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Histological and Morphometrical Studies on the Rat Nipple during the Reproductive Cycle

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Abstract: *Histological changes in the rat nipple during the reproductive cycle were observed. In virgin and the first half (days 5 and 10) of pregnancy, the nipple had a dull conical shape and the germinative layer of epidermis, thicker than that of the skin surrounding the nipple, deeply ingrew into the dermis in the basal region. From the second half (days 15 and 20) of pregnancy to the post-weaning period, the nipple appeared columnar in shape and many wrinkles were observed in the nipple wall especially during the lactating period. Collagen fibers longitudinally running in the nipple wall mainly comprised the dermis of the nipple and became loose during lactation. Small numbers of elastic fibers running parallel with smooth muscles were also observed in the nipple wall, and these increased in number and thickness from the second half of pregnancy, and most became frizzy structures during lactation. Around the lactiferous sinus, smooth muscle cells were arranged longitudinally but a few muscle cells were seen in a concentric layer, but during the lactating period the sinus was distended and many epithelial folds were observed. Morphometrical analysis indicated that the length of the nipple increased from the second half of pregnancy and reached the maximum on day 15 of lactation, approximately 3.7 times that in the virgin period. The outer diameter of the nipple and thickness of the nipple wall during lactation also reached approximately twice that in virgin. The size of the nipple decreased gradually after weaning. These observations suggest that the histological changes in the rat nipple during the reproductive cycle were mainly characterized by hyperplasia of the epidermis and hypertrophy of connective tissue in the dermis from the second half of pregnancy.*

Key words: *histology, morphometry, nipple, rat, reproductive cycle*

Introduction

The nipple is an organ unique to mammalian species and is indispensable for nursing pups via the mammary gland. In reproductive biology, however, it is usually just considered as a part of the mammary apparatus [10, 14]. Turner and Gomez [15], and Myers [8] examined the pre- and postnatal developments of the

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nipple in mice and rats, respectively, and described that the nipple differentiated from the 'milk point', the origin of the mammary apparatus. Despite a great number of histological and physiological studies on the mammary gland [2–4, 10, 14], there are only a few reports focusing on nipples in the classical literature [8, 15]. And these reports described the nipple as part of the duct system of the mammary gland.

During the female reproductive cycle, the mammary gland shows marked morphological changes both in parenchymal and stromal components, under the control of the pituitary, gonadal and placental hormones [1, 6, 9, 12]. We therefore performed histological and morphometrical observations to clarify the changes in the rat nipple during the reproductive cycle.

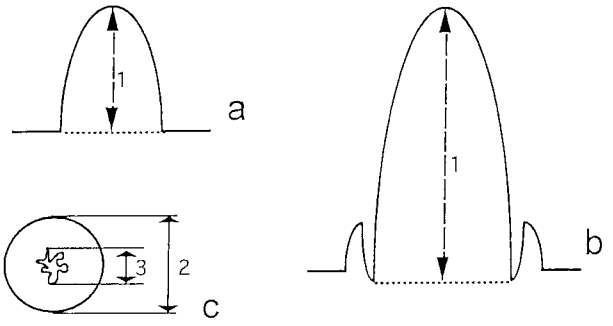

Fig. 1. Schematic illustration of the measuring points in morphometry. Longitudinal sections of the nipple from virgin to day 10 of pregnancy (a), after day 15 of pregnancy (b). Transverse section at the mid-level of the nipple (c). 1: Nipple length, 2: Outer diameter and 3: Inner diameter of nipple.

Materials and Methods

Animals: Wistar rats used in this study were bred and maintained as a closed colony in our laboratory. They were all housed in an environmentally controlled air-conditioned room (temperature: 23 ± 3°C, humidity: 60 ± 10%, light-dark cycle: 12–12 hr) and given a commercial diet (MF, Oriental Yeast Co., Tokyo) and tap water *ad libitum*. A total of 60 female rats (90-day-old) were mated with males of the same age, and the day on which spermatozoa were found in the vaginal lavage was called day 0 of pregnancy. They were divided into 12 groups, i.e. virgin, days 5, 10, 15 and 20 of pregnancy, days 5, 10, 15 and 20 of lactation, days 5, 10 and 15 of post-weaning. Each group consisted of 5 rats. At the lactating stage, each dam was housed with her 8 to 12 pups and separated from the young for 30 min before sampling.

Histology: Under diethyl ether and sodium pentobarbital anesthesia, the rats were perfused through the left ventricle with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The rat has 3 pairs of thoracic and 3 pairs of abdomino-inguinal nipples. The third thoracic and the first abdomino-inguinal nipples were further fixed with 10% neutral buffered formalin and then routinely embedded in paraffin. Serial sections were longitudinally or transversally cut at 6 μm and stained with hematoxylin and eosin (HE), Azan, elastica van Gieson or silver staining.

Morphometrical analysis: The measurement points are shown in Fig. 1. The width of the lactiferous sinus including the connective tissue (Fig. 1c) was considered to be the inner diameter of the nipple. Half of the outer diameter minus the inner diameter was defined as the thickness of the nipple wall. Each measurement was performed in HE-stained sections with an image analyzer (Nikon COSMOZONE Is, Tokyo). The data were statistically analyzed by paired *t*-test to compare the thoracic and the abdomino-inguinal nipples, and Fisher's PLSD law after dispersion analysis for comparison among the 12 stages.

Results

The results are given for the first abdomino-inguinal nipple, because no significant histological or morphometrical differences were observed between the third thoracic and the first abdomino-inguinal nipples during the reproductive cycle.

Histological observations

Virgin period: The nipple had a dull conical shape (Fig. 2a). Although the nipple wall was rarely wrinkled, there were approximately 10 dermal papillae in longitudinal sections. The epidermis of the nipple was thicker than that of the peripheral skin, but there were no hair follicles in the nipple wall. The basal layer of epidermis consisted of cuboidal cells, and ingrowth (described by Myers [8]) of the germinative layer was observed in the basal region of the nipple (Fig. 2a). The teat duct continued from the lactiferous sinus in

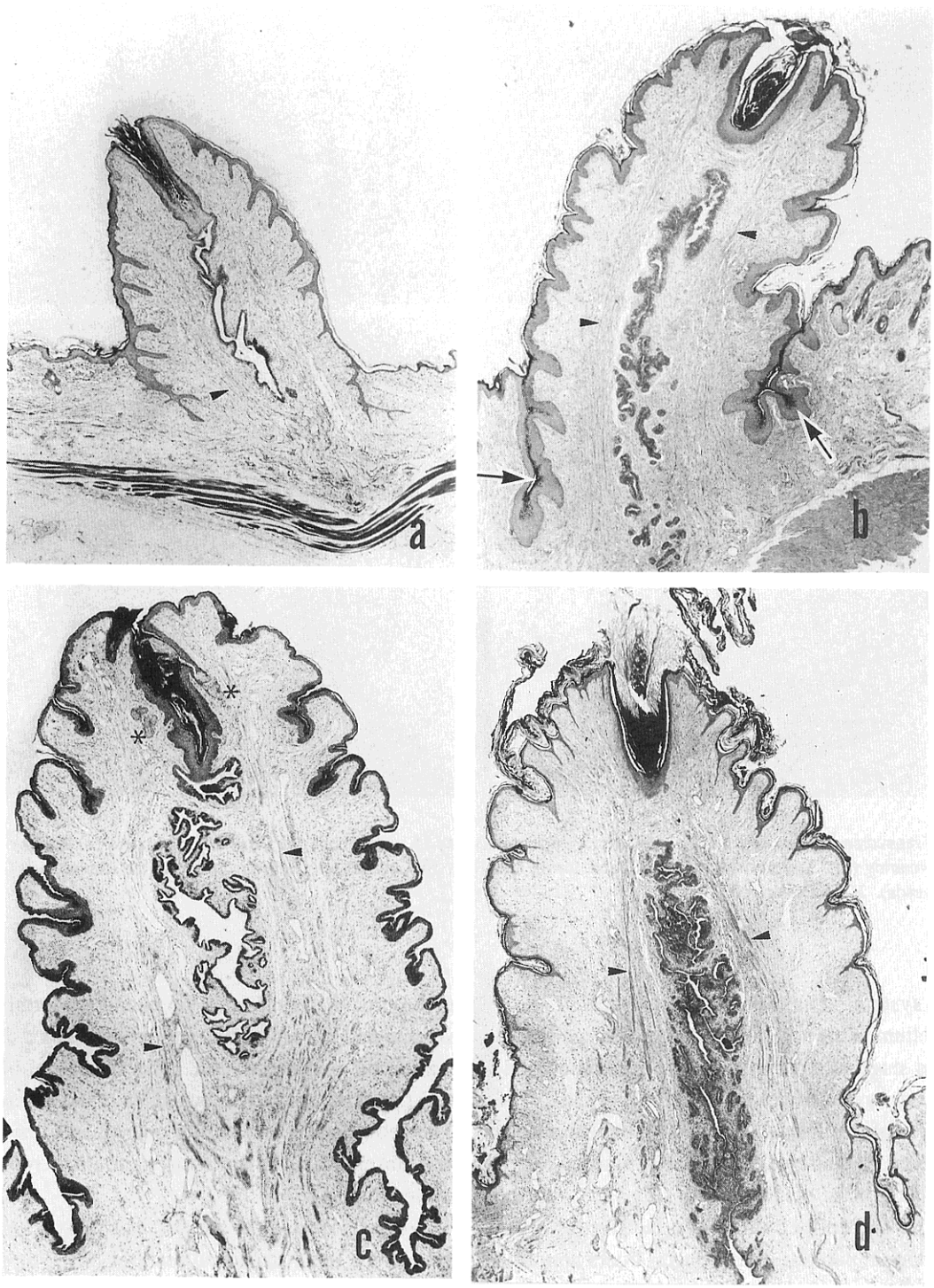

Fig. 2. Longitudinal sections of nipples in the virgin stage (a), day 15 of pregnancy (b), day 10 of lactation (c), and day 5 after weaning (d). The germinative layer of the epidermis ingrows toward the dermis in the basal region of the nipple in virgin and first half of pregnancy. The 'ingrowth' of the germinative layer forms a cleft during the second half of pregnancy (arrows). The reticular layer of the nipple contains smooth muscle cells arranged longitudinally (arrowheads). Sebaceous glands opening to the teat duct are found in the apex of the nipple (asterisks). HE staining. × 30.

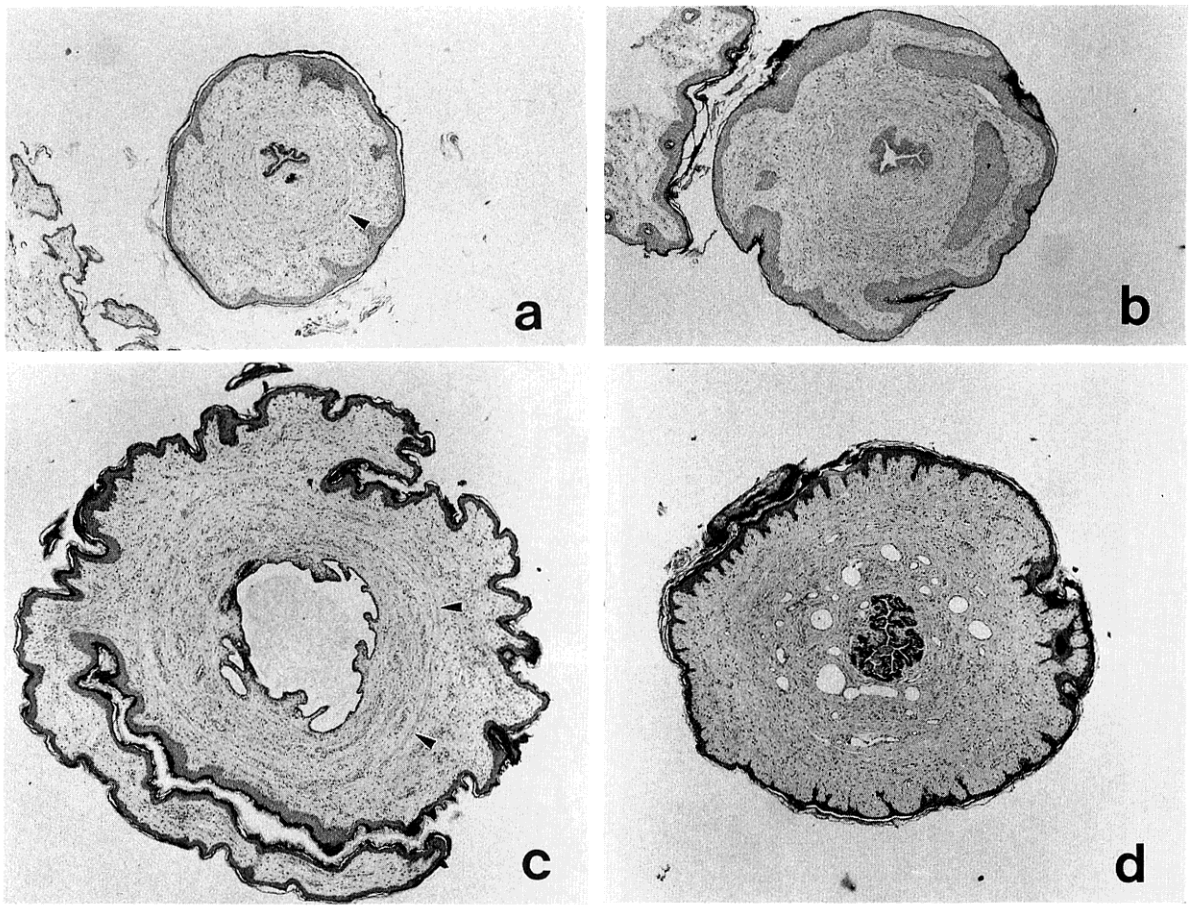

Fig. 3. Transverse sections of the nipples in the virgin stage (a), day 15 of pregnancy (b), day 10 of lactation (c) and day 5 after weaning (d). The lactiferous duct dilates during lactation. Smooth muscle cells surround the lactiferous duct (arrowheads). HE staining. × 35.

one ductal system. The teat duct had stratified squamous epithelium at the top of the nipple. The lactiferous sinus at this stage was narrow and the epithelium consisted of a few layers of columnar cells (Fig. 3a). The reticular layer of the dermis surrounding the lactiferous sinus consisted of dense collagen fibers running longitudinally to the axis of the nipple (Fig. 4a). In the stroma under the epithelium of the lactiferous sinus and in the dermal papillae, the collagen fibers appeared irregular and loose. Smooth muscle cells ran mainly longitudinally and some circular smooth muscles were seen to surround the longitudinal smooth muscles and the lactiferous sinus. Most elastic fibers accompanied longitudinal smooth muscles (Fig. 4a) and the others were irregularly oriented in the dermal papillae. Some elastic fibers also ran along the basal layer of the epithelium of the lactiferous sinus and epidermis. Blood vessels and nerves in the nipple were more developed than in the peripheral skin. The sinusoidal blood vessels ran along the lactiferous sinus in the reticular layer. The sebaceous gland which opened into the teat duct was observed at the apex throughout the reproductive cycle (Fig. 5a).

Pregnant period: In the first half (days 5 and 10) of pregnancy, the histological features of the nipples were similar to those in virgin, except that the nipples were more developed with regard to dermal papillae and penetration of the germinative layer in the basal region. The nipple became noticeably elongated and enlarged, and assumed a columnar shape in the second half (days 15 and 20) of pregnancy (Fig. 2b). The wrinkles in the nipple wall became clear, and about 15 wrinkles could

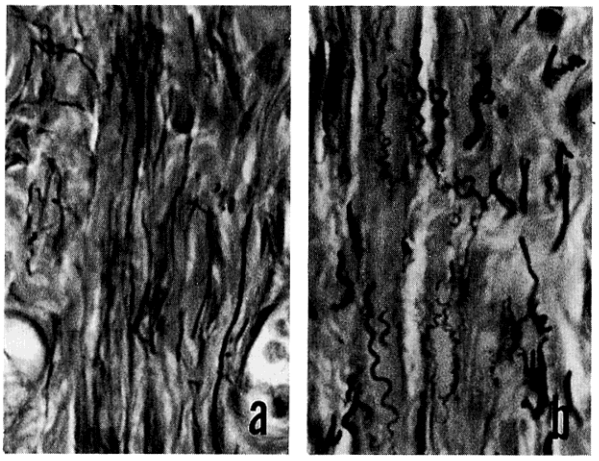

Fig. 4. Collagen and elastic fibers in the nipple walls in virgin (a), and on day 10 of lactation (b). As compared with the virgin stage, collagen fibers in the reticular layer are looser in the lactating stage, but those fibers in the dermal papillae are dense in the lactating and post-weaning stages. Elastic fibers are frizzy in the lactating and post-weaning stages, and thick fibers are found in the post-weaning stage. Elastica van Gieson staining. × 500.

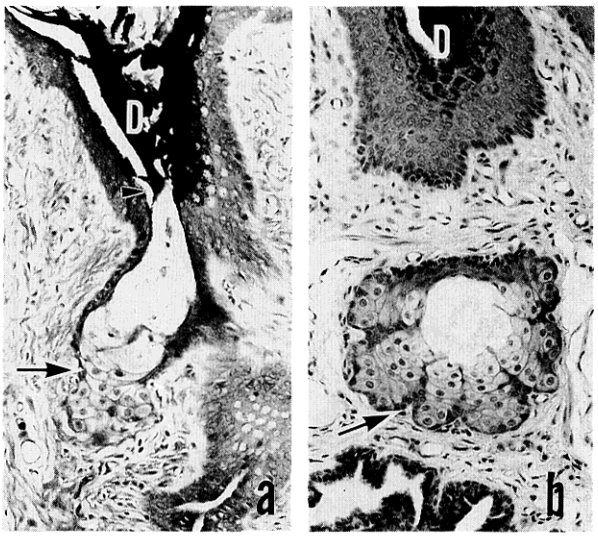

Fig. 5. Sebaceous glands in the virgin (a) and lactation (b) stages. Arrows; sebaceous gland, arrowhead; opening of the gland, D; teat duct. HE staining. × 150.

be counted in the second half of pregnancy. The germinative layer thickened and the cells in the basal layer became denser than in virgin. The epidermis of the teat duct was slightly thickened. The penetrating germinative layer in the base of the nipple formed a cleft. The lactiferous sinus was slightly dilated and the folds in the epithelium increased in number (Fig. 3b). Compared with the nipple of virgin rats, longer elastic fibers increased in number during the second half of pregnancy.

Lactation period: Throughout lactation, the nipple appeared barrel-shaped (Fig. 2c). The number of wrinkles in the nipple wall increased to approximately 30 and secondary wrinkles appeared. The thicknesses of the epidermis at the nipple wall and teat duct were almost the same as those during the second half of pregnancy. The sebaceous gland tended to be more developed during lactation (Fig. 5b). The cleft in the basal region of the nipple became deeper than during pregnancy. The lactiferous sinus lined by cuboidal epithelial cells was largely dilated (Fig. 3c). The folds in the sinus were well developed. Collagen fibers in the stroma of the lactiferous sinus and reticular layer of the connective tissue became loose, and they increased in the dermal papillae (Fig. 4b). Elastic fibers in these stages increased more than those in virgin and pregnant periods, and many frizzy fibers were found in the reticular layer (Fig. 4b). Smooth muscle cells and sinusoidal blood vessels in the reticular layer were well developed, and capillaries in the dermal papillae and under the lactiferous sinus increased in number. The nerves were similar to those in virgin.

Post-weaning period: The appearance of the nipple after weaning was similar to that during lactation, but the germinative layer of the epidermis on the nipple wall and teat duct were similar in thickness to those in virgin (Fig. 2d). On day 5 after weaning, the epidermis of the nipple contained a very thick corneum layer and the teat duct was slightly dilated. Although the lactiferous sinus became narrower than that during lactation (Fig. 3d), many folds in the epithelium remained on day 15 after weaning. Collagen fibers became denser than those during lactation. Elastic fibers on day 5 after weaning were similar to those during lactation, whereas shorter and thicker elastic fibers increased on days 10 and 15 after weaning.

Morphometrical observation

Figure 6 shows the changes in nipple length. The nipple length in virgin rats was 917.6 ± 108.5 μm (mean ± SE). In the first half of pregnancy, there was no significant difference from the virgin period. The nipple

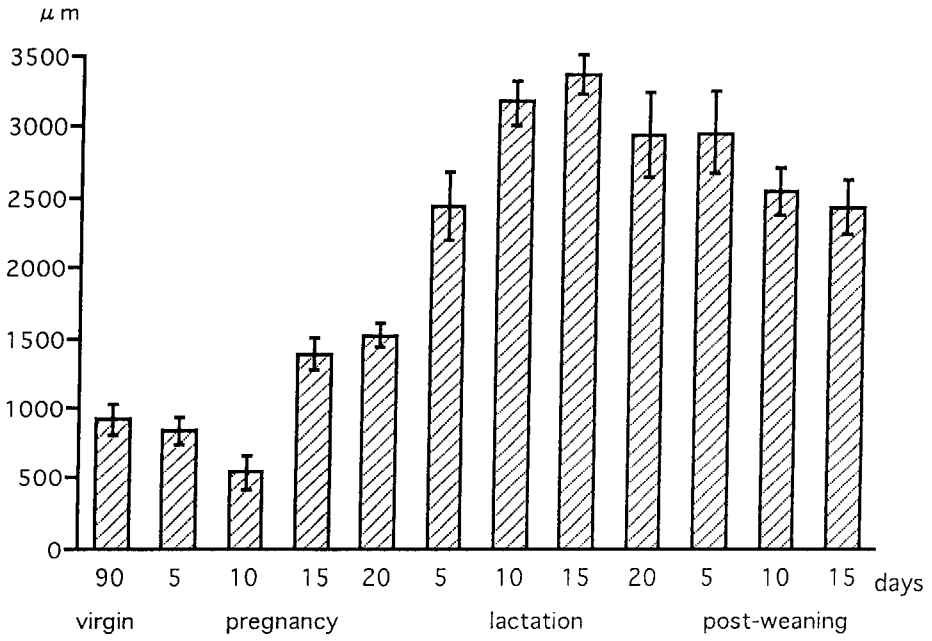

Fig. 6. Changes in nipple length. The nipples after day 5 of lactation are significantly longer than in virgin. There are significant differences between days 10 and 15 of pregnancy, day 20 of pregnancy and day 5 of lactation and days 5 and 10 of lactation. ($p<0.01$)

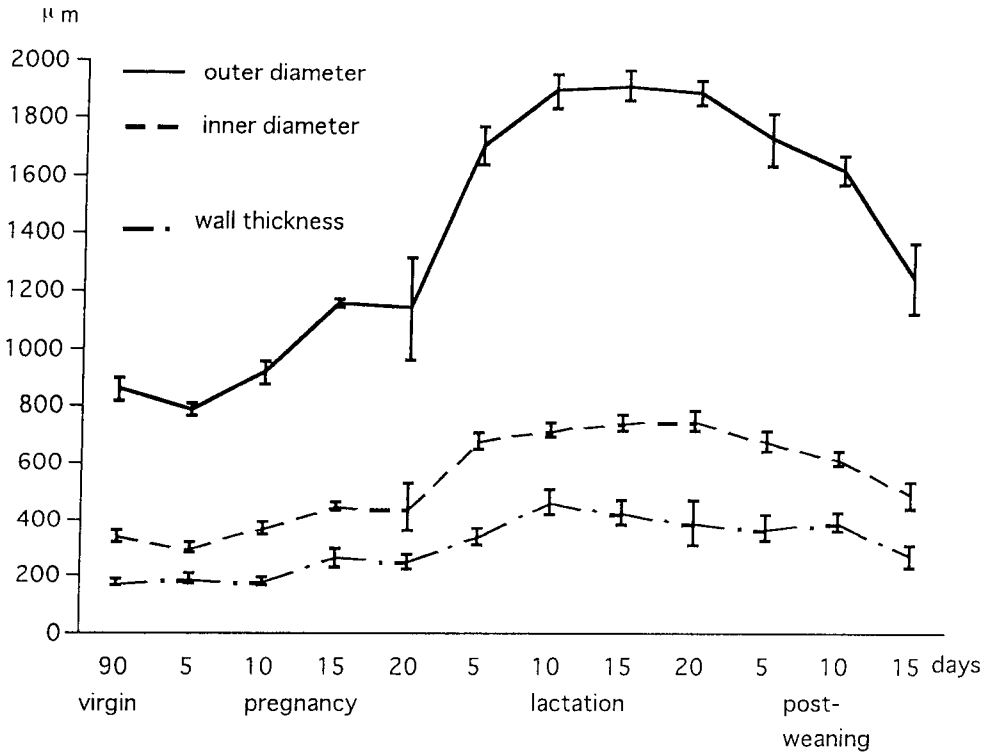

Fig. 7. Changes in nipple sizes in transverse sections. The outer diameter and wall thickness after day 5 of lactation are significantly larger than in virgin. The inner diameter from day 5 of lactation to day 10 after weaning are significantly larger than in virgin. There are significant differences in all parameters between day 20 of pregnancy and day 5 of lactation, and the outer diameter between days 10 and 15 after weaning. ($p<0.01$)

became noticeably elongated from day 15 of pregnancy. The length on day 15 of lactation reached the maximum (3378.3 ± 145.8 μm) which was about 3.7 times that in virgin, then decreased gradually. Figure 7 shows the changes in outer and inner diameter, and thickness of the nipple wall in the transverse sections. The outer diameter increased gradually with pregnancy and rapidly after day 5 of lactation. It reached its maximum (1918.2 ± 523.7 μm) on day 15 of lactation, and then gradually decreased. The changes in the inner diameter and thickness of the nipple wall showed the same tendency as in the outer diameter. They reached the maximum values on day 10 (inner diameter, 463.1 ± 43.4 μm) and day 20 (wall thickness, 751.4 ± 34.3 μm) of lactation, respectively.

Discussion

In the present study, the histological changes in the rat nipple during the reproductive cycle were first described by morphometry. It was clearly demonstrated that the nipple had a dull conical shape in the virgin period and during the first half of pregnancy. The nipple elongated rapidly during the second half of pregnancy and reached the maximal length on day 15 of lactation. This noticeable development of the nipple may be modified by some hormones, although the mechanical expansion by suckling probably contributes to this development during lactation. In the field of mammary gland research, it is well known that the formation of lobules and a decrease in the fat pad in the mammary gland of rats begins on day 10 of pregnancy and then gradually increases until parturition [16]. Tucker and Reece [13] reported that the maximum amount of proliferation in the mammary gland occurred in the second half of pregnancy. Munford [7] also showed that the number of nuclei per alveolar was markedly increased around the time of parturition, and this increase was steady during lactation. The morphological changes in the nipple observed in this study, for example the rapid elongation during the second half of pregnancy, are consistent with those of mammary parenchyma. It is known that many hormones, e.g. prolactin, progesterone, estrogen and other growth factors, control mammary development [4, 12]. Likewise, nipple development may be regulated by these hormones and growth factors.

The nipple wall is a continuation of the skin which is composed of the epidermis, dermis and subcutaneous tissue, but it differs from skin because the nipple wall contains little subcutaneous tissue. The nipple wall during lactation thickened, and many wrinkles arose from the epidermis. They frequently formed secondary wrinkles. The 'ingrowth' of the germinative layer found in the base of the nipple in the virgin period and during the first half of pregnancy formed a cleft from day 15 of pregnancy. A similar finding was reported by Turner and Gomez [15]. The wrinkles could help pups to firmly attach to the nipple and the cleft could relieve invasion in the maternal side by suckling. In our preliminary study with bromodeoxyuridine incorporation and its specific antibody, a number of S-phase cells were found in the epidermis at the base of the nipple on days 10 and 15 of pregnancy (unpublished data), suggesting that this region is the center of cell proliferation which may be responsive to hormones released during the first half of pregnancy.

Raaberg *et al.* [11] reported the existence of a sebaceous gland in the rat nipple and indicated that milk should contain cobalamin secreted by this gland. Cobalamin may play a role in defense against microorganisms. In this study, the sebaceous gland opening into the teat duct was also observed in the apex of the nipple, and was larger during lactation than the other periods. It probably has the ability to secrete a product into milk, but the function of the gland is not yet clear.

The dermis of the nipple was mainly composed of collagen fibers. From the virgin stage to the first half of pregnancy, the reticular layer contained dense collagen fibers and a few elastic fibers. During lactation, however, collagen fibers became loose and elastic fibers were increased in number and frizzed. Kuenzi and Sherwood [5] reported that the ratio of collagen fibers in the rat nipple, neutralized endogenous relaxin by monoclonal antibodies during the second half of pregnancy, was higher than that of control rats. But it was not clear from their results whether relaxin acts on collagen fibers directly or through indirect effects on other tissues. In this study, we demonstrated that the density of collagen fibers is different in the reticular layer from that in the dermal papillae of the rat nipple, so that there is a more complex regulatory system for collagen fiber development locally in the nipple.

The lactiferous sinus was narrow in virgin and the

first half of pregnancy, but was dilated during lactation. The epithelium of the sinus was flat and consisted of columnar cells in virgin and the first half of pregnancy, whereas it was folded and composed of cuboidal cells during lactation. These changes seem to maintain the form of the nipple and respond to dilation of the lactiferous sinus during lactation.

In summary, our observations clearly indicated marked changes in nipple structure during the rat reproductive cycle, which are mainly characterized by hyperplasia of the epidermis and hypertrophy of connective tissue in the dermis in the second half of pregnancy. All the structures, i.e. the wall, the ingrowth of the germinative layer, the dermis, and lactiferous sinus, appeared to change to adapt for nursing pups.

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