Ultrastructural Changes in Fat Cells and Blood Capillaries of the Mammary Gland in Starved Mice

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ABSTRACT. The effects of starvation on fat cells and blood capillaries of the first abdomino-inguinal mammary gland in mice were investigated by light and transmission electron microscopy. The body weight of starved mice abruptly decreased to approximately 70% of that of controls at 3 days of starvation and, thereafter, gradually decreased. In adipose tissues of mammary stroma, multilocular fat cells increased in number and clustered during starvation to a glandular appearance at 6 days. Collagen fibers increased in amount around mammary ducts and buds. By electron microscopy, multilocular fat cells possessed numerous mitochondria, small lipid droplets, and plasmalemmal vesicles, while endothelial cells of the blood capillaries showed numerous pinocytotic vesicles plus short marginal folds and microvillous processes. These observations prove that the number of pinocytotic vesicles in blood capillary endothelium is closely related with the increased amount of lipid of fat cells in the mammary gland during starvation.—KEY WORDS: mammary gland capillary, mouse, pinocytotic vesicle.

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It is well documented in mice that mammary buds differentiate and proliferate to form alveoli during pregnancy [1, 2, 11, 12], and that mammary fat cells decrease in number during pregnancy and are hardly detected during the lactating period [1, 9, 12]. Nishinakagawa *et al.* indicated that mammary adipose tissues are analogous to those of the peri-uterine and peri-ovarian regions [7] and that starvation might lead to the regression of adipose tissues [8]. In our previous study [5], changes in the microvasculature of the mammary gland during pregnancy and lactation were observed by transmission electron microscopy. We indicated that the density of pinocytotic vesicles and the length of marginal folds and microvillous processes of the endothelium in blood capillaries were closely related to the functional state of the mammary parenchyma. Additionally, it seems likely that changes in the microvasculature is also related to the increase and/or decrease of mammary adipose tissues. Therefore, the fasting experiment was carried out so that mammary fat cells could decrease in number in a short time as was seen in the late stage of pregnancy [3, 8, 9]. Although Nishinakagawa *et al.* [8] investigated the vasculature of mammary glands in starved mice, ultrastructural studies have not been carried out. In the present study, the effects of starvation on the parenchyma and blood capillaries of the mammary gland were investigated by light and transmission electron microscopy.

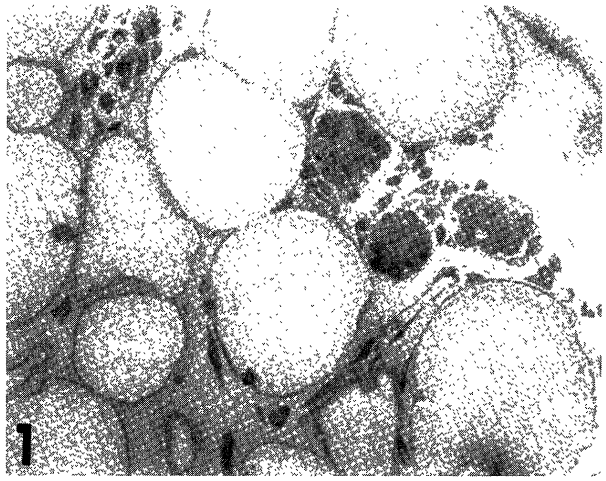
Twenty five JCL-ICR female mice (90 days old) were bred and maintained as a closed colony in our laboratory. The animals were starved for 1 to 6 days. Each group consisted of 3 (4 to 6 days of starvation) or more animals. Each mouse was caged in plastic cage with stainless steel mesh floor and never died during the experimental period. The room temperature was kept at 22±2°C during the experimental period. Control mice were supplied with a commercial diet (MF, Oriental Yeast Co., Ltd.) and water *ad libitum*, while starved mice received only water *ad libitum*.

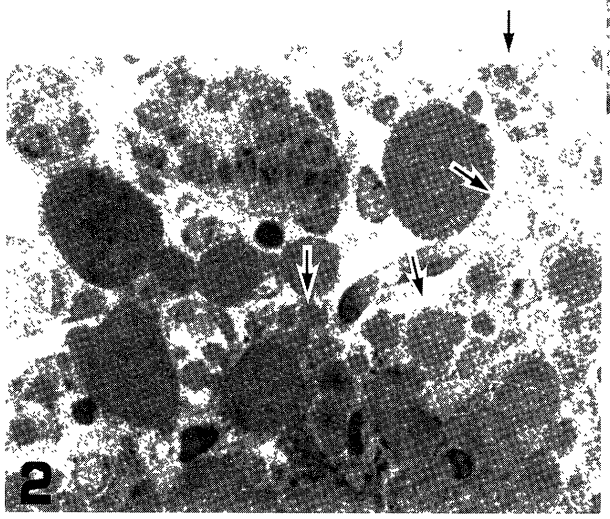
The mice were anesthetized by sodium pentobarbital before sacrifice. Tissues were prepared for light and transmission electron microscopy as described in our previous report [6].

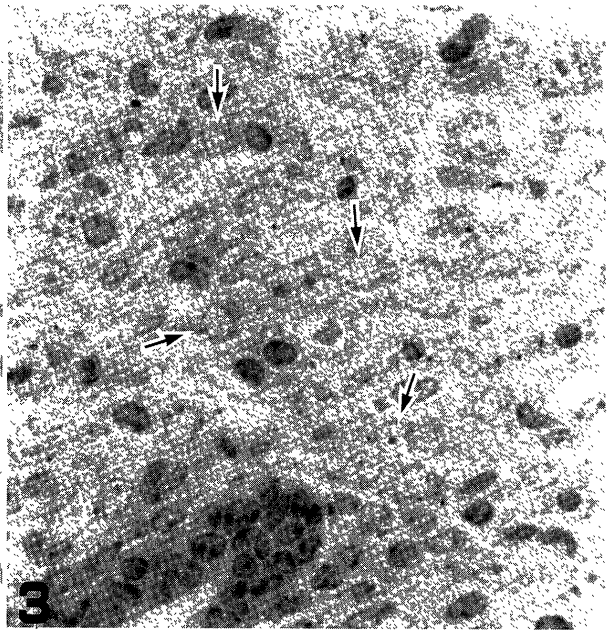
Body weight of starved mice abruptly decreased to approximately 70% of that of controls at 3 days of starvation and, thereafter, gradually decreased.

By light microscopic observation, the mammary ducts and buds of control mice were composed of columnar or cuboidal epithelial cells with a large, oval and lucent nucleus. In adipose tissues of the mammary stroma, most fat cells were of a unilocular type except for the regions surrounding the main vessel (Fig. 1). At 2 days of starvation, small unilocular fat cells were clustered in some regions. At 3 days of starvation, mammary parenchyma was composed of cuboidal epithelial cells with a oval lucent nucleus. Numerous multilocular fat cells were observed in clusters (Fig. 2). Collagen fibers increased in amount around the mammary ducts and buds. The stroma was largely occupied by multilocular fat cells at 5 days of starvation. At 6 days, the ducts and buds were morphologically similar to those in 3 days of starvation. Multilocular fat cells clustered in a glandular appearance (Fig. 3).

Electron microscopically, the epithelial cells of the mammary ducts and buds in control mice possessed a nucleus with an irregular contour, round or elongated mitochondria, numerous ribosomes, underdeveloped rough-surfaced endoplasmic reticulum, and Golgi apparatus (Fig. 4). The bud epithelium occasionally contained a few small lipid droplets. Fat cells had round or elongated mitochondria with lamella cristae and a few plasmalemmal vesicles (Fig. 5). A few pinocytotic vesicles, short marginal folds and microvillous processes were present in endothelial cells of blood capillaries (Fig. 6). At 1 day of starvation, multilocular fat cells with numerous plasmalemmal vesicles were detected. Endothelial cells of blood capillaries displayed similar morphology to that of control. At 3 days of starvation, some epithelial cells of ducts and buds included lipid droplets at the invagination of the nucleus. Multilocular fat cells contained varying cytoplasmic processes, numerous round mitochondria neighboring small lipid droplets, and abundant plas-







Figs. 1–3. Light micrographs of semithin sections stained with toluidine blue.

Fig. 1. Mammary gland in control. Fat cells are mostly of the unilocular type. × 700.

Fig. 2. Mammary gland at 3 days of starvation. Numerous multilocular fat cells (arrows) are accumulated. × 1,400.

Fig. 3. Mammary gland at 6 days of starvation. The stroma is occupied by multilocular fat cells (arrows) clustered in a glandular appearance. × 1,400.

malemmal vesicles (Fig. 7). Endothelium of blood capillaries possessed a large number of pinocytotic vesicles and short processes. After 4 days of starvation, lipid droplets in multilocular fat cells gradually decreased in diameter. At 6 days of starvation, most epithelial cells of ducts and buds had a few lipid droplets (Fig. 8). Some fat cells had large elongated mitochondria. Blood capillaries were characterized by the presence of more numerous pinocytotic vesicles, although marginal folds and microvillous processes remained short (Fig. 9).

Ultrastructural changes in mammary fat cells during starvation are similar to those of white and brown adipose cells located in other regions [4, 7, 10, 13, 14] and of mammary gland fat cells during lactation [3].

We have confirmed that endothelial cells of blood capillaries surrounding mammary adipocytes possess a higher density of pinocytotic vesicles with longer marginal folds and microvillous processes in late pregnant mice (unpublished data). However, it is still unknown whether changes in the length of those processes are affected by adipose tissues or neighboring development of alveolar epithelial cells. Under this condition, pinocytotic vesicles may transport lipids, while marginal folds and microvillous processes may play a partial role in the transportation of materials for synthesizing casein and lipids in adjacent alveolar epithelial cells. The present study showed that pinocytotic vesicles of blood capillary endothelium increased in number, while the length of marginal folds and microvillous processes remained short during starvation. Williamson [14] postulated that an abundance of pinocytotic vesicles might play a role in transporting free fatty acids from extravascular regions to the blood in starved rats. The present findings add support to this hypothesis.

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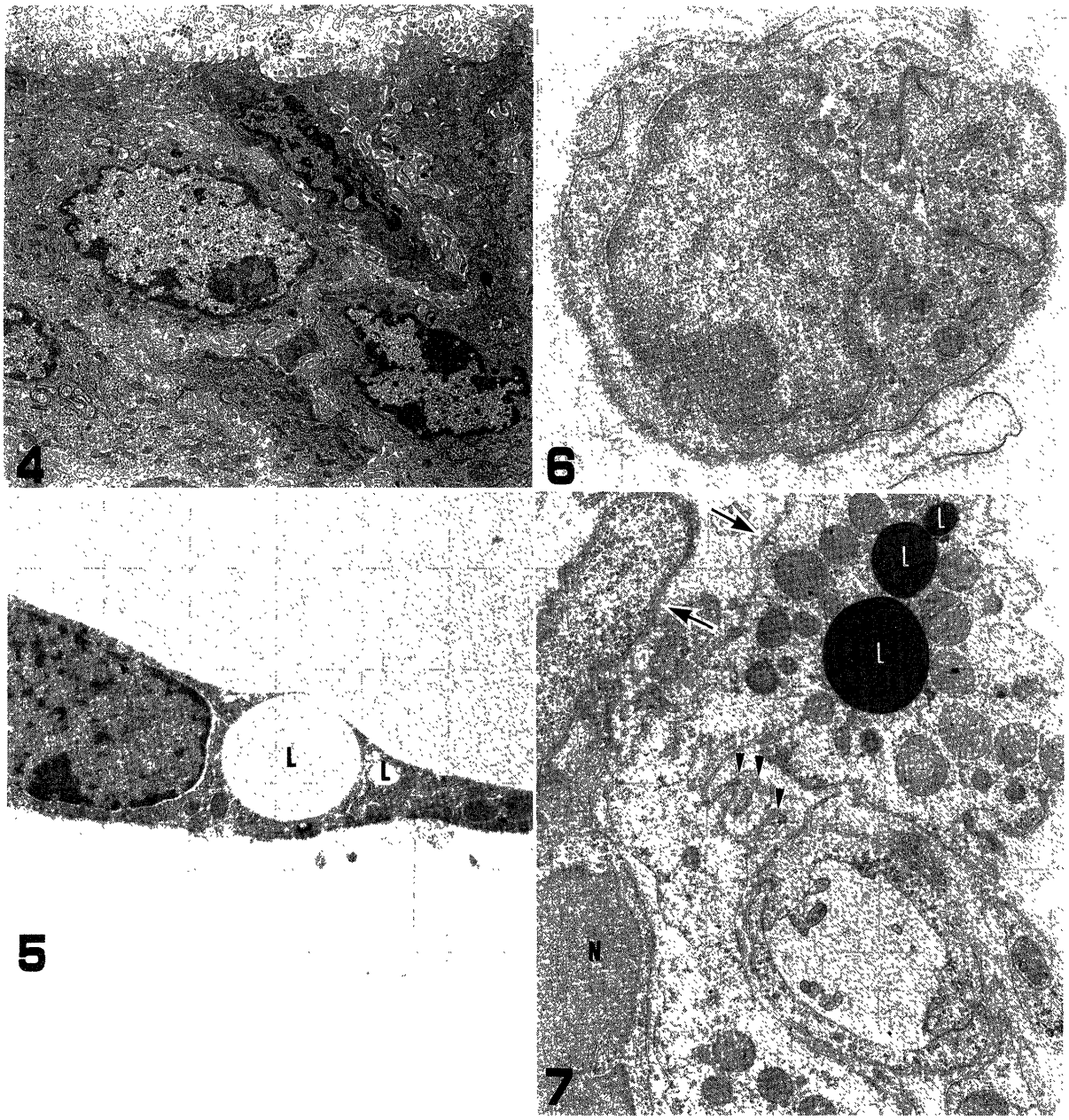

Figs. 4–9. Electron micrographs.

Fig. 4. Epithelial cells of duct in control. Few lipid droplets are seen. × 5,000.

Fig. 5. Fat cell in control. Small lipid droplets (L) and small mitochondria are seen. × 7,500.

Fig. 6. Capillary between unilocular fat cells in control. A short marginal fold and a small number of pinocytotic vesicles are seen. × 20,000.

Fig. 7. Lipid depleted adipocytes and capillary at 3 days of starvation. Adipocytes possess primary (arrows) and secondary (arrow heads) cytoplasmic processes, numerous round mitochondria around small lipid droplets (L) and abundant plasmalemmal vesicles. Endothelial cells of capillary contain a large number of pinocytotic vesicles. N; nucleus of adipocyte. × 7,500.

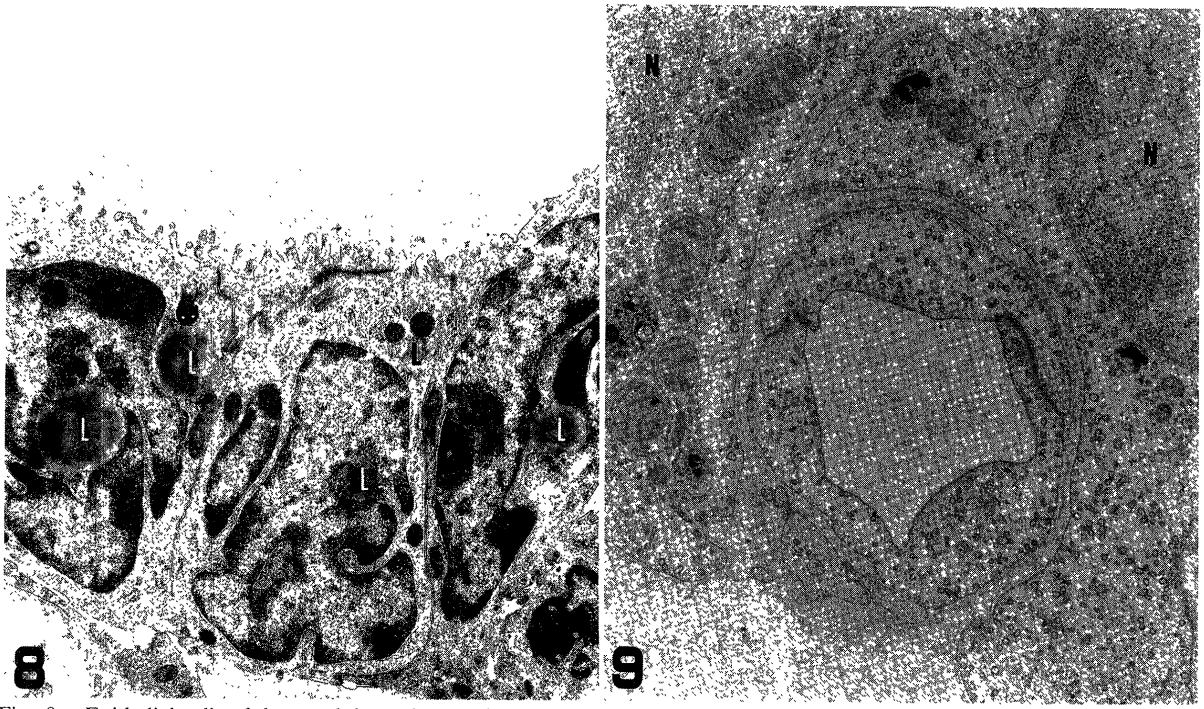

Fig. 8. Epithelial cells of duct at 6 days of starvation. Lipid droplets (L) at the invagination of the nucleus are seen. × 6,000.
Fig. 9. Capillary between two adipocytes at 6 days of starvation. Note numerous pinocytotic vesicles in endothelial cells. Adipocytes include numerous plasmalemmal vesicles, round and elongated mitochondria, but lack lipid droplets. N; nucleus of adipocyte. × 12,500.

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