Iron in the Lactating Mammary Gland of the Rat (34946)

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(Introduced by G. C. Ring)
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The physiological mechanism whereby iron is transferred from plasma to milk in the mammary gland of the lactating rat is of special interest because the iron content in rat milk is more than 10 times greater than in human or bovine milk and, furthermore, it is several times greater than the iron content of the blood plasma from which the milk iron is derived (1, 2). Experiments with tracer doses of ^{59}Fe injected intravenously into lactating rats showed that the transfer of ^{59}Fe from plasma to milk was a fast process which occurred exponentially and reached a plateau 2–4 hr after injection (3). When erythropoietic activity was suppressed in lactating rats by hypertransfusion, a rise in milk iron concentration occurred suggesting that the capacity of the mammary gland iron-transport system was not the limiting factor which determined milk iron concentration. Judging by the low specific activity of the mammary gland iron pool, it was suggested that most of the mammary gland iron is inactive in respect of secretion of iron into the milk (3). This mammary gland iron pool has an obscure role. Histochemically, iron deposition in the mammary glands of rats and mice is observed in virgin females as well as in females in early pregnancy (4, 5). This stainable iron, however, disappears completely in the last trimester of pregnancy and is absent during the entire course of lactation. The present work was designed to study this mammary gland iron pool both histochemically and by measurements of the iron content of milk and mammary gland and of various subcellular fractions of the mammary gland tissue.

Materials and Methods. Lactating albino rats, 2, 5, 10, 15, and 20 days postpartum and nursing a standard litter of eight, were injected intravenously with 8–10 μCi of tracer ^{59}Fe. Six hours later, they were milked manually and then killed with ether. The skin was removed and the mammary gland was dissected, removed, and weighed. A small aliquot of the gland was cut off for histochemical study. This aliquot was fixed in formalin and was then processed for sectioning and staining using the Prussian blue method. Before staining proceeded, the sections were pretreated with 20% hydrogen peroxide to "unmask" the iron–protein complexes (6). All glassware used was washed with acid to prevent iron contamination. The remainder of the gland tissue was divided into three portions. The first was homogenized with 0.25 *M* sucrose, and the homogenate was fractionated by differential centrifugation (7), resulting in nuclear, mitochondrial, ribosomal, and supernatant fractions. The fractions were counted for ^{59}Fe activity in a Packard automatic gamma counter. For the nonheme iron assay, another portion of the gland tissue was homogenized with normal saline and was assayed according to Kaldor's method (8). Measurement of the amount of milk retained in the mammary gland was done on the third portion of the gland by measuring the ratio of the lactose concentrations between the mammary gland homogenate and the milk samples, a method used by Slater (9). Milk iron concentrations were assayed by Ezekiel's method (10).

Results and Discussion. Table I shows the changes of milk iron and mammary gland nonheme storage iron at different stages of lactation in the rats. Contamination of the tissue iron due to the presence of milk iron was corrected for by measuring the amount

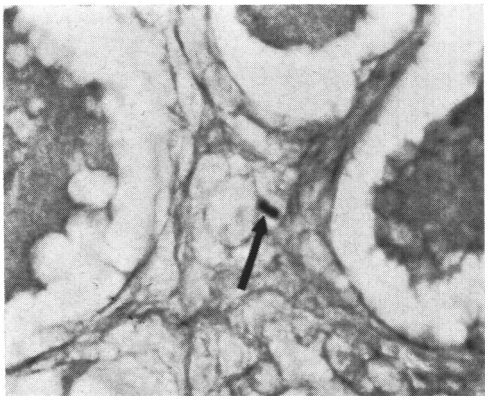

FIG. 1. The histological structure of the rat mammary gland on Day 5 of lactation. The dark spot (arrow) represents the iron granules which gave positive Prussian blue reaction. ×460.

of milk retained in the gland and the milk iron concentration. Despite the fact that milk iron concentrations were high and that half of the gross mammary gland weight was in the form of contained milk, the contamination from milk iron contributed only a small fraction. There was an increase in the mammary gland weight as lactation advanced; concurrently, the concentration of tissue iron dropped. Except for Day 2 of lactation, the total tissue iron remained fairly constant thereafter.

Figure 1 shows a typical histological structure of the rat mammary gland on Day 5 of lactation. The dark spot here indicated by arrow represents the iron granules which gave positive Prussian blue reaction. It was found that most of the iron granules were located in the connective tissue. This is different from the findings reported on virgin mice where the iron was located mostly inside the epithelial cells of the mammary gland (5). Whether such a difference can be attributed to the functional status of the gland or to a difference between mice and rats is uncertain, but judging from the sparse distribution of iron granules in the section, it is doubtful if the stainable iron could account for the entire mammary gland iron pool or even a large part of it.

The distribution of the intravenously injected ^{59}Fe in different subcellular fractions of the mammary gland is shown in Table II.

TABLE I. Changes of Milk Iron and Mammary Gland Nonheme Storage Iron at Different Stages of Lactation in Rats.[a]

Lactation (days)	No. of animals studied	Dam's body wt (g)	Milk		Mammary gland				
			Iron concn (μg/ml)	Lactose concn (mg/ml)	Weight (g)	Lactose concn (mg/g)	Per cent of milk retained	Uncorrected tissue iron (μg/g)	Uncorrected total iron (μg)	Corrected total iron (μg)
2	6	209 (± 14.1)	11.5 (± 1.15)	16.2 (± 1.04)	6.6 (± 0.58)	7.1 (± 0.84)	43.8 (± 1.89)	41.0 (± 5.11)	263 (± 29.9)	230 (± 31.7)
5	6	102 (± 4.0)	9.9 (± 0.43)	19.7 (± 1.40)	6.5 (± 0.64)	8.6 (± 0.94)	43.6 (± 1.20)	17.9 (± 1.56)	117 (± 14.6)	89 (± 15.8)
15	6	225 (± 7.5)	7.0 (± 1.72)	22.1 (± 1.70)	11.5 (± 0.83)	8.7 (± 0.67)	39.4 (± 2.48)	15.1 (± 0.89)	174 (± 17.6)	142 (± 18.8)
20	6	233 (± 7.4)	3.9 (± 0.18)	21.0 (± 0.98)	12.8 (± 0.85)	10.1 (± 0.49)	48.1 (± 2.34)	11.7 (± 0.64)	148 (± 8.0)	124 (± 10.2)

[a] The values are expressed in terms of mean ± standard error.

TABLE II. The Distribution of ^{59}Fe in Various Subcellular Fractions of the Rat Mammary Tissue After Intravenous ^{59}Fe Injection.

Animal no.	Lactation (days)	Percentage of ^{59}Fe found in: Nuclei	Mitochondria	Ribosomes	Supernatant fraction
1	2	5.1	5.8	6.7	82.6
2	2	3.0	6.2	10.6	81.2
3	5	3.5	7.0	10.3	79.2
4	5	1.8	5.8	8.4	86.0
5	10	0.5	9.7	5.6	84.2
6	10	0.5	5.3	9.2	85.0
7	15	1.8	4.9	9.4	84.9
8	15	4.0	5.5	9.2	81.3
9	20	3.0	7.0	10.3	79.6
10	20	4.0	6.5	9.5	80.0

At all stages of lactation the ^{59}Fe was found in the supernatant fraction accounted for some 80% of the total ^{59}Fe which had appeared in the gland tissue. Very little ^{59}Fe was detected in the nuclei or the cell debris, suggesting that the ^{59}Fe is most probably in association with the content of the vacuoles. Electron microscopic studies of the mammary gland of the mice by Wellings, Grunbaum, and Deome (11) had indicated that during the secretory phase the formed elements of the contents of the mammary epithelial cells are fat droplets and protein granules. The protein granules appeared inside the Golgi vacuoles and were believed to be the precursors of caseinogen. In a previous study of the distribution of iron in rat milk, most of the milk iron was located in the centrifugally separated casein fraction, especially in early lactation when the milk iron concentrations were high (12). It is, therefore, probable that most of the ^{59}Fe which enters the epithelial cells from the plasma is distributed inside the vacuoles and is in close association with the caseinogen.

Summary. Chemical analysis of lactating rat mammary glands showed the presence of considerable depositions of nonheme iron. Only a small quantity of this iron appears to be stainable by the Prussian blue method. The quantity and the distribution of this stainable iron outside the epithelial cells suggests that it does not represent the entire metabolic pool of the gland tissue. Subcellular fractionation of the lactating mammary gland 6 hr after intravenous injection of ^{59}Fe showed that most of the ^{59}Fe was located in the supernatant fraction probably in association with the caseinogen inside the vacuoles of the epithelial cells.

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