HEMODILUTION AND IRON METABOLISM
IN RATS DURING PREGNANCY

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Studies were made on the cellular components of the blood, the serum iron concentration, and the circulating blood and plasma volumes of rats in pregnancy and on the iron transport from the maternal blood to the conception products (uterus and its contents) using radioactive iron. Anemia of pregnancy developed and was most pronounced during late pregnancy when the conception products developed rapidly. The reticulocyte count did not increase significantly, so that there is no evidence for stimulation of hematopoiesis during pregnancy of rats. The circulating blood and plasma volumes increased significantly during late pregnancy, but the total hemoglobin and red cell volume did not increase.

The anemia in pregnancy of rats was due to hemodilution, but this hemodilution occurred in different grades in comparison with human pregnancy. The iron concentration of the serum decreased most during late pregnancy when development of the conception products was greatest and placental transport of iron to the fetus was largest.

It is now agreed that there are two main factors causing anemia in human pregnancy:

(1) An increase in plasma volume that outstrips the simultaneous increase in red-cell mass.

(2) Nutrient deficiency, especially, deficiency of iron and folic acid due to the increased demands of the fetus and of the pregnant woman and to impaired absorption during pregnancy.

Survey studies have shown that pregnancy wastage or anemia is much greater in socioeconomically less privileged groups and that maternal iron and folic acid deficiencies are much more prevalent in those populations (J).

In the laboratory experiments, anemia of pregnancy has been described in a number of widely divergent species of mammals. In general it has been con-
cluded that this anemia is due to a hemodilution, although the etiologic factors responsible for it remain unknown.

The works of Mitchell and Miller (2), Beard and Myers (3), and Van Donk et al. (4) on rats indicated that this anemia is not due to a lack of nutritional factors. Witten and Bradbury (5) reported hemodilution and decrease in the blood cell count in women after treatment with estrogen. Zarrow and Zarrow (6) demonstrated that treatment of rabbits with an ovarian extract produced a definite retention of water and anemia similar to that noted during pregnancy.

Newcomer (7) indicated that the placenta was the source of the factor responsible for the anemia of pregnancy in rats. Bond (8) measured the blood volume of pregnant rats and reported that the anemia was due to hemodilution, but he did not mention changes of blood constituents in relation to the development of conception products.

In this study anemia in pregnancy was examined in relation to the development of conception products and iron transport into conception products in rats. For this purpose we examined changes in the blood and plasma volumes and incorporation of radioactive iron (59Fe) into the conception products in relation to those weights during pregnancy in the rat.

MATERIALS AND METHODS

Female Wistar rats weighing 200 to 260 g were used. Rats were given laboratory stock diet and water ad libitum, and pregnant rats were housed in individual cages at 24°C. The beginning of gestation was taken as the time when spermatozoa were found in vaginal smears.

The red cell count, hemoglobin concentration, and hematocrit value were measured by standard methods. Reticulocyte counts were made on 2,000 red cells on wet preparations stained with brilliant cresyl blue and were expressed as percentages of red cells.

The color index of erythrocytes was calculated from the following formula (8):

$$\frac{g \text{ hemoglobin/dl blood of test rat}}{g \text{ hemoglobin/dl blood of normal rats}} \div \frac{\text{red cell count of test rat}}{\text{red cell count of normal rats}}$$

The average values of nonpregnant rats were taken as normal values.

The serum iron was determined by the o-phenanthroline method (9).

The plasma volume was measured by the dye-dilution method using T-1824 (10). The blood volume was calculated from the plasma volume and the hematocrit value. The total circulating hemoglobin (T-Hb) was calculated as the blood volume multiplied by the concentration of hemoglobin in the blood. The red cell volume (RCV) was calculated as the blood volume multiplied by the hematocrit value, but no correction was made by the ratio of the mean body hematocrit to that of the sample blood. Blood samples were taken by heart
puncture through the skin of the chest of rats under Nembutal anesthesia. For preparation of $^{59}$Fe-labeled plasma, pooled plasma taken from normal rats was incubated with $^{59}$Ferric citrate (specific activity 100 $\mu$Ci/$\mu$gFe, Dainabot, Tokyo) for 30 min at room temperature. Three days before the sacrifice of rats 0.4 ml (4 $\mu$Ci)/100 g of $^{59}$Fe-labeled plasma was injected into a tail vein under ether anesthesia. Animals were sacrificed by withdrawing blood from the heart as described above. Then the conception products (uterus and its contents) were rapidly removed and weighed.

The radioactivity (cpm) was counted in a well-type scintillation counter (Autogamma Spectrometer, Packard, Series, 5,000, U.S.A.).

Experiments on pregnant rats were made from day 9 to day 21 of gestation. The stage of gestation was classified as follows: Early pregnancy, from day 9 to day 12 of gestation, when the conception products are still small. Mid-pregnancy, from day 13 to day 17, when the conception products are moderate sized. Late pregnancy, from day 18 to term of gestation, when the conception products are well developed.

RESULTS

The conception products increased in weight very rapidly during late pregnancy. The mean weight of conception products at term was 60 g, which corresponded actually to the weight increase between days 9 and 21. Seventy percent of this increase occurred in the last 4 days of gestation, and one-third of the weight increase of total conception products was achieved in the last 24 hr of gestation (Fig. 1).

![Fig. 1](image)

Table 1. Body weight and conception products of pregnant rats.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Body weight (g)</th>
<th>Net body weight (g)</th>
<th>Conception products (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>200.4±4.0</td>
<td>216.1±4.1</td>
<td>0.73±0.30</td>
</tr>
<tr>
<td>Early pregnancy</td>
<td>217.1±4.1</td>
<td>213.3±4.8</td>
<td>8.05±1.10</td>
</tr>
<tr>
<td>Mid-pregnancy</td>
<td>221.4±5.0</td>
<td>220.5±3.0</td>
<td>38.21±3.37</td>
</tr>
<tr>
<td>Late pregnancy</td>
<td>255.6±5.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The red cell count, hemoglobin concentration, and hematocrit value gradually decreased during pregnancy, and especially during late pregnancy. These values may fall temporarily near the end of early pregnancy (Fig. 2).

Hematologic results on nonpregnant rats and rats at various stages of pregnancy are summarized in Table 2. Reductions in the red cell count, hemoglobin concentration, and hematocrit value during late pregnancy were 18.2% ($P<0.001$), 20% ($P<0.001$), and 20% ($P<0.001$), respectively, and moderate but significant
Fig. 1. Changes in weight of conception products and incorporation of $^{59}$Fe into maternal blood and the conception products. Points are mean values. Vertical bars are standard error of the mean above and below the mean values.

○—○ cpm of maternal blood, •—• cpm of conception products △—△ weight of conception products.

reductions of these values were also observed during early pregnancy ($P < 0.05$–$P < 0.001$).

The reticulocyte counts during pregnancy were not significantly different from those of nonpregnant rats. The color index (CI) of erythrocytes also did not change during pregnancy. The average serum iron concentration increased during early pregnancy but was not significantly different from that of nonpregnant rats. During late pregnancy it decreased to 37% of the value of nonpregnant rats ($P < 0.001$).

The circulating blood and plasma volumes increased during late pregnancy by 18.5% ($P < 0.001$) and 32.7% ($P < 0.001$), respectively. When calculated on the basis of the net body weight (total body weight minus weight of conception products), the increase of the circulating blood volume and plasma volume in
Fig. 2. Changes in red cell count, hemoglobin concentration, hematocrit, and serum iron during pregnancy. Points are mean values. Vertical bars are standard error of the mean above and below the mean values.

Table 2. Hematologic data

Values are means ± S.E. Numbers in parentheses are numbers of rats examined. Level of significance of difference from values of nonpregnant rats: a P<0.05, b P<0.01, c P<0.001.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Red cells (10⁶/mm³)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
<th>Reticulocytes (%)</th>
<th>C.I.</th>
<th>Serum iron (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>749 ± 16</td>
<td>13.5 ± 0.2</td>
<td>39.1 ± 0.7</td>
<td>2.7 ± 0.2</td>
<td>1.00</td>
<td>137 ± 15</td>
</tr>
<tr>
<td>Early pregnancy</td>
<td>683a ± 19</td>
<td>12.2b ± 0.2</td>
<td>34.9c ± 0.5</td>
<td>2.9 ± 0.3</td>
<td>0.98</td>
<td>171 ± 24</td>
</tr>
<tr>
<td>Mid-pregnancy</td>
<td>703 ± 21</td>
<td>12.5b ± 0.3</td>
<td>36.4a ± 0.9</td>
<td>3.5 ± 0.4</td>
<td>0.99</td>
<td>111 ± 13</td>
</tr>
<tr>
<td>Late pregnancy</td>
<td>613c ± 15</td>
<td>10.8c ± 0.5</td>
<td>31.0c ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>0.97</td>
<td>51c ± 8</td>
</tr>
</tbody>
</table>
Fig. 3. Changes in circulating blood and plasma volumes, total hemoglobin and red cell volume in pregnancy. Points are average values, calculated on the basis of the net body weight. For details, see text. Vertical bars are standard error of the mean above and below the mean values.

Table 3. Circulating blood and plasma volumes.

Values are means ± S.E. Numbers in parentheses indicate numbers of rats examined. C.B.V.: circulating blood volume, C.P.V.: circulating plasma volume. Net body weight means body weight minus weight of conception products. For explanation of symbols, see Table 2.

<table>
<thead>
<tr>
<th>Stage</th>
<th>C.B.V. (ml)</th>
<th>C.B.V./net body weight (ml/100 g)</th>
<th>C.P.V. (ml)</th>
<th>C.P.V./net body weight (ml/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>16.3</td>
<td>±0.3</td>
<td>9.9</td>
<td>±0.2</td>
</tr>
<tr>
<td>(8)</td>
<td></td>
<td>±0.3</td>
<td></td>
<td>±0.2</td>
</tr>
<tr>
<td>Early pregnancy</td>
<td>18.1</td>
<td>±0.8</td>
<td>11.7</td>
<td>±0.5</td>
</tr>
<tr>
<td>(13)</td>
<td></td>
<td>±0.8</td>
<td></td>
<td>±0.2</td>
</tr>
<tr>
<td>Mid-pregnancy</td>
<td>17.2</td>
<td>±0.9</td>
<td>10.9</td>
<td>±0.3</td>
</tr>
<tr>
<td>(9)</td>
<td></td>
<td>±0.9</td>
<td></td>
<td>±0.3</td>
</tr>
<tr>
<td>Late pregnancy</td>
<td>20.9a</td>
<td>±0.7</td>
<td>14.4c</td>
<td>±0.5</td>
</tr>
<tr>
<td>(20)</td>
<td></td>
<td>±0.7</td>
<td></td>
<td>±0.2</td>
</tr>
</tbody>
</table>

pregnancy were 11.8% (P<0.01) and 13.3% (P<0.001), respectively (Fig. 3 and Table 3). The total hemoglobin and red cell volume remained almost constant throughout pregnancy (Fig. 2 and Table 4). Incorporation of $^{59}$Fe into the conception products increased in proportion to their rapid developments, and total count of $^{59}$Fe in maternal blood decreased markedly in late pregnancy (Fig. 1).
DISCUSSION

Our experiments showed a decrease in the cell contents of the blood during pregnancy of rats. Anemia was most pronounced during late pregnancy. The erythrocyte count showed an 18% decrease from an average of nonpregnant animals, i.e., 7.47 million/mm³ to 6.13 million/mm³. This was paralleled with a concomitant decrease in the concentration of hemoglobin (20%), and hematocrit value (21%) (Table 2). The anemia is normochromic. The fact that erythrocyte count decreased in parallel with the hemoglobin content is also an indication of a lack of change in the color index during pregnancy.

The reticulocyte count was not found to increase markedly, in agreement with the report of Van Donk et al. (4), while it cannot be definitely concluded that hematopoiesis increased during pregnancy.

This fact is interesting in contrast to human pregnancy, where the reticulocyte count is reported to increase significantly during the third trimester of pregnancy (11). In late pregnancy the circulating blood and plasma volumes of rats increased by 11.8% and 13.3%, respectively, on the basis of the net body weight.

With pregnant women, larger increases in the blood volume (23%) and plasma volume (25-49%) have been reported (11, 12). Bond (8) reported a marked increase in the blood volume of rats during pregnancy. However, when he calculated the blood volume on the basis of the total body weight, including that of the uterus and its contents, he found that it did not change during pregnancy. We calculated the blood volume on the basis of the net body weight (i.e., total body weight minus weight of the uterus and its contents). It seems better to use this basis for calculating the blood volume in pregnant animals, because the dye (T-1824) is only found in the maternal circulation and does not pass through the placenta (13).
The total hemoglobin and red cell volume did not change during the pregnancy of rats (Table 4). These results show that development of anemia in rats during late pregnancy is due to hemodilution.

However, the characteristics of this anemia differ somewhat from those reported in pregnant women. In pregnant women, the total hemoglobin and red cell volume during the third trimester were reported to increase by 11% and 19%, respectively, as compared with controls (11).

Thus, in women anemia may largely be due to hemodilution which masks a concomitant increase in the cell content of the blood. In rats, hydremia may be less, but the increase in the plasma volume is not accompanied by an increase in the cell content, thus presenting anemia. Observations similar to those in rats have been reported in rabbits (14). In cows, increases in the blood and plasma volumes during pregnancy are not accompanied by a relative anemia (13).

These species differences in anemias of pregnancy may be caused by difference in hematopoietic capacity among different kinds of animals.

Hematopoiesis during pregnancy must also be considered in relation to iron metabolism. In this work, the iron content of the diet was sufficient to maintain normal hematopoiesis (ca. 50 mg/kg body weight/day).

Van Donk et al. (4) found that anemia of pregnancy in rats could not be corrected by additions of iron, copper, and other minerals to the diet. In our experiments, it was observed that incorporation of $^{59}$Fe into the placenta and fetus of rats increased markedly during late pregnancy (Fig. 1). Development of conception products was also very rapid during late pregnancy. Thus incorporation of iron may be accelerated by fetal and placental development at this stage.

The decrease in the serum iron concentration and $^{59}$Fe uptake in maternal blood during late pregnancy observed in this work can be attributed to increased iron transport to the placenta and fetus.

As the reticulocyte count did not increase even in the late stage of pregnancy when the serum iron concentration decreased significantly, these changes of iron metabolism during pregnancy do not stimulate reticulocytosis, as observed in human pregnancy. Thus the total hemoglobin and red cell volume did not increase during late pregnancy in rats, and the main cause of anemia in pregnant rats is not a decrease of iron in maternal blood.

REFERENCES

ANEMIA IN RATS DURING PREGNANCY

6) Zarrow, M. X. and Zarrow, I. G., Endocrinol., 52, 424 (1953).