Blood Formation in the Pregnant Mouse

By George J. Fruhman

Pregnancy represents a period during which the demands for oxygen and nutrients by rapidly growing fetuses and their associated tissues might be expected to evoke erythropoietic changes in the mother. Peripheral blood studies, however, have proven to be of limited value in the assessment of such changes because animals often display a "physiologic anemia" as the result of a disproportionate increase in plasma volume compared to red blood cell volume. This occurs in many species including man, rabbit, and rat. Clearly, direct examinations of the blood-forming tissues should furnish more meaningful data than those obtained solely by examining the peripheral blood. In this regard, many clinical reports concerning bone marrow during pregnancy have appeared but these are so contradictory that Wintrobe, in discussing erythropoiesis during pregnancy, concluded that "It would appear that, under normal circumstances, no important changes take place." Surprisingly little attention has been paid to the status of blood-forming tissues during pregnancy in laboratory animals. However, a recently published abstract indicates, as does this paper, that increased erythropoiesis does occur in pregnant mice.

Methods

Adult, female nulliparous CF No. 1 mice (Carworth Inc., New City, N. Y.) 4–6 months old and weighing 25–28 gm. were caged individually. They received mouse pellets (Rockland mouse diet, A. E. Staley Mfg. Co., Decatur, Ill.) and water ad libitum. Mice were mated by pairing one adult male of the same strain with one female. Each female was examined in the morning and, if a vaginal copulation plug was present, she was considered to be 1-day pregnant and was separated from the male. Gestation time in the mice examined was 20–21 days and only mice with 8–11 fetuses were used for detailed studies. Mice were examined at several stages of pregnancy; some studies also were made of lactating mice, 7 days post partum.

Tail blood was used for micro-hematocrit and reticulocyte determinations. Reticulocytes were stained with new methylene blue. The ratio of staining solution to blood was approximately 4:1 and this mixture was allowed to remain for 10 minutes in a capillary tube (0.9–1.1 mm. inner diam.). It was then remixed and streaked directly from the capillary tube to the slide. Such air-dried specimens were cytologically superior to those prepared by making conventional smears. Plasma volume was determined by the Evans Blue (T1824) technique. Touch preparations of fresh spleen and tibial bone marrow were stained with a Wright-Giemsa mixture buffered at pH 6.6. Cellularity of tibial bone marrow was determined with the aid of an electronic particle counter.

Mice were injected subcutaneously with approximately 0.1 μc 59Fe citrate at 6–7 p.m. and...
BLOOD FORMATION IN THE PREGNANT MOUSE

Fig. 1.—Effects of pregnancy on body weight; \(^{59}\)Fe uptake in uterus, placentae, and fetuses; reticulocyte percentages; and hematocrit percentages. Each point represents an average of 6 mice. The broken horizontal line represents the mean value of a group of virgin mice. Vertical bars delineate ± 1 Standard Error of the Mean.

were decapitated 15 hours later. Radioiron uptake was assessed in (1) a pooled sample of both tibiae and femora, (2) spleen, and (3) the uterus and its contained placentae and fetuses. In order to compensate for the significant amounts of radioiron taken up by the fetuses and their associated tissues, the following formula was used to calculate the \(^{59}\)Fe uptake by the erythropoietic tissues.

\[
\frac{\% \text{ of injected dose in bone marrow or spleen}}{(\% \text{ of injected dose})} = \frac{\% \text{ of injected dose in bone marrow or spleen}}{100\% - \% \text{ of injected dose in uterus, placentae and fetuses}}
\]

Additional details of the methods used have been described previously.\(^6\) Samples of spleen and liver were fixed in an alcohol-formalin-acetic acid mixture, embedded in paraffin, sectioned at 8 \(\mu\), and stained with hematoxylin and eosin.

RESULTS

Body Weight and \(^{59}\)Fe in Uterus, Placentae and Fetuses

Total body weight was increased significantly at 6 days after insemination. The most marked changes, however, occurred between days 9 and 18 when the pregnant mice gained about 5 gms. per day (Fig. 1).

Concomitant examinations of the uterus and its contents revealed that \(^{59}\)Fe uptake in these tissues began to rise sharply after 9-12 days and reached a peak at 18 days post coitum (Fig. 1). During the final week of pregnancy, over 50 percent of the radioiron injected into the mother was found in the fetuses and their associated tissues and, of this fraction, up to 65 percent was found in the fetuses themselves.

Peripheral Blood

The hematocrit values fell as pregnancy progressed, going from 48 percent before pregnancy to less than 37 percent at parturition (Fig. 1). The steepest
Table 1.—Plasma Volume in Virgin and Pregnant Mice
(Means ± Standard Errors)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Mice</th>
<th>Plasma Vol., ml.</th>
</tr>
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<tbody>
<tr>
<td>Virgin</td>
<td>6</td>
<td>1.65 ± 0.045</td>
</tr>
<tr>
<td>Pregnant, 18–20 days</td>
<td>6</td>
<td>2.96 ± 0.081*</td>
</tr>
</tbody>
</table>

* p < 0.01

decline began after day 9 and coincided with the period when total body weight increased most rapidly. Seven days after parturition, hematocrit levels had risen to 41.5 ± 0.42%. Plasma volumes were measured on day 18 and compared with those of virgin mice. At this stage of pregnancy, the average plasma volume (Table 1) was almost 80 percent higher than that of the virgin mice.

Reticulocyte percentages rose sharply after day 6, remained high between days 9–15, and then fell to control levels (Fig. 1). At the height of the response, the average value rose to over 7 percent and consisted in great part of young reticulocytes as judged by the richness of their stained reticulum. Lactating mice, examined 7 days post partum, displayed a marked secondary reticulocytosis (6.8 ± 0.88%).

**Bone Marrow**

Nucleated erythroid cell percentages increased during pregnancy, reached a plateau after 9 to 12 days and then declined and remained near control levels during the remaining days of pregnancy (Fig. 2). Seven days after parturition, there was a second increase in the percentage of nucleated erythroid cells which then averaged 36 ± 2 percent. Quantitative tibial marrow counts (Table 2), taken in conjunction with the percentage values, indicated that the erythroid hyperplasia of pregnancy was real and was not merely the result of a shifting erythroid/myeloid ratio. Furthermore, the significant decrease in marrow cellularity seen on day 18 is evidence that the erythroid hypoplasia was greater than percentage values alone would indicate. Grossly, the marrow was yellow during the terminal stages of pregnancy in contrast to the normal pink appearance. More bone spicules than normal also were present. Although quantitative counts were not made, increased numbers of plasmocytes were noted in marrow prints taken from animals in the latter half of pregnancy and these cells were sometimes seen in clusters of 2 or 3.

This clearcut morphologic evidence of erythropoietic stimulation during pregnancy was not mirrored by corresponding ferrokinetic studies (Fig. 2). Thus at the height of erythroid hyperplasia, the 15-hr. radioiron uptake was actually subnormal. On the other hand, the erythroid hypoplasia noted during terminal stages of pregnancy was accompanied by 59Fe uptake values so low that in some cases they were barely discernible.

**Spleen**

Splenic weight increased steadily as pregnancy progressed, reached a high plateau after 12–15 days, and then decreased again (Fig. 2). Seven days after parturition, splenic weight averaged 130 ± 9 mg., which was significantly
Examination of liver sections did not reveal erythropoiesis in this site during pregnancy. Generally, splenic weight correlated well with the amount of splenic erythropoiesis present as assessed from sections, prints and radioiron uptake data. Erythroid hyperplasia was most marked at 12-15 days of pregnancy. In histological sections, the red pulp appeared to be filled with large clusters of nucleated erythroid cells. In many areas, the cells of the red pulp impinged upon the white pulp and even appeared to invade the white pulp. Examination of spleen prints confirmed the erythroid nature of the red pulp response; erythroid cells were seen in all stages of development. Splenic involution occurred between day 15 and the time of parturition, and a significant loss of splenic weight was attributable, in large measure, to depletion of the red pulp. Scattered islands of nucleated erythroid cells remained and were composed primarily of late normoblasts with pyknotic nuclei. Relative numbers of neutrophilic and eosinophilic myelocytes and plasmocytes were increased during the third third of pregnancy and abnormally large concentrations of megakaryocytes occurred in some areas of the red pulp. Inasmuch as the splenic mass was shrinking at this time, the increases in these cells may have been more apparent than real; no measurements were made of their absolute numbers.

Radioiron uptake by the spleen rose during the first 15 days of pregnancy and then fell during the remaining days (Fig. 2), paralleling the changes in spleen weight and degree of erythropoiesis.

**Liver**

Examination of liver sections did not reveal erythropoiesis in this site during pregnancy.

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**Fig. 2.**—Effects of pregnancy on $^{59}$Fe uptake in spleen and bone marrow, numbers of marrow erythroid cells and spleen weight. Each point represents an average of 6 mice. The broken horizontal line represents the mean value for a group of virgin mice. Vertical bars delineate ±1 Standard Error of the Mean. Changes in $^{59}$Fe uptake in spleen and bone marrow have been corrected to compensate for uptake by the fetuses and associated tissues. See text for details.
Table 2.—Cellularity of Tibial Marrow in Female CF #1 Mice (Means ± Standard Errors)

<table>
<thead>
<tr>
<th>Days Pregnant</th>
<th>No. of Mice</th>
<th>Nucleated cells harvested, millions</th>
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<tbody>
<tr>
<td>1–3</td>
<td>6</td>
<td>11.4 ± 0.27</td>
</tr>
<tr>
<td>6–9</td>
<td>6</td>
<td>12.0 ± 0.47</td>
</tr>
<tr>
<td>15–17</td>
<td>6</td>
<td>10.1 ± 1.04</td>
</tr>
<tr>
<td>18–21</td>
<td>6</td>
<td>8.8 ± 0.56*</td>
</tr>
<tr>
<td>7 Days post partum</td>
<td>6</td>
<td>14.8 ± 0.34</td>
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*p < .01 when compared to mice 1-3 days pregnant.

pregnancy. An unexpected observation was the appearance, from mid-pregnancy to parturition, of scattered mitotic figures among the hepatocytes.

Additional Observations

Some mice which had vaginal copulation plugs and were, therefore, judged to have been inseminated did not develop fetuses. In these animals, there was no increase in either splenic or bone marrow erythropoiesis; there was no decrease in hematocrit or increase in reticulocytes.

In general, those mice bearing fewer than normal numbers of fetuses showed significantly less erythropoietic stimulation than did those with larger litters.

Discussion

Marked increases in erythropoiesis occurred in pregnant mice during the first 15 days of gestation and this was followed by an equally significant drop to normal or below normal levels during the last 4 or 5 days of pregnancy. The spleen played a key role in these changes, and this is in agreement with a recent report of Fowler and Nash. Following radiation-induced destruction of the bone marrow, the spleen functions as an important erythropoietic organ. More recently, a disassociation between the marrow and the splenic responses to radiation also has been described in which rats displayed massive splenic erythropoiesis which was not accompanied by bone marrow recovery. The spleen also responds sensitively to more subtle, physiologic stimuli. For example, when starved mice were refed, it was the spleen rather than the bone marrow which displayed the more rapid and larger erythropoietic response. Similarly, splenic erythropoiesis was heightened during estrus in mice at a time when comparable changes were undetectable in the marrow. Taken in conjunction with the present work, these findings are compatible with the idea that the spleen may be the primary organ of erythropoietic homeostasis in the mouse.

While the most striking erythropoietic changes were noted in the spleen, the marrow displayed somewhat similar morphologic changes. It should be noted, however, that an apparent discrepancy between morphologic and ferrokinetic results occurred. During mid-pregnancy, Fe uptake by the marrow fell at times when the numbers of nucleated erythrocytes rose, and the reasons for this are not immediately apparent. One factor may be related to the pre-
BLOOD FORMATION IN THE PREGNANT MOUSE

Carious state of iron balance during pregnancy. It has been reported that iron deficiency will cause $^{59}$Fe to be incorporated into marrow earlier than normal and this mechanism may have been operative in the present experiments. On the other hand, it also appears possible that a static, morphologic evaluation of marrow cellularity may not be an accurate index of erythroid cell proliferation. Further studies will be required to resolve this problem.

No histologic evidence of extramedullary erythropoiesis in the liver was seen. An unexpected finding was the presence of mitosing hepatocytes. This may be referable to an increase in circulating sex hormones. In this regard, Allan reported that estradiol injections increased the incidence of binucleated cells in rabbit liver and that combinations of estrogen and progesterone potentiated this effect.

Despite the numerous hormonal influences on blood formation, it is possible that the oxygen requirement of the fetuses and their associated tissues may be the primary determinant of the erythropoietic changes in the pregnant mouse. Investigations in other species, including sheep and rabbit, indicate the rapidity with which increasing amounts of oxygen are diverted to the growing fetuses. In mice, bearing as many as 11 fetuses, it is possible to envision that this could cause hypoxia in the mother and thus lead to increased erythropoiesis. Both erythropoiesis and circulating reticulocyte numbers fell after 15 days of gestation in the face of a dropping hematocrit. If oxygen levels are of primary importance, one should expect to see a reversal of maternal hypoxia during this phase of gestation. Barcroft's data on a small number of sheep indicate that the rate at which fetal oxygen consumption increased began to slacken toward the end of pregnancy. The occurrence of a comparable slowing of fetal oxygen consumption in mice could cause an abatement of the maternal hypoxia and lead to a decline in erythropoiesis. This hypothesis follows the well-accepted idea that hypoxia is the fundamental stimulus for erythropoiesis and that it acts through a humoral factor, erythropoietin, that is produced either in or with the help of the kidneys.

SUMMARY

Pregnancy in mice was accompanied by increased erythropoiesis which was most marked in the spleen and was reflected peripherally by increased percentages of circulating erythrocytes. During the last four or five days of pregnancy, there was an equally significant drop in erythropoiesis to normal or below normal values and this occurred despite a falling hematocrit. It is suggested that the changing oxygen requirement of the fetuses may be the primary determinant of these erythropoietic changes.

SUMMARIO IN INTERLINGUA

Pregnantia in muses esseva accompaniate de un augmento del erythropoiese. Isto esseva le plus marcate in le splen. Periphericamente illo esseva reflectite per un augmento del procentage de erythrocytos in le circulation. Durante le ultime quatro o cinque dies del pregnantia, il occurreva un similemente significative declino del erythropoiese usque ad o mesmo ad infra valores normal. Isto occurreva in especto de un descendente hematocrite. Es formulate le these que le variationes in le requirementes de oxygeno del parte del fetos es le determinante primari in iste alterationes erythropoietic.
ACKNOWLEDGMENT

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REFERENCES

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