normoblasts increased during pregnancy to reach a highly significant peak of 19.93 per cent during the third trimester as compared with the mean value of 13.47 per cent in the non-pregnant group. After delivery these cells decreased somewhat, but, at six weeks postpartum showed a significant increase to 20.60 per cent when compared with the non-pregnant normal. The orthochromatic normoblasts showed a similar but not statistically significant trend during pregnancy, the first four puerperal days and at six weeks postpartum. During the third trimester, 4.8 mitotic figures were found per 500 cells counted which was highly significant when compared with 3.1 for the non-pregnant normal. A significant increase was also found during the fifth to the eighth puerperal days and at six weeks postpartum. In figure 3 it may be observed that the early normoblasts increased more rapidly during the first part of pregnancy than did the late
Table 1: Sternal Marrow Differential Counts: Mean Values, Standard Deviations and Standard Errors in Pregnancy Compared with Normal Non-Pregnant Women.

<table>
<thead>
<tr>
<th>CELL TYPE (%)</th>
<th>NORMAL</th>
<th>1ST TRIMESTER</th>
<th>2ND TRIMESTER</th>
<th>3RD TRIMESTER</th>
<th>4TH DAY</th>
<th>5TH DAY</th>
<th>6TH DAY</th>
<th>6 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>S.D.</td>
<td>M</td>
<td>S.D.</td>
<td>M</td>
<td>S.D.</td>
<td>M</td>
<td>S.D.</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>0.48</td>
<td>0.31</td>
<td>0.52</td>
<td>0.38</td>
<td>0.36</td>
<td>0.06</td>
<td>0.30</td>
<td>0.06</td>
</tr>
<tr>
<td>Progranulocytes</td>
<td>1.24</td>
<td>0.50</td>
<td>1.60</td>
<td>0.69</td>
<td>1.82</td>
<td>0.31</td>
<td>1.58</td>
<td>0.29</td>
</tr>
<tr>
<td>Early myelocytes</td>
<td>3.57</td>
<td>0.64</td>
<td>4.02</td>
<td>0.43</td>
<td>4.12</td>
<td>0.34</td>
<td>4.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Late myelocytes</td>
<td>1.07</td>
<td>0.56</td>
<td>1.33</td>
<td>0.67</td>
<td>1.43</td>
<td>0.37</td>
<td>1.30</td>
<td>0.31</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>2.16</td>
<td>0.61</td>
<td>2.26</td>
<td>0.68</td>
<td>2.34</td>
<td>0.38</td>
<td>2.22</td>
<td>0.36</td>
</tr>
<tr>
<td>Eosinophilic myelocytes</td>
<td>1.15</td>
<td>0.51</td>
<td>1.24</td>
<td>0.55</td>
<td>1.47</td>
<td>0.37</td>
<td>1.33</td>
<td>0.34</td>
</tr>
<tr>
<td>Eosinophilic metamyelocytes</td>
<td>1.67</td>
<td>0.66</td>
<td>2.13</td>
<td>0.74</td>
<td>2.39</td>
<td>0.46</td>
<td>2.20</td>
<td>0.42</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.12</td>
<td>0.07</td>
<td>0.18</td>
<td>0.14</td>
<td>0.22</td>
<td>0.13</td>
<td>0.25</td>
<td>0.18</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.85</td>
<td>0.61</td>
<td>0.90</td>
<td>0.64</td>
<td>0.94</td>
<td>0.61</td>
<td>0.97</td>
<td>0.61</td>
</tr>
<tr>
<td>MEGAKARYOCYTES</td>
<td>0.18</td>
<td>0.08</td>
<td>0.20</td>
<td>0.09</td>
<td>0.29</td>
<td>0.10</td>
<td>0.32</td>
<td>0.11</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.17</td>
<td>0.08</td>
<td>0.19</td>
<td>0.10</td>
<td>0.28</td>
<td>0.12</td>
<td>0.23</td>
<td>0.13</td>
</tr>
<tr>
<td>Reticulum cells (Phagocytic)</td>
<td>0.06</td>
<td>0.03</td>
<td>0.07</td>
<td>0.04</td>
<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Reticulum cells (Non-Phagocytic)</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Mitoses</td>
<td>1.87</td>
<td>1.32</td>
<td>1.67</td>
<td>1.45</td>
<td>2.00</td>
<td>1.35</td>
<td>2.00</td>
<td>1.35</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>0.54</td>
<td>0.35</td>
<td>0.60</td>
<td>0.37</td>
<td>0.63</td>
<td>0.39</td>
<td>0.63</td>
<td>0.39</td>
</tr>
<tr>
<td>Basophilic normoblasts</td>
<td>1.06</td>
<td>0.40</td>
<td>1.10</td>
<td>0.43</td>
<td>1.21</td>
<td>0.44</td>
<td>1.21</td>
<td>0.44</td>
</tr>
<tr>
<td>Polychromatic normoblasts</td>
<td>15.67</td>
<td>7.22</td>
<td>15.97</td>
<td>7.48</td>
<td>16.16</td>
<td>7.64</td>
<td>16.16</td>
<td>7.64</td>
</tr>
<tr>
<td>Orthochromatic normoblasts</td>
<td>8.19</td>
<td>4.20</td>
<td>8.25</td>
<td>4.30</td>
<td>8.34</td>
<td>4.35</td>
<td>8.34</td>
<td>4.35</td>
</tr>
<tr>
<td>Mitoses</td>
<td>3.10</td>
<td>1.62</td>
<td>3.40</td>
<td>1.72</td>
<td>3.57</td>
<td>1.89</td>
<td>3.57</td>
<td>1.89</td>
</tr>
<tr>
<td>Ery. Ratio</td>
<td>0.37</td>
<td>0.04</td>
<td>0.41</td>
<td>0.05</td>
<td>0.43</td>
<td>0.05</td>
<td>0.43</td>
<td>0.05</td>
</tr>
</tbody>
</table>
TABLE 2.—P-Values* of Sternal Marrow Differential Counts Comparing the Three Trimesters of Pregnancy, Puerperium and 6 Weeks Post Partum with Normal Non-Pregnant Women

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>1st Tri.</th>
<th>2nd Tri.</th>
<th>3rd Tri.</th>
<th>1-IV Days</th>
<th>V-VIII Days</th>
<th>6 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Progranulocytes</td>
<td>0.01</td>
<td>0.05</td>
<td>—</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Early Myelocytes</td>
<td>0.001</td>
<td>0.01</td>
<td>—</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Late Myelocytes</td>
<td>—</td>
<td>—</td>
<td>0.001</td>
<td>0.01</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>0.05</td>
<td>0.05</td>
<td>0.02</td>
<td>0.01</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
<td>0.02</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.05</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>—</td>
</tr>
<tr>
<td>Monoocytes</td>
<td>0.001</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma Cells</td>
<td>—</td>
<td>—</td>
<td>0.05</td>
<td>0.001</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Reticulum Cells (phagocytic)</td>
<td>—</td>
<td>—</td>
<td>0.01</td>
<td>0.02</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Reticulum Cells (non-phagocytic)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.02</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mitoses</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.02</td>
<td>0.01</td>
<td>—</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Basophilic</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normoblasts</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Polychromatic</td>
<td>0.05</td>
<td>—</td>
<td>0.001</td>
<td>0.001</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Orthochromatic</td>
<td>—</td>
<td>—</td>
<td>0.05</td>
<td>0.02</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mitoses</td>
<td>—</td>
<td>—</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>—</td>
</tr>
</tbody>
</table>

* P-values found are less or approximately equal to the values stated. P-values greater than 0.05 are not shown in this table.

normoblasts. The above findings would suggest that the quantitative increase of erythropoiesis is associated with a relative increase of the immature forms of the normoblastic red cell series. The early red cell precursors (erythrogones + pronormoblasts + basophilic normoblasts) were calculated as the percentages of the total nucleated red cells present. Comparison between means of the early red cell precursors found in the bone marrows of the normal non-pregnant group and those of all groups studied, showed that there is a relative increase of immature red cells (shift to the left) during all three trimesters of pregnancy, the first 8 days postpartum and at six weeks postpartum. This increase of immature cells was most prominent and was statistically significant (P = 0.01) in the second trimester.

In figure 4 the erythropoietic-leukogenic ratio, obtained from the bone marrow differential counts and the hemoglobin concentration of the peripheral blood, are plotted for all groups studied. The erythropoietic-leukogenic ratio was calculated by dividing the percentage of all nucleated red cells by the total percentage of all white cells counted, after subtracting the lymphocytes and monocytes. In general, there seems to be a reciprocal relationship between the increase of erythropoiesis as expressed by the erythropoietic-leukogenic ratio and the decrease of hemoglobin occurring during pregnancy. A similar reciprocal relationship was found postpartum.
Fig. 2.—The proportions of the red cell precursors and their mitoses in the bone marrow of pregnant and non-pregnant women.

Fig. 3.—The proportional relation between the immature and mature normoblasts in the bone marrow of pregnant and non-pregnant women.
Fig. 4.—The relation of the erythrogenic-leukogenic ratio of the bone marrow and the hemoglobin value of the peripheral blood (pregnant, non-pregnant and puerperal women).

Fig. 5.—The proportions of the neutrophilic precursors and their mitoses in the bone marrow of pregnant and non-pregnant women.
The results of the mean differential counts of the white cell series are shown in tables 1 and 2 and figures 5 and 6. Although the myeloblasts increased in numbers during pregnancy and during the puerperium, this trend was not statistically significant. The progranulocytes increased significantly during the first trimester ($P < 0.01$). The slight increase during the second and third trimesters was not statistically significant. These cells showed a highly significant increase throughout the puerperium ($P = 0.001$). The early myelocytes showed the same trends as the progranulocytes. During the first and second trimesters these cells increased significantly, but decreased to approximately normal during the third trimester. They were significantly increased throughout the puerperium.

The late myelocytes and metamyelocytes decreased during pregnancy, rose slightly, although not to the non-pregnant normal level, during the early puerperium and showed a highly significant decrease at six weeks postpartum. Although the decrease of metamyelocytes during the first three trimesters of pregnancy was of dubious statistical significance, the trend was essentially the same as that of the late myelocytes. Granulocytic mitoses increased during pregnancy and the puerperium; this increase, however, was only statistically significant at six weeks postpartum. Eosinophilic granulocytes showed no consistent trend and consequently it is impossible to state that they differed from the non-pregnant normal during pregnancy and the puerperium.
The lymphocytes showed a decrease which was highly significant during the last two trimesters of pregnancy and during the early puerperium. At six weeks postpartum they had risen to the non-pregnant normal range. The monocytes showed no consistent or important quantitative changes during pregnancy or the puerperium. The plasma cells showed a statistically significant increase during the latter part of the pregnancy, a highly significant increase during the early puerperium, which was maximal five to eight days postpartum and they were still significantly increased at six weeks postpartum. The reticulum cells showed a change similar to that of plasma cells during late pregnancy and the early puerperium. Increased phagocytosis by the reticulum cells was only found during the third trimester. All reticulum cells were increased during the early puerperium but had returned to the non-pregnant normal range at six weeks postpartum.

In general, the results of the statistical analysis confirmed the conclusions drawn from the observations mentioned at the beginning of this section.

Discussion

Review of Literature

In these studies the bone marrow showed an increase of normoblastic erythropoiesis while the hemoglobin concentration, the red cell count and the hematocrit of the peripheral blood showed the usual decrease during pregnancy. When normal red cells are transfused into normal pregnant women their survival time is the same as when transfused into normal non-pregnant subjects and consequently it seems unlikely that pregnancy alters the life span of the red cell. Routine supplements of iron, vitamin C and vitamin B complex, which did not contain folic acid or vitamin B12, were taken antepartum by the patients of this series. Their hemoglobin and red cell values did not differ significantly from those of patients to whom iron was not given. The morphology of the red cells in the blood and of their precursors in the bone marrow was indistinguishable from that found in the non-pregnant normal. It would seem apparent that the increase of normoblastic erythropoiesis in the bone marrow results in the increase of total circulating red cell volume and of total hemoglobin mass which has been observed during pregnancy and which is masked by the relatively greater increase of plasma volume.

The increased erythropoiesis which develops between the eighth day and sixth week postpartum is more difficult to explain because of the absence of observations during this period. The loss of blood at delivery and the rapid decrease of total circulating hemoglobin and of red cell mass may be responsible for the subsequent increase of erythropoietic activity.

It was observed that as erythropoiesis increased during pregnancy and at six weeks postpartum, there was an apparent decrease of late granulocytes. Had stab and polymorphonuclear cells been included it is possible that the late granulocytes may not have shown this apparent decrease. Figures 5 and 6 also show an increase of the immature granulocytic precursors, which suggests that during pregnancy and the puerperium there is a shift to the left of granulocytes. This suggestion is supported statistically by comparing the immature granulocytic precursors (myeloblasts, progranulocytes, early myelocytes) expressed as per cent
of total granulocytes, found during pregnancy and the puerperium with those of the non-pregnant normal. By this method it may be concluded that there is an increase of immature granulocytic cells in all three trimesters of pregnancy, early in the puerperium and at six weeks postpartum. Statistical analysis showed that this shift to the left is highly significant (P = 0.001) in the first trimester of pregnancy, is significant in the second trimester (P = <0.01) and is not significant statistically in the third trimester (P = >0.05). During the first eight puerperal days and at six weeks postpartum there was a highly significant increase of immature granulocytes (P = 0.001) as compared with the non-pregnant normal.

This increase of the more immature granulocytes, the increase of mitotic figures, the observed increase of marrow cellularity during pregnancy and the early puerperum, and the leukocytosis of the peripheral blood support the conclusion that granulopoiesis is increased during pregnancy and the puerperium.

In attempting to compare the bone marrow findings of normal non-pregnant women with those of normal pregnant and puerperal women, it is recognized that there are many factors which limit the accuracy and the range of usefulness of such a study. The following variables and sources of error were carefully considered: bone marrow aspiration versus trephine biopsy; the morphologic classification of cells; the number of cells differentiated in each count and the cells included or excluded from the differential counts; the performance of the counts from random areas or from areas of bone marrow particles; the number of bone marrow aspirations used for statistical analysis, the methods of statistical analysis and the validity of their application to this study.

Not only would it have been impractical to perform trephine biopsy in these studies but we would agree with others that the aspiration method permits better cytologic differentiation. The amount of material aspirated was limited to 0.2 ml. to keep dilution of the sample with blood at a minimum. The differential counts were performed in the areas of stained particles in cover slip preparations in order to obtain the most constant and most accurate results.

Although comparison of the total nucleated cell counts of the bone marrow from puerperal women with those of the non-pregnant normal group showed a definite trend, they were not utilized in the statistical analysis because the wide spread of the individual counts seemed to substantiate the conclusion of Reich and Kolb that total cell counts of aspirated marrow samples are inaccurate. Stab and polymorphonuclear neutrophils were excluded from the differential counts, and both lymphocytes and monocytes were excluded in calculating the erythrogenic-leukogenic ratio in order to decrease the variation resulting from mixture of marrow and blood. It is difficult to determine whether the decreased lymphocytes in marrow aspirates during pregnancy and the early puerperium were due to decrease of the lymphocytes in blood and/or in bone marrow.

Previous reported studies of the bone marrow in normal pregnancy and the puerperium are summarized in table 3. Unfortunately the results of most of these observations are not strictly comparable because of variations of technic, of amount of aspirate, of methods of analysis, of morphologic terminology and of selection of normal non-pregnant controls. Thus, for his non-pregnant normal controls, Daniachij used the bone marrow findings of a mixed group of females and males of various ages as reported by Arinkin while Hansen used the find-
ings of similar groups as reported by Segerdahl and by Nordenson. Markoff and Wolff and Limarzi gave no details regarding their findings in the non-pregnant normal. All but one of the eight pregnant women studied by Forsell were in the fourth month of pregnancy. Pignoli studied 20 healthy women during pregnancy and the puerperium and used the marrow aspirations of these same 20 women at three months postpartum as the non-pregnant controls.

Pitts and Packham aspirated 10 ml. of material, Daniachij 0.5 to 1.0 ml.; Wolff and Limarzi prepared their differential smears from theuffy coat of centrifuged oxalated aspirates; the remainder of the authors aspirated less than 0.3 ml. of material.

Leitner counted 300 cells on some occasions, but in other instances counted 500 cells. Markoff performed no differential counts. Hansen, and Wolff and Limarzi failed to state the number of cells counted in each differential study; all other authors counted 500 or more cells in each differential. All authors included stab and polymorphonuclear neutrophils, eosinophils, basophils, lymphocytes and monocytes in their differential counts. Most authors failed to state whether their differential counts were performed upon random cells or upon bone marrow particles. Pitts and Packham performed total nucleated cell counts of the bone marrow aspirates. Wolff and Limarzi measured the packed cell volume of the buffy coat.

Some authors failed to describe their criteria for morphologic classification. Thus, Daniachij claims to have found megaloblasts in the normal bone marrow of pregnancy which reached a maximum of 1 per cent during the third trimester. It is probable that his so-called megaloblasts were the proerythroblasts, macro-normoblasts or erythroblasts of others, as no other author found megaloblasts in the bone marrow during normal pregnancy or the puerperium.
All authors found an increase of cellularity of the bone marrow during pregnancy, with the exception of Forsell who observed no variation from the non-pregnant normal.

Callender was the only author who subjected her studies to statistical analysis and this analysis failed to show significant hyperplasia of any cell type or a shift to the left of the red cell series during pregnancy or the puerperium. When, however, she studied hematoxylin and eosin sections obtained from the same marrow aspirates, she noted some increase of cellularity in the late weeks of pregnancy and in the early puerperium and also observed clusters of early nucleated red cells. Pitts and Packham found a general hyperplasia of the bone marrow during pregnancy which involved all cells equally. As previously noted, there was considerable hemodilution of the marrow and it is noteworthy that their stab and polymorphonuclear neutrophils, lymphocytes and disintegrated cells constituted ±75 per cent of all cells counted. Consequently, small quantitative changes of other marrow cells may have been masked. Hansen and Markoff found erythropoietic hyperplasia to be more marked than granulopoietic hyperplasia, whereas Daniachij and Pignoli observed the opposite. Several authors observed increased immaturity of granulopoiesis. Markoff, Hansen, Pignoli and Callender noted the presence of larger erythroblast precursors which were variably called macroblasts, macropronormoblasts or proerythroblasts. None of these cells, however, were thought to belong to the megaloblastic series. Most noted an increased anisocytosis and some observed asynchronism of the granulocytic precursors. Increase of eosinophilic granulocytes and decrease of lymphocytes were noted by the occasional author. Three authors noted an increase of plasma cells. Wolff and Limarzi found a striking increase of megakaryocytes during pregnancy and for some three months postpartum.

Only four of these authors commented on the appearance of the bone marrow after delivery. Markoff stated that it did not differ from the non-pregnant normal, but did not indicate at what stage postpartum these observations were made. Daniachij and Pignoli observed a partial but incomplete return of the marrow toward the non-pregnant normal at 6 and 10 days postpartum respectively. Wolff and Limarzi observed persistence of the marrow hyperplasia for three months postpartum.

In the course of our studies the marrows of three postpartum patients showed qualitative morphologic abnormalities not observed in the remainder of the cases, and consequently, were excluded from the normal series. All three patients were examined on the 4th or 5th day postpartum. They did not differ medically, obstetrically or hematologically from the other women studied except one patient whose mean corpuscular red cell volume was 97 cu. micra. All other hematologic determinations were well within the normal range.

The bone marrow of one of these patients showed an increase of basophilic red cell precursors to 4 per cent, a few intermediate megaloblasts, a few intermediate macrometamyelocytes and macrostabs on the fifth postpartum day. The bone marrow of the second patient contained 4.4 per cent basophilic normoblasts, and a very occasional intermediate macrometamyelocyte and macrostab; erythropoiesis was entirely normoblastic. The blood and bone marrow of the third pa-
tient were entirely normal for the early puerperium except for the presence of a very occasional intermediate macrometamyelocyte and intermediate macrostab in the bone marrow.

It is our belief that the marrow findings in these three patients represent the earliest transition toward megaloblastosis and that the development of megaloblastic anemia was prevented by termination of the pregnancies. These changes and their possible pathogenesis have been discussed in a previous paper.17

SUMMARY AND CONCLUSIONS

1. The morphologic findings of sternal bone marrow aspirates obtained from thirty healthy women during each of the three trimesters of normal pregnancy, from ten healthy women on each of the first eight puerperal days and from thirty healthy women at six weeks postpartum were compared with the findings of marrow aspirates obtained from 30 non-pregnant normal women of childbearing age.

2. Statistical analysis of the results of differential counts of these aspirates confirmed the impressions obtained from examination of the bone marrow aspirates and their differential smears.

3. Significant quantitative changes occurred in the cellular components of the bone marrow during normal pregnancy, which were maximal in the third trimester. There was a significant increase of normoblastic erythropoiesis and, to a lesser extent, of granulopoiesis during pregnancy, which was associated with an increase of immature cells (shift to the left) of both erythropoietic and of granulopoietic tissues.

4. During the first eight puerperal days the increase of erythropoiesis and of granulopoiesis diminished but did not return to normal non-pregnant normal values. At six weeks postpartum both erythropoiesis and granulopoiesis were more active than in the early puerperium and were significantly increased as compared with the findings in the marrows of normal non-pregnant women.

5. There was some increase of plasma cells and of reticulum cells in the bone marrow during pregnancy and the early puerperium.

6. No megaloblastic or macrogranulocytic changes were found in the two hundred bone marrow aspirates taken from pregnant and puerperal women or in the thirty aspirates taken from non-pregnant normal women.

7. The marrow findings of three women in the early puerperium were reported separately from the above groups. An occasional intermediate macrometamyelocyte was found in the marrows of these patients and in one a few nucleated red cells showed the earliest transitional changes toward megaloblasts. It is believed that these changes represent the earliest morphologic evidence of a deficiency of one or more of the growth factors necessary for normal hemopoiesis.

SUMMARIO IN INTERLINGUA

1. Le constatationes morphologic in aspiratos de medulla ossee del sterno obtenite ab trenta normal feminas durante cata un del tres trimestres de normal pregnantias, ab dece normal feminas a cata un del prime octo dies puerperal, e ab trenta normal feminas sex septimanas post parto eseva comparate con le constatationes in aspiratos de medulla obtenite ab trenta normal feminas non-gravide de etates fertile.
2. Le analyse statistic del risultatos de numeraciones differential in iste aspiratos confirmava le impressiones colligite in le examine del aspiratos de medulla ossee e de lor frottis differential.

3. Significative alteratiorie quantitative occurreva in le componentes cellular del medulla ossee in le curso de pregnantias normal. Illos attingeva lor maximos durante le tertie trimestre. Il occurreva un augmento significative del erythropoiese normoblastic e a grades minus extense del granulopoiese. Isto esseva associat con un augmento de cellulas immatur (deviation sinistrorse) in le tessutos erythropoietie e granulopoietic.

4. Durante le prime octo dies puerperal il augmento del erythropoiese e del granulopoiese redescendeva sed non retornava a normal valores non-pregnante. Sex septimanas post parto le erythropoiese e etiam le granulopoiese esseva plus active que durante le prime periodo puerperal. Illos mostrava augmentos significative in comparation con le constatationes in le medullas de normal feminas non-pregnante.

5. Occurreva un certe augmento de cellulas de plasma e de cellulas de reticulo in le medulla ossee durante le pregnantia e le prime periodo puerperal.

6. Nulle alteratiorie megaloblastic o macrogranulocytic esseva trovate in le duo centos aspiratos de medulla ossee obtenite ab feminas pregnanta e puerperal o in le trenta aspiratos obtenite ab normal feminas non-pregnante.

7. Le constatationes in le medulla de tres feminas durante le prime periodo puerperal esseva reportate separatamente. Sporadic macrometamitocytos intermediate esseva trovate in le medullas de iste patientes. In un de illas, un parve numero de nucleate erythrocytos mostrava le prime comeniciamento del alteratiories transitional verso le forma megaloblastic. Es stipulate que iste alteratiories representa le plus precoce signos morphologic de un carenia de un o plures del factores crescential que es necessari in le hematopoiese normal.

REFERENCES


Louis Lowenstein and Catherina A. Bramlage

The Bone Marrow in Pregnancy and the Puerperium

LOUIS LOWENSTEIN, CATHERINA A. BRAMLAGE and MADELEINE LALONDE