Iron Metabolism of the Lactating Mouse (35705)

TATT-TUCK LOH
(Introduced by G. C. Ring)
Physiology Department, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

In the rat, plasma volume (1), red cell volume, and plasma total iron-binding capacity (TIBC) (2) become elevated during pregnancy and remain increased throughout lactation. The rise in the plasma volume, however, exceeds that in the red cell volume and consequently, the hemoglobin concentration of the circulating blood is reduced (2). Due to the continuous secretion of high concentration of iron into milk, the body iron stores become depleted during lactation (3). Comparatively very little information is available regarding the iron metabolism of the lactating mouse.

The object of the present work was to find out whether any significant differences in hematological measurements and in body iron stores occur between lactating and nonlactating mice. High concentrations of iron in milk and in the lactating mammary gland were reported in a few species, *i.e.*, in rats and quokkas (4, 5). The iron content of mouse milk and its relation to the mammary gland iron deposition were also studied.

Materials and Methods. A total of 36 lactating albino mice, having litter size of six sucklings, were studied in batches of six on Days 0, 2, 5, 10, 15, and 20 of lactation, respectively. Six hours after their litters were removed, they were weighed and milked manually while under ether anaesthesia. A few minutes prior to milking, 1 to 2 units of oxytocin were injected subcutaneously to facilitate the milking process. The milk samples in aliquots of either 50- or 100-μl vol were assayed for iron using a modification of Ezekiel's method (6). After milking the blood was removed by direct cardiac puncture with a syringe and needle moistened with heparin solution. The mammary gland, liver, and spleen of the animal were then dissected, weighed, and stored at $-16°$ until assayed. On the freshly drawn blood sample, hemoglobin was estimated by the cyanmethemoglobin method (7) and the hematocrit value was estimated by centrifuging in a microhematocrit tube. The remaining portion of blood was centrifuged at 2000*g* for 15 min to obtain plasma. Plasma iron and total iron-binding capacity (TIBC) were assayed immediately by the method of Watkins and Butler (8). Total nonhemoglobin iron in the mammary gland, liver, and spleen were determined using aliquots of aqueous homogenates by a method described by Kaldor (9).

For comparison, all measurements, except for milk iron, were performed on six adult virgin female mice of 2½ months of age and on another six females on days 13–15 of gestation.

Results. The highest body weight was recorded for the 13- to 15-day pregnant females. This weight, however, included the weight of the products of conception, fetuses and placentae. The body weight of the lactating animals rose progressively and at Day 15 postpartum was almost twice the weight of virgin females. Liver weight and the weight of the mammary gland showed a progressive rise during pregnancy and lactation, with the highest values recorded at Day 10 for both tissues. At that point the weight of the mammary gland was almost 10-fold that of the weight of the gland in the virgin animals and more than three times the weight of the gland at parturition. The weight of the spleen was highest in the pregnant mice and diminished during lactation.

Figure 1 shows the plasma iron and total iron-binding capacity values (TIBC), hemoglobin concentrations and hematocrit values of virgin and pregnant females, and of lactating dams. Plasma iron concentration was about 200 μg/100 ml plasma throughout lac-

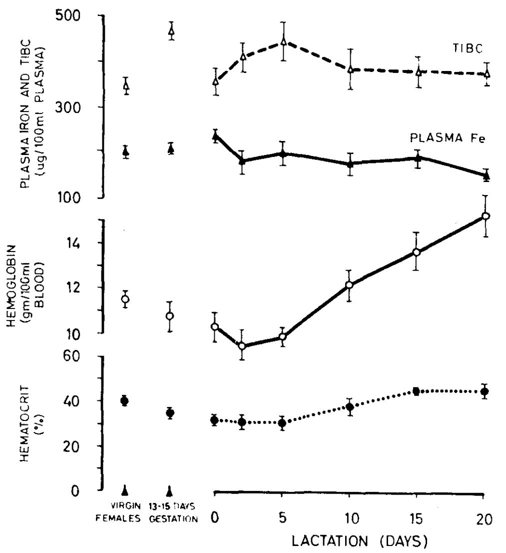

FIG. 1. Changes in plasma iron and total iron-binding capacity, hemoglobin concentrations, and hematocrit values in virgin, pregnant, and lactating mice. Each plot represents the mean of estimations on six animals and the vertical bar corresponds to one standard error of the mean.

tation except at parturition, and these values were comparable with those of virgin and pregnant mice. At parturition, the plasma iron value was significantly higher than at any other time tested ($p<.05$). Plasma TIBC was significantly higher in the pregnant animals compared with virgin mice ($p<.01$). At parturition, the TIBC which was 355 μg/100 ml, was low, but rose to a maximal value of 445 μg/100 ml at Day 5 of lactation. Thereafter, the value dropped to about 380 μg/100 ml and remained at this level. Hemoglobin concentration was lower in pregnant than in virgin animals and diminished further during early lactation. Nevertheless, from Day 10 of lactation onward, the hemoglobin concentrations of the lactating mice exceeded those of the virgin females and reached a value of 15.3 g/100 ml at Day 20. The hematocrit values followed a similar pattern to that seen in hemoglobin. They showed a minimum value at Day 5 of lactation, but eventually exceeded the virgin values after Day 15 of lactation.

Figure 2 shows the milk iron concentrations and the nonhemoglobin storage iron in the liver, spleen, and mammary gland in the different groups of animals. The iron stores in the liver and spleen were significantly depleted at parturition, the values being only one half and one quarter that of the virgin animals, respectively ($p<.01$). However, during lactation there was relatively little change in these iron stores. On the other hand, there was an increase in the iron content of the lactating mammary gland compared with the nonlactating gland. Nevertheless, this increase in iron content was not significant statistically. An extremely high iron content (38 μg/ml milk) was found in mouse milk at the beginning of lactation. The milk iron content dropped to 15 μg/ml on Day 10 and thereafter, the milk iron concentrations changed little.

Discussion. As a result of an increase of food intake and appetite, a progressive gain in body weight during lactation was reported in rats (10). The present studies show a similar trend in the lactating mouse. A maximal body weight occurs around midlactation (Table I). This gain in body weight is accounted for only to a small extent by the

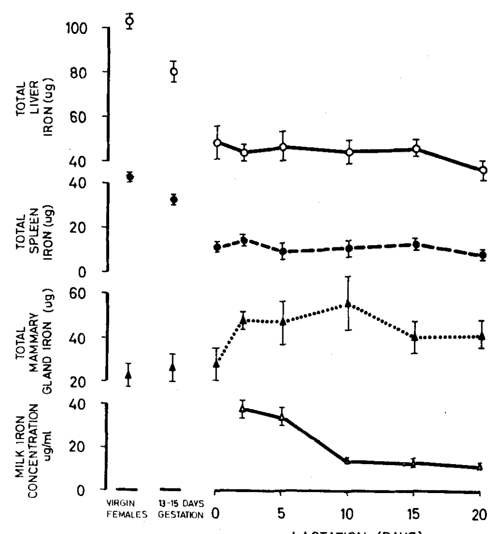

FIG. 2. Changes in nonhemoglobin iron content in livers, spleens, and mammary glands in virgin, pregnant, and lactating mice and in milk iron concentrations at different stages of lactation. Each plot represents the mean of estimations of six animals and the vertical bar corresponds to one standard error of the mean.

TABLE I. Variations in Weights of Whole Body, Liver, Spleen, and Mammary Gland in Virgin, Pregnant, and Lactating Mice.[a]

Weights (g)	Virgin females	Pregnant females	Lactating dams on days 0	2	5	10	15	20
Whole body	22.7 (± 2.4)	29.6 (± 2.8)	24.5 (± 2.9)	26.8 (± 2.5)	29.0 (± 2.5)	35.4 (± 6.1)	39.3 (± 5.0)	28.3 (± 6.8)
Liver	1.15 (± .24)	1.26 (± .28)	1.56 (± .17)	1.53 (± .38)	1.66 (± .39)	2.16 (± .40)	1.89 (± .51)	1.69 (± .25)
Spleen	0.14 (± .04)	0.20 (± .11)	0.16 (± .03)	0.18 (± .10)	0.18 (± .08)	0.15 (± .05)	0.13 (± .07)	0.13 (± .03)
Mammary gland	0.35 (± .31)	0.85 (± .45)	1.04 (± .38)	1.18 (± .45)	1.59 (± .60)	3.39 (± 1.31)	2.49 (± 1.30)	1.40 (± .12)

[a] The results are expressed as the mean ± standard deviation.

increase in the weight of liver and mammary gland.

As in the rat (2, 3), the blood hemoglobin concentration in the mouse decreases toward the end of pregnancy and continues to fall during early lactation. However, it rises gradually as lactation progresses and by Day 10 of lactation it exceeds the virgin concentration. In the rat, the fall in hemoglobin during early lactation is probably due to the fact that the expansion rate of the plasma volume exceeds that of red cell volume (1, 2). Whether such an explanation applies to the mouse is not known.

At parturition, the plasma iron content is significantly higher than during gestation and after lactation begins (Fig. 1). This fall occurs at a time when the fetus is no longer withdrawing iron from the plasma. As in humans (11), rats and rabbits (2), there is a significant elevation of the plasma TIBC in the last third of pregnancy in mice. At parturition, the plasma TIBC drops but rises again to a maximal value at Day 5 of lactation. The rise in the plasma TIBC during the first 5 days postpartum coincides with the depression in hemoglobin concentrations and the depletion of body iron stores suggesting that a mild stage of iron deficiency may prevail during early lactation. In the human, Beutler and his associates (12) observed that the plasma TIBC is a reliable index of iron deficiency only when the hemoglobin level is below 9 g per 100 ml.

Liver and spleen iron content of the lactating mouse are about half of the values in virgins indicating utilization of iron from body iron stores occurs during gestation. It is not possible to tell from the present study where and how the iron is channelled. Despite the continuous secretion of high concentrations of iron into milk, there is no further fall in body iron stores and plasma iron content during the entire course of lactation. Rather, after Day 10 of lactation the hemoglobin concentrations exceed those of virgin females. This suggests that an effective iron-balancing mechanism may be in operation during lactation. By what mechanism this is achieved is not clear.

As in the quokka and rat (4), mouse milk is secreted with an extremely high concentration of iron. Since the amount of milk produced is unknown, the total loss of iron through milk from the lactating mouse cannot be calculated here. However, judging from the gain in body weight and the total body iron of the suckling mouse, the iron loss through milk is quite considerable (13). The major source of milk iron is presumably from the plasma (14). As in the lactating rat (5), a considerable deposit of iron in the lactating mammary gland is found in the mouse. The significance of this mammary gland iron pool with respect to iron secretion into mouse milk is doubtful. In the rat, most of the iron in the mammary gland iron pool plays an unimportant role in the milk iron secretion (14).

Summary. The hemoglobin, plasma iron, and total iron-binding capacity (TIBC), and iron content in livers, spleens, mammary glands, and milk of mice were measured at different stages of lactation. The results were compared with values obtained on virgin and pregnant mice. There was a drop in liver and spleen iron throughout gestation. Nevertheless, the plasma iron and TIBC values were not different from the nonlactating figures. Despite a significant loss of iron through milk, there was no further fall in the body iron stores and plasma iron content during the entire course of lactation. Rather, after Day 10 of lactation, the hemoglobin concentration exceeded those of virgin females. This suggests the operation of a very effective iron-balancing mechanism during lactation.

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