Effect of Different Intensities of Iron-Deficient Anemia in Pregnant Rats on Maternal Tissue Iron and Fetal Development

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Summary Effect of the different intensities of iron-deficient anemia in pregnant rats on the maternal tissue iron and the fetal development was investigated.

The different intensities of iron deficiency were produced by changing period of feeding on the iron depleted diet (0.38 mg/100 g diet) prior to gestation. The anemic rats were divided into three groups with the hemoglobin levels of 12, 10 and 8 g/100 ml on the first day of gestation. Then, rats of each group were fed on the iron adequate and on the depleted diets during gestation. The whole body weights of the three deficient animals were lower than those of the corresponding controls on day-21 of gestation. Food intakes of the three deficient groups tended to be lower than those of corresponding controls. The values of hemoglobin (Hb), hematocrit (Ht) and red blood cells (RBC) decreased with the progress of pregnancy, and the hypochromic anemia was observed in all deficient animals. Iron contents of various tissues and the ratio of ferritin iron to total iron in liver and spleen of each deficient group were also apparently lower than those of corresponding controls. The numbers of placenta of the deficient groups were similar to those of corresponding controls. The litter size of severe anemic-animals was less than those of light and moderate anemic-animals, and the average body weight of fetus in severe anemic-animals was much lower than those of other groups. These results suggested that a higher severity of anemia in the mother at the beginning of pregnancy may result in a more frequent resorption of the fetus but the anemic status did not affect the ability of gestation itself.

Key Words iron deficiency, anemia, hemoglobin, pregnant rats, tissue iron, fetal development, resorption

The Committee on Animal Nutrition of National Research Council (NRC) (1) proposed that the iron requirement for growth and maximal hemoglobin level in the rat is about 35 mg Fe/kg diet but did not indicate the requirement for reproduction.

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Some investigators have indicated the different requirement for pregnant rats. McCall et al. (2) found that a level of 240 mg Fe/kg diet was needed during pregnancy and lactation. However, Ahlstrom and Jantti (3) indicated that fetal resorptions were observed in rats fed on 198 mg Fe/kg diet during gestation, and suggested that the level of 198 mg/kg diet was excessive. On the other hand, Lin and Kirksey (4) indicated that the iron requirement for young pregnant rats was different from that for adult pregnant rats, and the requirement for young pregnant and adult pregnant rats was 50–250 and 10–50 mg/kg diet, respectively. They also found that fetal development was impaired by maternal iron deficiency. Depletion of iron stores has also been reported to occur at normal pregnancy in the rats due to the transfer of large quantities of iron from maternal stores to the fetus during the final 6 days of gestation (5, 6). Murry and Stein indicated that fetus obtained more iron from maternal absorption with diminishing iron stores during pregnancy (7).

In a previous paper, we showed that when the iron intake of the dams was restricted only during gestation, the fetal weight gain was normal (8). These findings strongly suggest that maternal iron store at the beginning of pregnancy may play a significant role in the development of fetus. However, the effect of intensity of iron deficiency before pregnancy on the fetal development was unknown.

This study was designed to investigate the relationship between the intensity of maternal iron deficient anemia and fetal development by measuring hematological parameters, iron contents in serum and tissues in dams, litter size and fetus weight.

MATERIALS AND METHODS

Treatment of animals. Three groups of female Sprague-Dawley rats were used, each consisting of 12 rats aged 5 weeks for group-I and 4 weeks for group-II and -III.

Rats of the group-I, -II and -III were fed on iron depleted diet (0.38 mg Fe/100 g diet) for 3, 4 and 5 weeks respectively as shown in Fig. 1. The group-I, -II and

| Groups | Animals     | Period | Mating | Experimental groups                      |
|--------|-------------|--------|--------|-----------------------------------------|
| I      | 5 weeks rats| 3 weeks| Mating | Iron adequate diet (Control I)           |
|        |             |        |        | Iron depleted diet (Deficiency I)        |
| II     | 4 weeks rats| 4 weeks| Mating | Iron adequate diet (Control II)          |
|        |             |        |        | Iron depleted diet (Deficiency II)       |
| III    | 4 weeks rats| 5 weeks| Mating | Iron adequate diet (Control III)         |
|        |             |        |        | Iron depleted diet (Deficiency III)      |

Fig. 1. Experimental schedule.
Table 1. Composition of experimental diets.

| Ingredients          | Control* (g/kg) | Iron depleted* (g/kg) |
|----------------------|-----------------|-----------------------|
| Casein               | 150             | 150                   |
| α-Corn starch        | 68              | 68                    |
| Sucrose              | 650             | 650                   |
| Soybean oil          | 50              | 50                    |
| Salt mixture         | 50^b            | 50^c                  |
| Cellulose powder     | 20              | 20                    |
| Vitamine mixture     | 10              | 10                    |
| Choline chloride     | 2               | 2                     |

*Iron contents in control and depleted diets were 4.10 and 0.38 mg/100 g diet, respectively. ^b Harper, A. E. (1959): J. Nutr., 68, 405–418. ^c Ferric citrate was omitted from Harper’s salt mixture.*

-III were designated light, moderate and severe anemic-rats, respectively. Then, the virgin rats weighing 175 to 190 g were mated with males of the same strain. The day of insemination was determined by daily examination of vaginal smears for spermatozoa. The day observed sperm is called the day-1 of gestation. Each group was divided into two groups of which 6 rats were continued to be fed on the iron depleted diet and another 6 rats were fed on the control diet containing adequate amount of iron as shown in Table 1. All rats were maintained with diet and iron-free deionized water ad libitum throughout the experimental period.

*Analytical procedure.* A measured quantity of blood was taken from the tail vein on day-1, -12 and -21 of gestation. The Hb level was measured by the method of cyanmethemoglobin, and Ht and RBC was estimated with a high speed microhematocrit centrifuge and a Microcellcounter (Toa Medical Electr. Co.), respectively. Mean corpuscular hemoglobin concentration (MCHC) was calculated from these values. The rats were killed by decapitation on day-21 of gestation after fasting for 24 h. The uteri were opened, the fetal membranes were divided and the umbilical vessels were severed. The numbers of fetus and placentae were counted. The fetus and the combined placentae with fetal membranes from each litter were weighed, and stored together as a pool. The maternal tissues such as liver, spleen and kidney were removed and weighed, also skeletal muscle (thigh muscle), intestine and cerebrum were removed. The liver was perfused through the portal vein with cold 0.9% NaCl solution before isolation. For the estimation of dietary iron an aliquot amount of sample (10 g) was dry-ashed under 600°C overnight in the electric heated oven.

For the estimation of tissue iron samples were wet-ashed with nitric acid. The iron contents of these samples were determined atomic-spectrophotometrically. The plasma iron was estimated by the method of the International Committee for
Table 2. Body weight gain and food intake in iron deficient rats.

| Groups          | Day 1  | Day 6  | Day 12 | Day 15 | Day 18 | Day 20 | Day 1–15 (g/day) | Day 16–20 (g/day) |
|-----------------|--------|--------|--------|--------|--------|--------|------------------|------------------|
| Control I       | 188 ± 3.0 | 208 ± 4.9 | 235 ± 7.0 | 250 ± 6.7 | 276 ± 7.3 | 306 ± 7.7 | 16.1 ± 0.71   | 18.8 ± 0.52     |
| Deficiency I    | 190 ± 2.9 | 211 ± 4.4 | 247 ± 3.9 | 251 ± 4.3 | 270 ± 4.5 | 281 ± 5.4 | 16.8 ± 1.01   | 18.3 ± 0.41     |
| Control II      | 180 ± 6.8 | 197 ± 6.0 | 219 ± 8.8 | 239 ± 14.0 | 262 ± 16.1 | 286 ± 16.1 | 16.6 ± 0.69   | 18.1 ± 0.30     |
| Deficiency II   | 184 ± 4.8 | 192 ± 5.2 | 217 ± 6.7 | 234 ± 6.9 | 250 ± 6.6 | 268 ± 7.9 | 15.3 ± 0.60   | 17.0 ± 0.37     |
| Control III     | 177 ± 4.6 | 188 ± 3.8 | 209 ± 8.2 | 225 ± 9.8 | 246 ± 12.4 | 261 ± 14.0 | 12.9 ± 0.65   | 17.8 ± 1.68     |
| Deficiency III  | 180 ± 3.8 | 194 ± 4.5 | 216 ± 4.6 | 226 ± 4.6 | 244 ± 5.8 | 250 ± 7.7 | 13.5 ± 0.25   | 16.7 ± 0.65     |

Each value represents M ± SE for 6 rats.
Table 3. Changes in hemoglobin, hematocrit and red blood cells in iron-depleted pregnant rats.

| Groups       | Day 1          | Day 12         | Day 21          |
|--------------|----------------|----------------|-----------------|
|              | Hb (g/100 ml)  | Ht (%)         | RBC (×10^6/ mm³) | Hb (g/100 ml)  | Ht (%)         | RBC (×10^6/ mm³) | Hb (g/100 ml)  | Ht (%)         | RBC (×10^6/ mm³) |
| Control I    | 12.2 ± 0.50    | 38.5 ± 0.6     | 648 ± 17        | 13.3 ± 0.40     | 42.5 ± 1.3     | 714 ± 17        | 12.3 ± 0.40    | 36.6 ± 0.9     | 639 ± 13        |
| Deficiency I | 11.9 ± 0.63    | 37.3 ± 1.3     | 692 ± 17        | 10.5 ± 0.33*    | 33.0 ± 0.6**   | 628 ± 23**      | 6.9 ± 0.21**   | 28.0 ± 1.2**   | 505 ± 26**      |
| Control II   | 10.6 ± 0.07    | 33.6 ± 0.6     | 563 ± 5         | 14.2 ± 0.60     | 44.8 ± 0.6     | 653 ± 26        | 12.7 ± 0.81    | 40.2 ± 1.4     | 611 ± 36        |
| Deficiency II| 10.7 ± 0.21    | 34.1 ± 0.3     | 557 ± 4         | 8.9 ± 0.38**    | 27.2 ± 1.4**   | 407 ± 16**      | 6.7 ± 0.40**   | 23.5 ± 1.3**   | 340 ± 18**      |
| Control III  | 7.5 ± 0.41     | 30.0 ± 1.4     | 440 ± 23        | 13.5 ± 0.41     | 44.7 ± 0.8     | 705 ± 24        | 11.8 ± 0.59    | 39.3 ± 1.5     | 619 ± 31        |
| Deficiency III| 8.6 ± 0.50    | 31.8 ± 2.1     | 466 ± 29        | 6.9 ± 0.38**    | 25.0 ± 1.1**   | 382 ± 21**      | 4.7 ± 0.42**   | 18.7 ± 1.1**   | 259 ± 21**      |

Each value represents M ± SE for 6 rats. Significantly different from respective control group, *p < 0.05, **p < 0.01.
Standardization in Hematology (ICSH)(9) modified by Matsubara using the detergent and reducing agent. The details of estimation were as follows. An aliquot of plasma (0.5 ml) was added to 2.0 ml of the reducing solution, in which 0.3 g sodium laurylsulfate and 0.5 ml thioglycolic acid were dissolved in 100 ml of 0.2 M acetate buffer, pH 4.1, and then 0.05 ml of bathophenanthroline sulfonic acid solution (1 g/100 ml) was added. The absorbance at 535 nm was read. Total iron binding capacity (TIBC) was estimated by the method of Ramsay (10). Ferritin iron in liver and spleen was separated by the method of Drysdale and Ramsay (11), and the supernatant containing ferritin iron was heated at 75°C for 10 min to remove contaminated hemoglobin. Iron concentration in the supernatant was determined atomic-spectrophotometrically.

RESULTS

As shown in Table 2, no significant difference was observed in the body weight between control and deficient animals of group-I, -II and -III until day-15 of gestation, but thereafter the weight of the deficient rats in each group tended to be slightly lower than that of the control animals. The food intake was not significantly different from each other.

Tables 3 and 4 showed the hematological data. The values of Hb, Ht, RBC and MCHC of deficient animals in each group were markedly lower than those of controls throughout the gestation period. The values of Hb, Ht and RBC of control groups restored to almost normal levels on day-12 of gestation, but again lowered in the late pregnancy. The decrease of MCHC in deficient groups suggests that the hypochromic and typical iron deficient anemia occurred. No significant difference between the control and deficient animals in the three groups was observed in weight of tissues such as liver, spleen and kidney. The serum iron contents and

| Groups       | MCHC (%)          |
|--------------|-------------------|
|              | Day 1     | Day 12    | Day 21    |
| Control I    | 31.7 ± 0.72 | 31.3 ± 0.60 | 33.6 ± 0.40 |
| Deficiency I | 31.9 ± 0.79 | 31.7 ± 1.06 | 24.6 ± 0.97* |
| Control II   | 31.5 ± 0.84 | 31.7 ± 1.15 | 31.6 ± 1.00 |
| Deficiency II| 31.4 ± 0.38 | 32.5 ± 1.32 | 28.5 ± 0.81** |
| Control III  | 25.0 ± 0.57 | 30.3 ± 0.85 | 30.0 ± 0.74 |
| Deficiency III| 27.0 ± 1.19| 27.5 ± 0.70** | 25.1 ± 0.82* |

Each value represents M ± SE for 6 rats. Significantly different from respective control group, *p < 0.01, **p < 0.05.
Table 5. Serum iron concentration and iron content in various tissues in pregnant rats fed an iron depleted diet.

| Groups      | Serum | Liver | Spleen |
|-------------|-------|-------|--------|
|             | Iron  | TIBC* | Saturation | Total-Fe (µg/wet tissue wt.) | FN-Fe* | FN-Fe/T.Fe (%) | Total-Fe (µg/g tissue wt.) | FN-Fe* | FN-Fe/T.Fe (%) |
| Control I   | 56.3 ± 6.8 | 431.6 ± 38.5 | 13.0 | 519.7 ± 69.0 | 203.1 ± 21.9 | 39.1 | 211.0 ± 19.1 | 64.2 ± 4.5 | 30.2 |
| Deficiency I| 27.9 ± 4.9* | 735.5 ± 39.5* | 3.9 | 383.0 ± 30.0* | 58.1 ± 3.0* | 16.2 | 132.2 ± 14.5* | 18.0 ± 0.8* | 13.1 |
| Control II  | 56.9 ± 8.3 | 401.4 ± 35.6 | 14.0 | 498.4 ± 39.1 | 180.3 ± 20.2 | 36.0 | 192.1 ± 20.7 | 55.0 ± 4.4 | 28.6 |
| Deficiency II| 30.3 ± 5.7* | 887.3 ± 34.0* | 3.4 | 344.5 ± 24.8* | 56.4 ± 8.2* | 16.4 | 128.7 ± 16.0* | 16.2 ± 3.6* | 12.1 |
| Control III | 55.5 ± 7.3 | 395.3 ± 23.7 | 14.0 | 333.5 ± 29.3 | 119.3 ± 18.2 | 35.7 | 118.1 ± 16.8 | 35.6 ± 4.8 | 30.1 |
| Deficiency III| 33.6 ± 4.9* | 841.4 ± 40.5* | 4.0 | 239.2 ± 14.1* | 39.5 ± 4.4* | 16.5 | 79.5 ± 8.9* | 9.5 ± 2.1* | 11.9 |

Each value represents M ± SE for 6 rats. *TIBC, total iron binding capacity; FN-Fe, ferritin iron. *Significantly different from respective control group, p < 0.01.
Table 6. Iron content in various tissues in pregnant rats fed an iron depleted diet.

| Groups       | Kidney (µg/tissue wt.) | Muscle (µg/g) | Intestine (µg/g) | Cerebrum (µg/g) | Uterus (µg/g) | Placenta (µg/g) |
|--------------|------------------------|---------------|------------------|-----------------|---------------|-----------------|
| Control I    | 101.1 ± 9.6*           | 20.7 ± 0.8*   | 24.0 ± 1.8*      | 25.5 ± 2.6*     | 52.7 ± 2.2*   | 123.6 ± 13.2b   |
| Deficiency I | 64.3 ± 3.6*            | 8.4 ± 0.8*    | 9.5 ± 1.5*       | 16.3 ± 1.4*     | 41.0 ± 3.5*   | 96.2 ± 8.2*     |
| Control II   | 90.7 ± 8.1             | 19.1 ± 2.6    | 16.0 ± 1.2       | 17.7 ± 1.7      | 49.0 ± 3.9    | 121.3 ± 8.6     |
| Deficiency II| 68.2 ± 6.9*            | 9.3 ± 0.5*    | 11.0 ± 0.6*      | 14.1 ± 0.7**    | 41.0 ± 4.2**  | 95.9 ± 8.6*     |
| Control III  | 74.6 ± 6.8             | 11.1 ± 0.8    | 14.7 ± 0.9       | 21.2 ± 0.8      | 41.8 ± 1.9    | 99.2 ± 5.3      |
| Deficiency III| 55.1 ± 2.4*           | 8.9 ± 1.0**   | 9.4 ± 0.6*       | 15.2 ± 1.0**    | 33.9 ± 4.6**  | 77.7 ± 7.0*     |

Each value represents M ± SE, *n = 6 rats, b n = 10 samples. Significantly different from respective control group, *p < 0.01, **p < 0.05.
Table 7. Reproductive performances in rats fed an iron depleted diet.

| Groups       | Final body wt. | Wt. of conception | Litter size | Wt. of a fetus |
|--------------|----------------|-------------------|-------------|---------------|
| Control I    | 302 ± 8.8      | 280 ± 5.5         | 11.0 ± 0.6  | 10.8 ± 0.5    |
| Deficiency I | 95.8 ± 3.8     | 27.4 ± 3.9*       | 8.0 ± 1.6   | 4.81 ± 0.19   |
| Control II   | 279 ± 19.9     | 260 ± 8.2         | 8.8 ± 1.7   | 8.7 ± 1.8     |
| Deficiency II| 48.2 ± 9.7     | 27.8 ± 3.9*       | 8.2 ± 1.7   | 4.78 ± 0.27   |
| Control III  | 227 ± 14.9     | 224 ± 6.6         | 9.8 ± 0.6   | 3.87 ± 0.19   |
| Deficiency III| 58.2 ± 5.5    | 58.2 ± 3.9**      | 9.8 ± 0.6   | 2.94 ± 0.31** |

Each value represent M ± SE for 6 maternal rats. * Two (°) and three (**) maternal rats did not produce fetus. Significantly different from respective control group. °p<0.01, **p<0.05.
transferrin saturation of the deficient animals were lower than those of corresponding control groups, while the TIBC values of the deficient groups were markedly higher than those of controls (Table 5). Either total and ferritin iron contents or the ratio of ferritin iron to total iron (FN-Fe/Total-Fe) in liver and spleen of the deficient animals were apparently lower than those of controls as shown in Table 5.

As shown in Table 6, the total iron contents in kidney, muscle, intestine, cerebrum, uterus and placenta of deficient rats were also lower than those of the controls in each group. Weights of conception products and placenta from three deficient groups were lower than those of the corresponding controls (Table 7), and higher severity of iron deficiency in the dams may result in lighter weight of conception products. The placenta numbers of deficient rats, however, were almost similar to corresponding controls. Each dam in group-I (light anemia) had five fetuses in average, either the litter size or fetus weight was lower than those of the control, since normal average fetus number is considered to be about ten, approximately five or so would be resorbed. The more severe iron deficiency in the dams the more the rate of fetal resorption occurred. Two dams among six rats of group-II (moderate anemia) had no fetus. The litter size and fetus weight of the remaining four dams was 2.0 and 1.65 g in average, respectively. Three dams among six rats of group-III (severe anemia) had no fetus and the litter size and fetus weight of remaining three dams was 1.2 and 0.88 g in average, respectively.

DISCUSSION

Anemia frequently observed in pregnant women is considered as a normal physiological change (12). Sturgeon (13), however, pointed out that many anemic women have iron deficiency. Severe anemia affects not only the physiological status of mother but also the growth of fetus and new born infant (14). Thus, the improvement of anemia in women is an important problem to maintain the health of pregnant women and normal growth of fetus (15).

NRC (1) proposed that the iron requirement of rat is about 35 mg/kg diet, but have not indicated the requirement for reproduction. McCall et al. and Ahlstrom and Jantti claimed the iron requirements for reproduction presented by 28–58 and 240 mg/kg diet are different from each other (2, 3). On the other hand, Lin and Kirksey (4) indicated that the requirement for young and adult pregnant rats was 50–250 and 10–50 mg/kg diet, respectively. Thus, it appears likely that the young pregnant rats may need more iron than the adults. It seems likely that such difference in iron requirement was related to the difference in iron store between young and adult rats. From these finding, it may raise a question whether or not the retardation of fetal development is dependent on the intensity of anemic status of pregnant rat at the beginning of gestation. The present experiments were carried out to examine the effect of different intensities of maternal iron deficiency in pregnant rats on the tissue iron of dams and on the fetal development. Iron-depleted status of these animals was induced by feeding an iron depleted diet for various period prior
to pregnancy (Fig. 1). Even rats with severely iron-deficient anemia prior to pregnancy were able to achieve gestation. The values of Hb, Ht and RBC at the beginning of pregnancy (day-1) in all animals were low. But those values of control groups increased by feeding iron adequate diet during pregnancy, and the fetus development were almost normal. On the other hand, those values of all deficient groups decreased by further feeding iron depleted diet during pregnancy.

The Hb levels of deficient group-I, -II and -III on day-21 was remarkably lowered by 6.9, 6.7 and 4.7g/100ml, respectively. The remarkable decrease in MCHC in all deficient animals on day-21 of gestation suggested that the hypochromic anemia occurred. The decrease in serum iron and transferrin saturation were observed, and the increase in TIBC was also observed in the deficient animals. These results were similar to those of previous papers (4,16).

The observations of Kaufman and Wyllie (17, 18) have reported that iron transfer from maternal body to fetus increased in the late gestation with maximum transport on day-20 or -21 of gestation. They also reported that maternal tissue iron decreased in the late pregnancy, and the contents of ferritin iron in liver and spleen were markedly decreased. It seems that the fetal resorption observed in the present study mainly occurred in late gestation.

In a previous paper (8), we have reported that when iron intake of the dams was restricted during only pregnancy period, the fetal weight gain was normal and the maternal Hb level on day-21 of gestation was 12.0 g/100 ml, while the maternal Hb level of deficient group-I in this paper was 6.9 g/100 ml. The maternal tissue iron contents (liver, spleen and muscle) in the deficient animals of this study were also markedly lower than those in the deficient animals of the previous study.

Murry and Stein (7) reported that the fetuses obtained more iron from maternal absorption and less from maternal store with diminishing iron store. In contrast to previous papers (7, 8, 19), the maternal tissue iron was markedly lowered at the beginning of gestation in this paper. It appears likely that the more maternal absorbed iron must be required for the normal development of fetus under these conditions, and the subsequent feeding on the iron depleted diet during pregnancy may result in the severe impairment of fetus development. This hypothesis was confirmed by the results obtained here.

Deficiency of oxygen supply to fetus also may be an important factor in causing fetal resorption and growth retardation in iron deficient anemic-dams. Iron in animals is a constituent of hemoglobin, myoglobin and cytochromes, which are important in oxygen transport and respiration (20). Various enzymes containing iron also play important roles in biological oxidation in cells. Therefore, an iron-deficient status may induce not only anemia but also various metabolic lesions in many tissues. For example, Beutler (21) found a reduction in the concentrations of cytochrome c in the liver and kidneys of mildly anemic-rats. From these findings together with the results obtained here, it appears likely that the impairment of fetal development during gestation observed in severe anemic-dams may result from the lesion of respiration and biological oxidation caused by the reduction of oxygen supply.
supply to fetal tissues.

Wigglesworth (22) showed that the uterine blood supply was partially obstructed by ligature of a uterine artery in the pregnant rats on day-17 of gestation, resulting in that the average fetal weights on day-21 or -22 were lower than those of the dams without ligature. In the present study, the severe anemia in the dams seems to cause a decrease of oxygen supply to fetuses as well as in the case of partial obstruction of uterine arteries.

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