Permeability of Mammary Gland Capillaries to Ferritin in Mice

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ABSTRACT. The permeability of mammary gland capillaries to ferritin was investigated by transmission electron microscopy. In virgin mice, the concentration of tracers in perivascular spaces increased with the advance of time after injection. The ferritin never passed through the intercellular junction of the endothelial cells. In the early to middle stages of lactation, numerous ferritin particles were observed in the basal cytoplasm of alveolar epithelial cells after only 1 min of circulation. These findings indicate that a large amount of materials quickly pass through the capillary walls during these periods for milk production. The number of vesicles labelled with ferritin per μm^2 of endothelial cytoplasm decreased remarkably in the late stage of lactation. This result suggests that the permeability of vesicles declines along with the degeneration of alveolar cells.—KEY WORDS: capillary, ferritin, mammary gland, mouse, permeability.

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The mammary gland develops remarkably during pregnancy and lactation [5, 13, 22]. The blood vessels also develop conspicuously in conjunction with mammogenesis and lactogenesis [6, 13, 21, 22] because a large quantity of blood is required to produce milk [9]. The development of blood vessels may be accompanied by changes in the permeability of mammary gland capillaries.

The permeability of blood capillaries has been investigated in many organs using various tracers such as carbon, ferritin, hemoglobin, tannic acid, peroxidase and others [1, 3, 10, 15, 16, 18, 20, 23]. To our best knowledge, however, there are no reports on the permeability of mammary gland capillaries.

Although it has been thought that pinocytotic vesicles are associated with transendothelial transport of macromolecules [1, 16], the changes in permeability have not yet been reported in mammary gland capillaries in relation to pinocytotic and coated vesicles. In our previous study, the density of pinocytotic vesicles of the mammary gland capillaries reached the peak during lactation, indicating its close relationship with the functional state of mammary parenchyma [11]. In the present study, we attempted to elucidate the changes in permeability of mammary gland capillaries using ferritin as a tracer in different physiological states of the gland at various times after injection.

MATERIALS AND METHODS

Thirty seven JCL-ICR female mice were purchased from Japan CLEA Co., Ltd., Tokyo, Japan, to use in this study as materials. The animals were in one of the following physiological stages: virgin (70–90 day-old), pregnant (10 and 18 days of pregnancy), lactating (5, 10, 15 and 20 days after partum), or post-weaning (10 days after weaning). During lactation each mouse was housed with 8 to 10 pups. All mice were supplied with food and water *ad libitum*.

For transmission electron microscopy (TEM), type I horse spleen ferritin (10% solution in 0.15 M NaCl, Sigma) was used as a tracer. Ferritin (100 mg) was infused for 1 min into the caudal vein of the mice and allowed to circulate for an appropriate time. The animals were sacrificed by cervical dislocation to remove the first abdomino-inguinal mammary glands. The glands were cut into small pieces and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer for 4 hr at 4°C. They were rinsed in the same buffer and post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 to 3 hr at 4°C. Being rinsed in the same buffer and distilled water, the samples were stained with uranyl acetate saturated in 50% ethanol, dehydrated in a graded series of ethanol and embedded in Epon 812. Thin sections were cut on an ultramicrotome, non-stained or lightly stained with uranyl acetate and lead citrate, and observed with a JEM-1200 EX and a H-7000 KU transmission electron microscope at 80 kV and 75 kV, respectively.

For morphometry, more than 2 animals were used in each of the following stages: virgin, pregnant (10, 18 days of gestation), lactating (10, 20 days post partum), or post-weaning (10 days after weaning). More than 20 cross sections of the mammary gland capillaries were obtained from different animals and tissue blocks. TEM micrographs of capillaries were taken at a magnification of × 20,000 and enlarged to give working prints of × 40,000. Ferritin injection was not assumed to cause artificial contraction of blood capillaries because no degranular mast cells were observed in the mammary gland throughout the experiments. Cytoplasmic vesicles were counted on enlarged photographs to calculate the percentage of vesicles with ferritin particles. The area of endothelial cytoplasm without the nuclear region was measured by an image analyzer (Kontron MOP-10). The density of vesicles was calculated as the number of vesicles per μm^2 of endothelial cytoplasm. The percentage of vesicles with ferritin particles was multiplied by the density of vesicles to show the density of vesicles labelled with ferritin. The

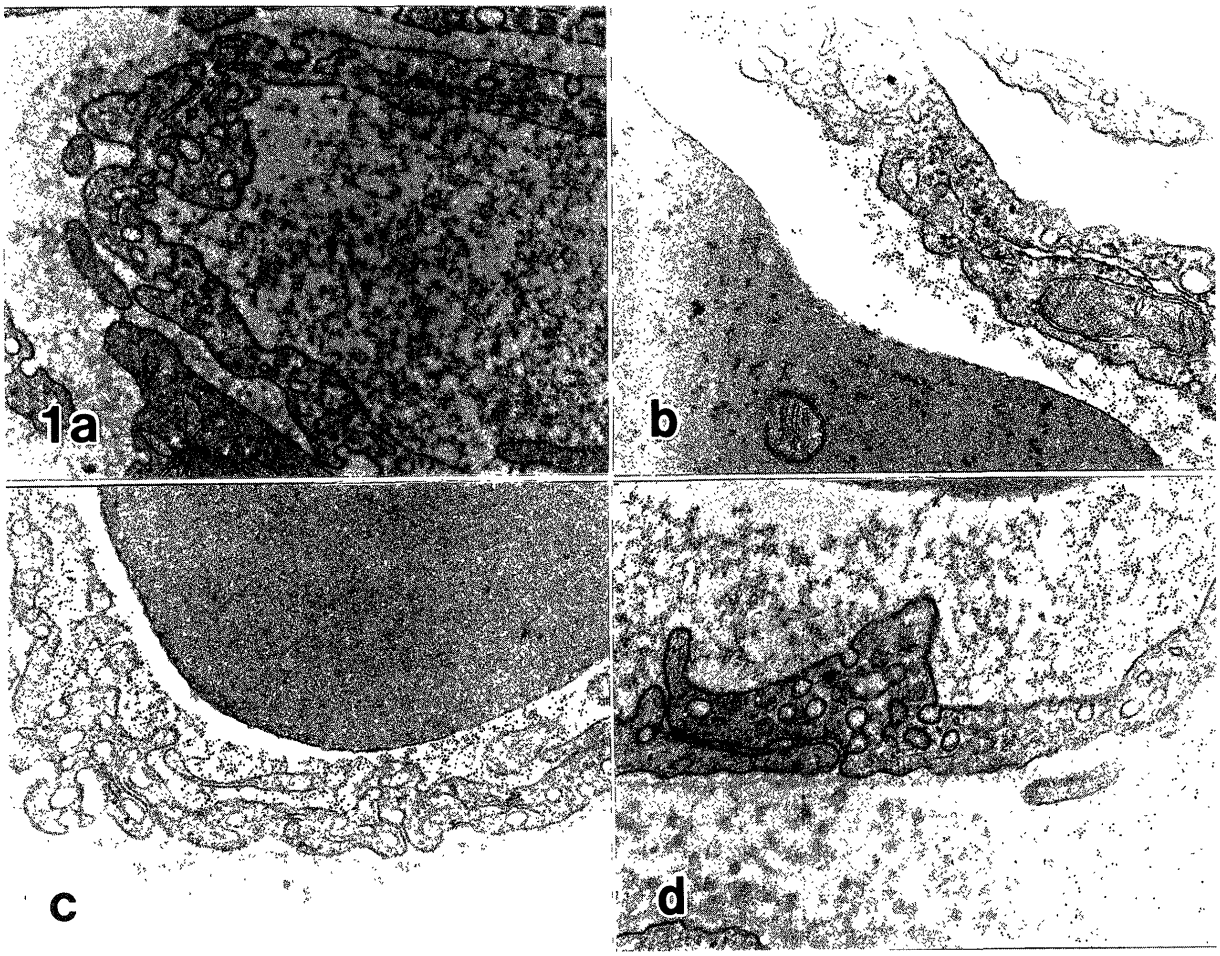

Fig. 1. Transmission electron micrographs of capillaries around the mammary duct in virgin mice. × 40,000. a) At 1 min after injection, ferritin particles are observed in luminal pinocytotic vesicles and coated vesicles and pits of luminal plasma membrane. b) At 3 min after injection, ferritin particles are seen in the multivesicular body. c) At 5 min after injection, many pinocytotic vesicles and coated vesicles labelled with ferritin particles are seen in the cytoplasm of the endothelium. d) At 10 min after injection, numerous ferritin particles are observed in the perivascular space. The intercellular junction does not allow ferritin to pass through.

data were statistically analyzed by Student's *t*-test.

RESULTS

Virgin mice: At 1 min after injection, ferritin particles were observed in a few cytotic vesicles near the luminal surface (Fig. 1a). Multivesicular bodies with particles were recognized at 3 min (Fig. 1b). Even at 5 min, ferritin particles were observed in a large number of abluminal cytotic vesicles, but not discharged in the perivascular space (Fig. 1c). At 10 min, numerous ferritin particles were seen in the perivascular space (Fig. 1d). Intercellular junctions of endothelium showed no permeability to ferritin.

Pregnancy: At 3 min after injection, the percentage of vesicles labelled with ferritin particles was higher in 10-day-pregnant mice than in virgin mice. Some of abluminal vesicles were labelled with ferritin. Two min later, numerous ferritin particles were discharged into the perivascular space (Fig. 2).

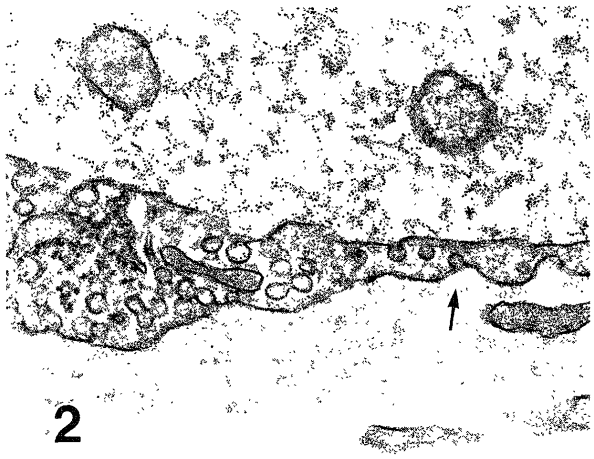

Fig. 2. Capillary surrounding a mammary alveolus at 10 days of pregnancy. At 5 min after injection, a few ferritin particles are observed in the perivascular space. An arrow indicates ferritin discharged into the perivascular space. × 40,000.

In 18-day-pregnant mice, ferritin particles were found in the perivascular space at 3 min after injection. At 5 min, a few particles were observed in the basal infoldings of alveolar epithelial cells around capillaries (Fig. 3).

Lactation: In mice on day 5 and 10 of lactation, ferritin particles were observed not only in approximately 30% of cytotic vesicles but also in the perivascular space at 1 min after injection. Some particles were also observed in the basal infoldings of alveolar epithelial cells (Fig. 4). At both 3 and 5 min after injection, the vesicles containing ferritin particles were recognizable in the basal cytoplasm of alveolar epithelial cells (Fig. 5). In fenestrated capillaries surrounding the mammary alveoli, ferritin particles passed through the diaphragm and were discharged into the perivascular space (Fig. 6). An apparent transendothelial channel composed of one to three vesicles was observed in the endothelium with a thinner wall (Fig. 7). At 15 and 20 days of lactation, fewer ferritin particles were observed in the perivascular space at 3 min after injection, although numerous vesicles contained ferritin particles. At 5 min, ferritin particles between basal infoldings remained in less numbers as compared with those on day 5 and 10, whereas neumerous particles were observed in the perivascular space (Fig. 8).

Post-weaning: At 10 days after weaning, vesicles were still capable of ferritin uptake. At 3 min after injection, ferritin particles were seen in the luminal and abluminal vesicles. At 5 min, the particles were observed not only in cytotic vesicles but also in the multivesicular body and the perivascular space (Fig. 9). These findings were similar to those in virgin mice.

Morphometry: The percentage of vesicles with ferritin particles and the number of those per μm^2 of endothelial cytoplasm are shown in Figs. 10 and 11, respectively. The percentage of labelled vesicles at 5 min after injection was higher than that of 3 min after injection in each stage. The percentages at both 3 and 5 min after injection reached the peak in mice at 10 days of lactation, but reduced by half in mice at 20 days of lactation. The density of ferritin-labelled vesicles also reached the peak at both 3 and 5 min after injection in mice at 10 days of lactation. In mice at 20 days of lactation, however, the density of ferritin-labelled vesicles decreased significantly at 5 min after injection in comparison with that at 3 min.

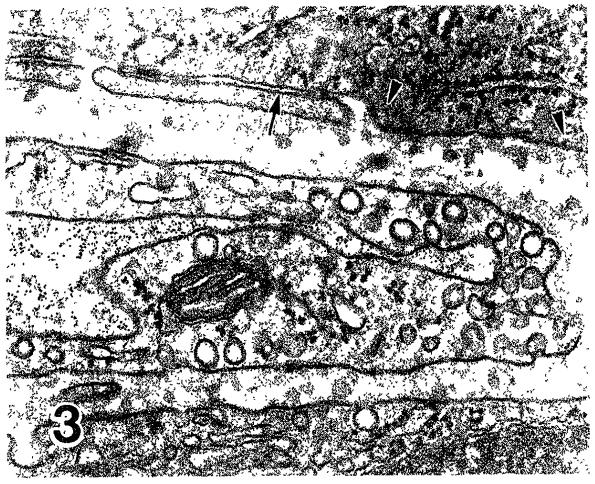

Fig. 3. Capillary between the alveoli at 18 days of pregnancy. At 5 min after injection, ferritin particles are observed in the perivascular space, the space between the marginal folds, the cytoplasm of the alveolar epithelial cell (arrow), and the vesicles (arrowheads) of alveolar epithelium. × 40,000.

DISCUSSION

In the present study, the capillary permeability to ferritin changed in relation to various physiological states of the mammary gland. During the early to middle stages of lactation, ferritin particles were observed in the spaces among basal infoldings of alveolar epithelial cells even after a short circulation time. The percentage and density of ferritin-labelled vesicles reached the peak in mice at 10 days of lactation. These findings indicate that the permeability of mammary gland capillaries remarkably increases during this period for alveolar epithelial cells to take up the materials for milk production actively. In the middle stage of lactation, the percentage of vesicles with ferritin rapidly reached the peak, approximately 30% at 1 min after injection, and did not increase significantly thereafter. These results may support that the transporting activity of vesicles is especially high in the middle stage of lactation [7, 11].

In mice at 20 days of lactation when the mammary alveola were remarkably degenerated, the percentage and density of ferritin-labelled vesicles reduced to about a half of those just prior to the previous stage, although the density of vesicles still remained the highest value at this period [11]. In addition, Iwamatsu *et al.* [9] have reported that the diameter of blood vessels supplying the first abdomino-inguinal mammary gland reach the peak during lactation. These reports and the present results suggest that the function of vesicles may decrease in the late stage of lactation.

In the present study, no ferritin particles passed through the intercellular junction of endothelial cells of the blood capillary. This finding was consistent with the previous reports [1, 4, 12, 17, 19] in that the clefts between capillary endothelial cells were impermeable to macromolecules such as ferritin with a diameter of 11 nm. The present results also support the hypothesis that the clefts serve as a small pore [2, 24].

In our previous report [11], the capillary endothelium was described to have a thinner wall during lactation. This structural feature was reconfirmed in the present study. Transendothelial channels composed of one to three vesicles are frequently formed in a thinner wall where the materials are easily allowed to pass through [8, 14, 20].

The permeability of mammary gland capillaries gradually increased after the beginning of pregnancy, reached the peak in the middle stage of lactation, and then suddenly decreased in the late stage of lactation. These changes in permeability seem to run parallel with the physiological states of the mammary gland.

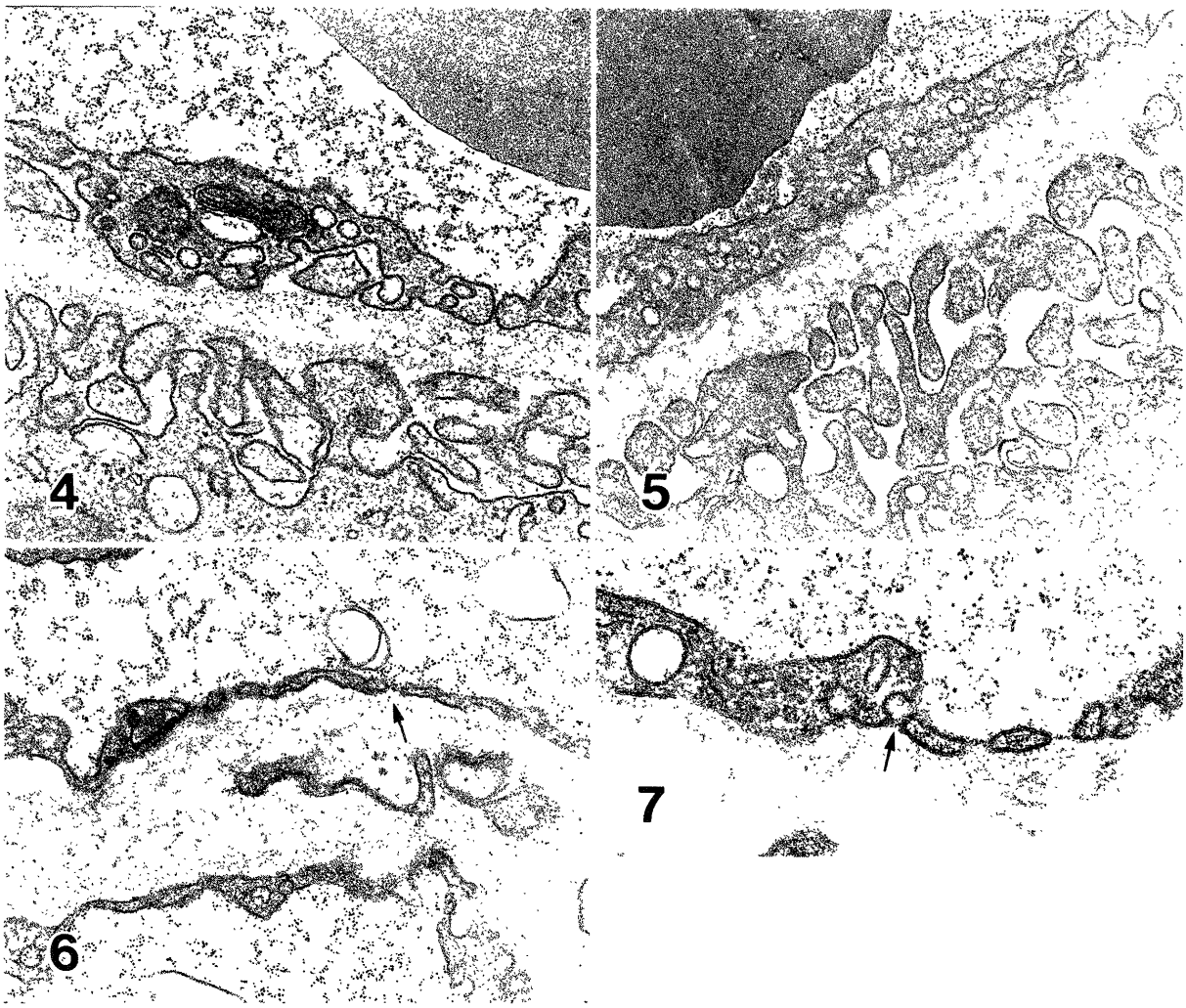

Fig. 4. Capillary wall beneath the alveolar epithelial cell at 10 days of lactation. At 1 min after injection, ferritin particles are observed in the perivascular space, the spaces among basal infoldings, and the vesicles of alveolar epithelium. × 40,000.
Fig. 5. Capillary beneath the alveolar epithelial cell at 10 days of lactation. At 3 min after injection, ferritin particles are taken up by the vesicles of the alveolar epithelial cytoplasm. × 40,000.
Fig. 6. Fenestrated capillaries around the mammary alveoli at 10 days of lactation. At 5 min after injection, ferritin particles pass through the diaphragm and are discharged into the perivascular space (arrow). × 40,000.
Fig. 7. A transendothelial channel formed by one vesicle (arrow) at 5 days of lactation. × 60,000.

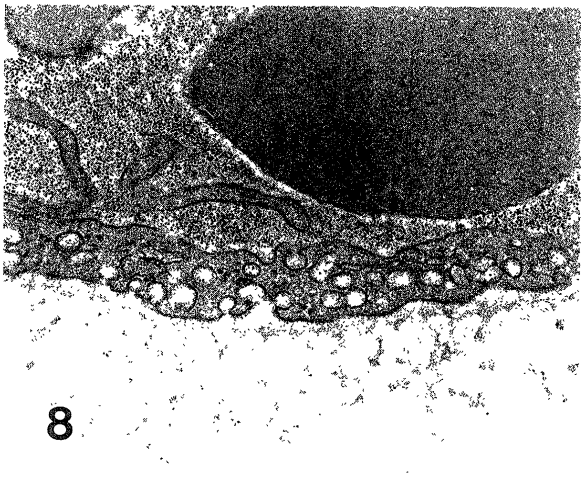

Fig. 8. Capillaries around an alveolus at 20 days of lactation. At 3 min after injection, a few ferritin particles are seen in the perivascular space. × 40,000.

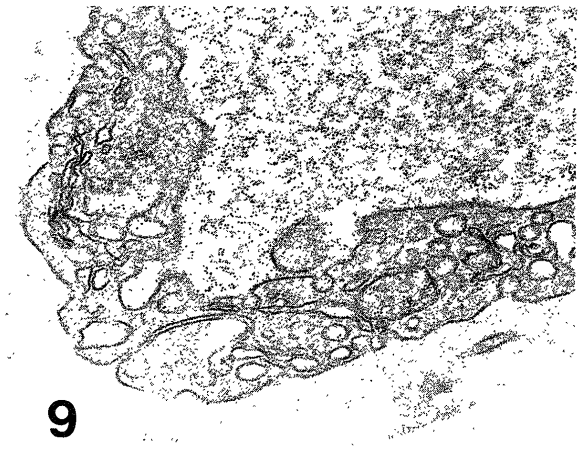

Fig. 9. Capillary surrounding the mammary duct at 10 days after weaning. At 5 min after injection, ferritin particles are seen in the luminal or abluminal pinocytotic vesicles, coated vesicles, and the multivesicular body. A few particles are also observed in the perivascular space. × 40,000.

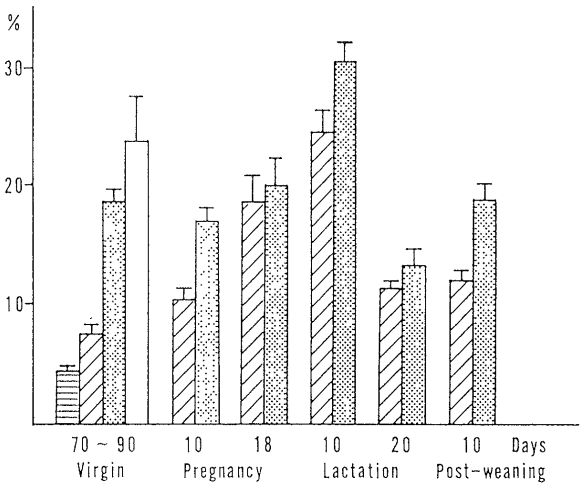

Fig. 10. Changes in the percentage of pinocytotic vesicles and coated vesicles labelled with ferritin particles at 1; ▤, 3; ▨, 5; □, and 10; □ min after injection. Each value indicates mean ± SEM.

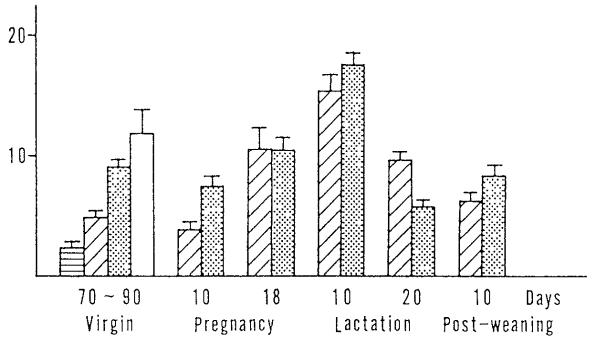

Fig. 11. Changes in the number of vesicles labelled with ferritin particles per μm^2 of endothelium cytoplasm at 1; ▤, 3; ▨, 5; □, and 10; □ min after injection. Each value indicates mean ± SEM.

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