

## A Scanning Electron Microscopic Study of Dermal Collagen Fibers in Growing Mink

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**Abstract** Morphological changes in dermal collagen fibers in growing mink were investigated using a scanning electron microscope (SEM).

The SEM view of dermal collagen fibers varied throughout the newborn, growing and mature skin phases. The reticular layer of newborn mink dermis was plate-like, and appears to be composed of fine collagen fibers, proteoglycans and glycoproteins. With mink growth, randomly entangled dermal collagen fibers, which were relatively fine and curled or waved, showed regularity of the three-dimensional network.

A three-dimensional network of thick collagen fibers was observed in the mature mink dermis, and the fiber arrangement altered in relation to the depth from the epidermis, i. e., in the upper dermis, many fibers were arranged diagonally to the skin surface, whereas in the mid and deep dermis, thick fibers were conspicuously arranged parallel to the skin surface. The collagen fiber network was formed by repeated branching and anastomosis of the fibers.

There were thin elastic fibers in the dermal-epidermal junction and between the collagen fibers and skin appendages.

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### INTRODUCTION

In mammals, collagen is the most abundant structural constituent of the dermis, accounting for approximately 70~75% of the dry weight of the skin. However the content of skin collagen is not constant throughout life, and age-related qualitative and quantitative changes have been reported (KORN and SCHNIDER, 1989). In the mink, the skin varies both biochemically and histologically, that is, the dermal thickness and collagen content change not only with growth but also the hair cycle, which includes seasonal molting (NISHIUMI *et al.*, 1986; KONDO and NISHIUMI, 1991).

It is also well known that dermal collagen fibers present a three-dimensional organization. Changes in the quality and quantity of collagen are naturally correlated with collagen fibrillar organization. However, the arrangement of collagen fibers in the mink dermis is far fully understood and, in particular, their three-dimensional organization has yet to be analysed.

The present study reports the age-related changes in the three-dimensional arrangement of collagen fibers in the dermis of mink, as revealed by scanning electron microscopy

(SEM). Although the structure of connective tissue fibers as revealed by SEM is disturbed by the presence of hampering cellular and extracellular amorphous elements such as glycosaminoglycans, these substances were removed by the method of MEYER *et al.* (1982).

## MATERIALS AND METHODS

Male Sapphire mink, all originating from the same sire, born around May 8, 1985, and raised under identical conditions were selected and sacrificed at a rate of one animal per week until December 6, 1985. The animals were frozen immediately after sacrifice in order to prevent as much as possible changes in the structural elements of the skin that body temperature might cause.

Skin samples were excised from the dorsal region after hair removal. Each sample, measuring approximately  $5 \times 5 \times 3$  mm, was immersed and frozen in OCT compound (Tissue-tek, MILES), and sagittal and horizontal sections were cut at a thickness of 50~60  $\mu$ m on a cryostat (DAMON) at  $-20^{\circ}\text{C}$ . The sections were then placed on a circular coverglass 10 mm in diameter.

In order to treat the sections for SEM, we applied a modification of the enzyme treatment method of MEYER *et al.* (1982). The sections were incubated with 0.1% hyaluronidase (type IV-S, SIGMA) in 50 mM Tris-maleate buffer (TMB, pH 5.4) for 5 h at  $37^{\circ}\text{C}$  and afterwards rinsed in TMB twice for 2 min. The sections were incubated with 0.1% bacterial crude  $\alpha$ -amylase (type IX-A, SIGMA) in 50 mM TMB for 12 h at  $37^{\circ}\text{C}$  and rinsed in distilled water twice for 2 min. They were then fixed with 10% formalin and serially dehydrated in ethanol (70~100%). To minimize distortion, the dehydrated sections were immersed in xylene (TSUJI *et al.*, 1979) and finally dried in a desiccator.

The specimens were attached to brass standard stubs with silver paint (Q. D. Colloidal Silver, BIO-RAD). The prepared stubs were then coated with a thin layer of gold using an ion-sputtering apparatus (JFC-1100, JEOL). Observations were made with a JEOL JSM-T20 scanning electron microscope operated at 19 kV.

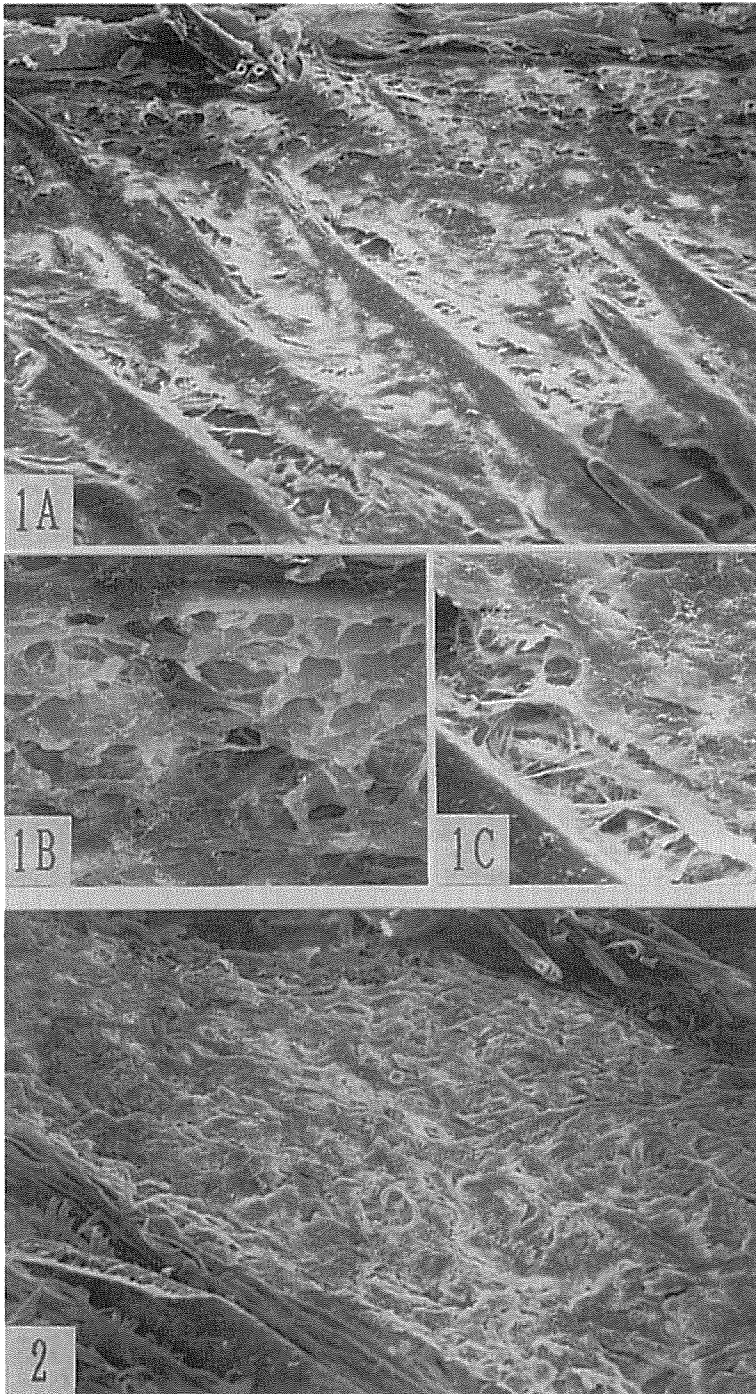
## RESULTS

### *View of dermis in infancy*

The SEM view of newborn mink dermis showed a flat appearance without any clear fibrous structure (Fig. 1A). The squashed mid and deep dermis lost its solidity, and the surface of the coverglass was evident in some places. On the other hand, the upper dermis below the epidermis appears sponge-like but its framework was membranous (Fig. 1B). Detailed observation revealed fine fibrillar networks in the sponge-like structure (Fig. 1B), and also thin fibers were observed around the hair follicles (Fig. 1C). There were two kinds of fiber, one extending straight from the hair follicle to the dermis and the other looped in the dermis (Fig. 1C).

### *View of dermis during growth*

As shown in Fig. 2, the solidity of skin sections increased with mink growth until the 10th week. The fibrous structure of collagen in the dermis was partially evident, but most of the dermis was covered with amorphous substances. A sponge-like structure in the upper dermis below the epidermis was observed, but the three-dimensional organization and/or ar-



- Fig. 1. Scanning electron micrographs of sagittal sections of infant mink dermis (2 weeks old). Lack of a clear fibrous structure in the whole dermis (A,  $\times 150$ ), a sponge-like structure of the fine fibrillar network in the upper dermis (B,  $\times 450$ ), and thin fibers around a hair follicle in the mid dermis (C,  $\times 450$ ) are evident.
- Fig. 2. Scanning electron micrograph of a sagittal section of growing mink dermis (8 weeks old). The fibrous structure of collagen is partially evident.  $\times 200$

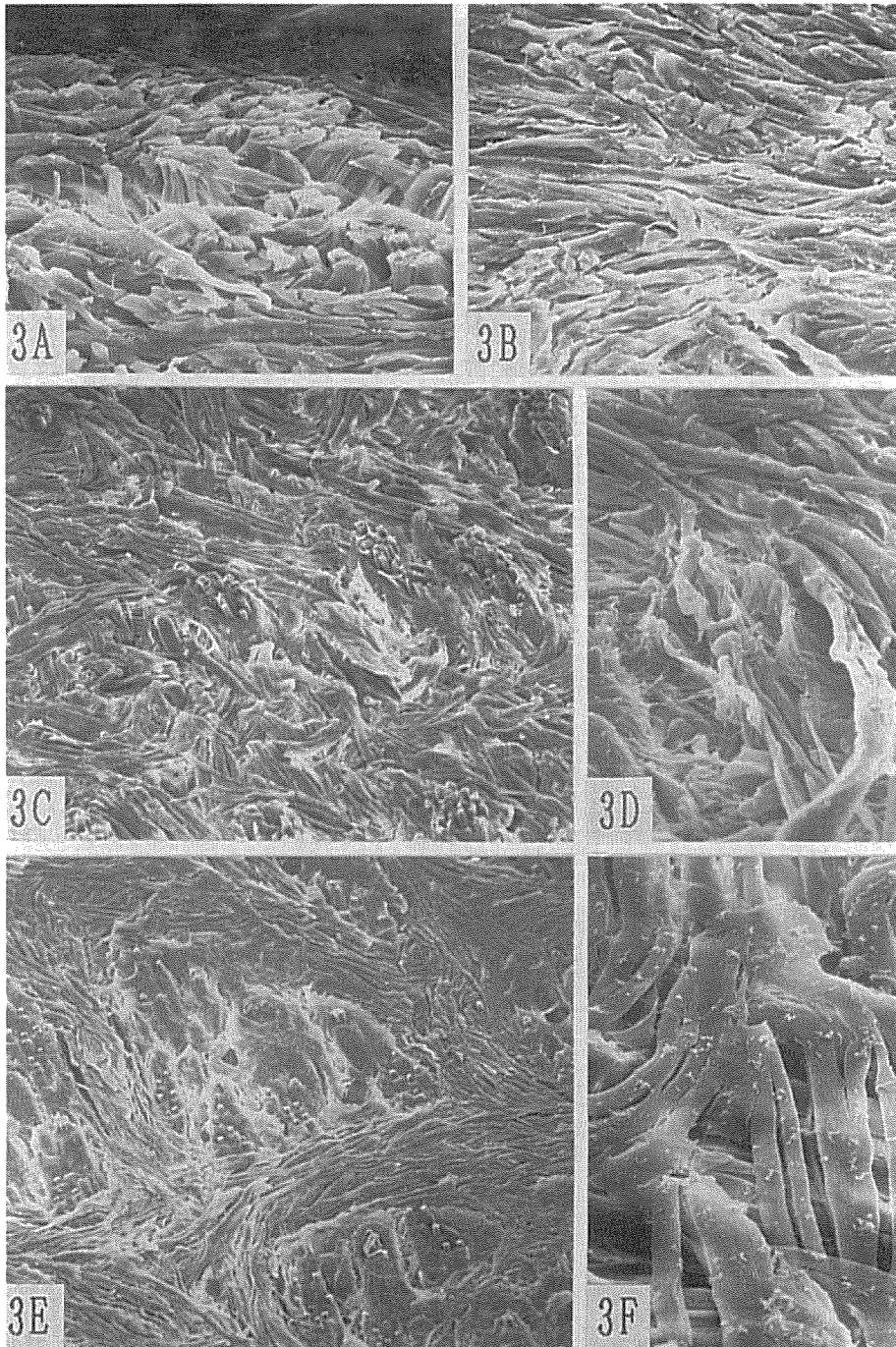


Fig. 3. Scanning electron micrographs of mature mink dermis (12 weeks old). In sagittal sections (A and B), collagen fibers cross each other in two main directions, which run diagonally to the skin surface in the upper dermis (A,  $\times 450$ ), and collagen fibers are arranged parallel to the skin surface in the mid and deep dermis (B,  $\times 300$ ). In horizontal sections (C, D, E and F), one direction of the collagen fibers in the upper dermis is parallel to the hair follicle, and other crosses it almost at right angles (C,  $\times 150$ ); branching and anastomosis of these fibers are evident at high magnification (D,  $\times 875$ ). The orientation of collagen fibers in the mid and deep dermis is predominantly horizontal, and the collagen fiber bundles with branching and anastomosis are interwoven among hair follicle groups (E,  $\times 80$ ; F,  $\times 440$ ).



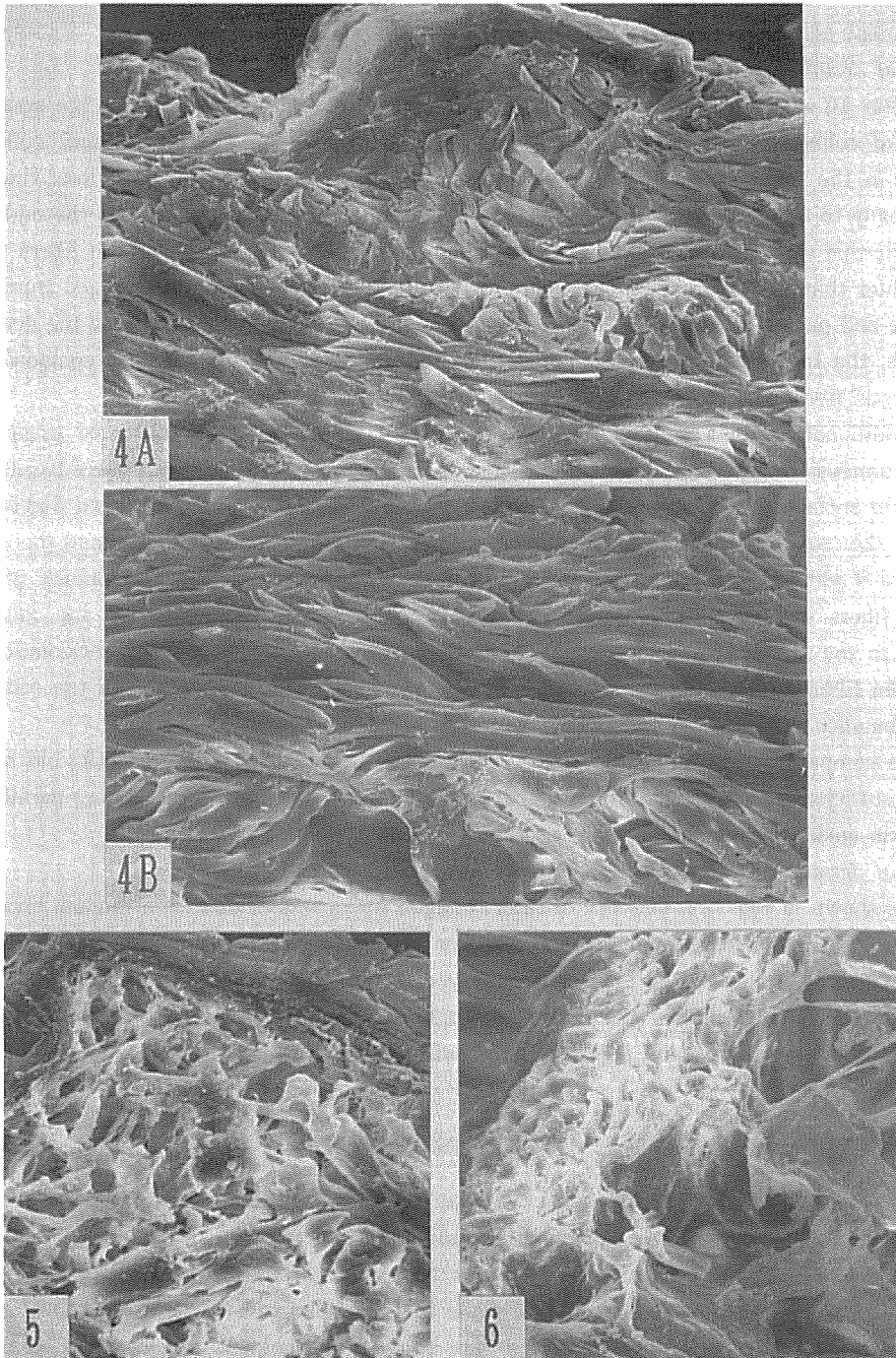


Fig. 4. Scanning electron micrographs of sagittal sections of sufficiently mature mink dermis (30 weeks old). A thicker collagen fiber arrangement parallel to the skin surface and a decrease in the interfiber space are evident in both the upper dermis (A,  $\times 450$ ) and mid and deep dermis (B,  $\times 450$ ).

Fig. 5. Scanning electron micrograph of a sagittal section of the dermal-epidermal junction. Extremely thin elastic fibers are connected with dermal collagen fibers. 14 weeks old.  $\times 700$

Fig. 6. Scanning electron micrograph of a sagittal section of the subcutis. A twist of fine collagen fibers is evident. 24 weeks old.  $\times 350$

rangement of collagen fibers was not evident.

#### *View of dermis at maturity*

After 10 weeks of age, the dermis was filled with collagen fibers, although the arrangement of collagen fibers varied with depth from the dermis. In sagittal sections, collagen fibers in the upper dermis generally crossed each other in two main directions running diagonally to the skin surface (Fig. 3A), and almost all of the collagen fibers in the mid and deep dermis were arranged parallel to the skin surface (Fig. 3B). The collagen fibers were straighter than hitherto, and single fibers, or smaller fiber bundles, often emerged from one bundle and passed into another, forming a closely interwoven dense network. In the deepest dermis, the transitional region of the dermis and subcutis, plate-like bodies composed of numerous fibers side by side were observed.

Additional sections parallel to the skin surface were studied in order to grasp the three-dimensional network of collagen fibers. In the upper dermis, collagen fibers parallel to the skin surface crossed each other, forming a lattice, while the fibers arranged diagonally to the skin surface showed two directions; one was parallel to the hair follicle and the other crossed it almost at right angles (Fig. 3C). In addition, branching and anastomosis of collagen fibers were observed, as in the sagittal sections (Fig. 3D). Therefore, the collagen fibers in the upper dermis showed a complicated stereolattice network. The orientation of collagen fibers in the mid and deep dermis was predominantly horizontal, and the collagen fiber bundles were interwoven among hair follicle groups (Figs. 3E and 3F).

As compared with the situation of 12 weeks of age, the dermis in 30 weeks old mink, when sufficiently mature, had thicker and more abundant collagen fibers running parallel to the skin surface, and the interfiber space decreased (Figs. 4A and 4B).

#### *View of dermal-epidermal junction and subcutis*

As shown in Fig. 5, a network of finer collagen fibers was evident as a random arrangement at the dermal-epidermal junction, and the extremely thin fibers which connected dermal collagen fibers with the epidermis appeared to be elastic fibers.

Although penetration of the dermis through the subcutis was observed as a band by light microscopy, this region appeared as a twist of fine collagen fibers, like a rope, using SEM (Fig. 6).

## DISCUSSION

The present SEM study has confirmed the alterations in the shape and arrangement of dermal collagen fibers during the growth of mink.

In newborn mink dermis, fewer collagen fiber bundles were demonstrated in a light microscopic study (NISHIUMI *et al.*, 1986), while the plate-like substances composed of a felt adhering to much thinner collagen fibers were apparently dominant in this region by SEM. Recently it was reported that hyaluronic acid, which is the most common glycosaminoglycan in the dermis, was combined with collagen fibers and glycoproteins such as fibronectin (ISEMURA *et al.*, 1982), and that collagen fibers formed plate-like substances in the presence of large quantities of hyaluronic acid (DOCHERTY *et al.*, 1989). It was also recognized that a felt-like membranous organization of collagen fibrils was built up in the presence of proteoglycans or glycosaminoglycans *in vitro* (VOGEL *et al.*, 1984). In addition, the highest pro-

teoglycan content was found in newborn mink skin (NISHUMI *et al.*, 1991). These findings suggest that the plate-like substance in newborn mink dermis may be built up by a close combination of collagen fibers with proteoglycans and glycoproteins, and therefore this substance may have been difficult to dissolve, even the enzymes we used.

With mink growth, the plate-like substances in the dermis were transformed into a regular three-dimensional network of collagen fibers via randomly entangled collagen fibers, which were relatively fine and curled or waved. Although the three-dimensional network was maintained after the age of 10 weeks, an increase of collagen fiber diameter and a decrease of the interfiber space were observed, confirming the morphological changes in collagen fibers with skin aging (PFALLER *et al.*, 1979; LAVKER *et al.*, 1987).

The present study also showed that the collagen fiber arrangement producing the three-dimensional network in mature mink dermis altered in relation to depth from the epidermis. The arrangement of dermal collagen fibers has been investigated extensively, and is reported to depend mostly on the two-dimensional construction of a scissors lattice or a three-dimensional stereolattice network, but is still under discussion (GIBSON *et al.*, 1965; BROWN, 1972; PFALLER *et al.*, 1979; MEYER *et al.*, 1982; MEYER and NEURAND, 1987; LAVKER *et al.*, 1987). According to MEYER *et al.* (1982) and MEYER and NEURAND (1987), the bulk of the dermis is dominated by a massive three-dimensional network of collagen fibers, which cross each other in two directions. The present study showed that in the upper dermis, collagen fibers ran diagonally across each other, as in previous reports, whereas those in the mid and deep dermis were conspicuously arranged in parallel to the skin surface.

The collagen fiber network in mink dermis is not formed by piles of sheets, but by the three-dimensional architecture of collagen fibers crossing each other, with repeated branching and anastomosis. This architecture appears to contribute to the increase in solidity of the mink dermis in spite of its relative thinness in priming time and then to subsequent to increase in strength of mink fur skin.

It is evident that the elasticity, power to absorb shock, extensibility and tension of the dermis are not completely dependent on the collagen fiber network, as mentioned above. This is especially true when considering that the longitudinal extensibility of collagen fibers and fibrils is not very great (ELDEN, 1980). It is the elastic component, i. e., the elastic fibers forming a wide-meshed sponge throughout the whole dermis, that creates constant tension of the skin (MEYER *et al.*, 1981). Although the elastic fibers constitute 2~5% of the dry weight of the dermis (KLIGMAN and BALIN, 1989), it has been observed by SEM that the elastic fibers occupy a considerable amount of the dermal compartment (TSUJI *et al.*, 1979; MEYER *et al.*, 1981), suggesting that the elastic fibers are extremely light. Our investigation of elastic fibers using conventional orcein-stained sections (KONDO *et al.*, 1991) gave an impression that the fibers were rather sparser than those mentioned in the previous SEM reports. It seems that the collagen fibers, making up the great bulk of the dermis, evidently mask the minor elastic component. Therefore, it was difficult in our SEM observations to identify the elastic fibers, since the dermal collagen fibers were not removed. IMAYAMA and BRAVERMAN (1989) illustrated the increasingly complicated entanglement of both collagen and elastic fibers with aging, and proposed that this fiber entanglement enabled the dermis to sustain a higher stress. In the present SEM observations, this entanglement could not be

confirmed, although very fine elastic fibers were arranged perpendicularly to the skin surface at the dermal-epidermal junction and horizontally between collagen fibers and skin appendages, e. g. hair follicles and skin glands. It is assumed that the elastic fibers at the dermal-epidermal junction may function as a cushion against external pressure, although the roles of these fibers in this region are still uncertain. On the other hand, the elastic fibers interconnecting the skin appendages with collagen fibers appear to play a role in the anchorage and maintenance of these appendages.

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## ミンクの成長における真皮コラーゲン線維の走査型電子顕微鏡観察

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ミンクの成長における真皮コラーゲン線維の形態学的変化を, 走査型電子顕微鏡 (SEM)を用いて研究した。

SEM 観察から, 真皮コラーゲン線維は, 出生直後, 皮膚成長期, 皮膚成熟期の3つの段階を経て変化することが示された。出生直後のミンク真皮はプレート状物質が中心であり, プロテオグリカンや糖タンパク質とコラーゲン線維が密接に結合してプレート状物質を形成したものと考えられる。ミンクの成長に伴い, 真皮コラーゲン線維は, 比較的細い, 曲がりくねった線維がランダムに絡まり合った様子に変化した後, 規則正しい三次元的ネットワークを示した。

コラーゲンの三次元的ネットワークは, 成熟した真皮に観察され, そのときのコラーゲン線維の配向状態は, 真皮の深さの程度により異なった。すなわち, 真皮上部のコラーゲン線維は皮膚表面に対して斜めに配向し, 一方真皮中部から下部では, 比較的太いコラーゲン線維が皮膚表面に対して平行に配向した。各々のコラーゲン線維は, さらに, 分岐・合流して, 三次元的ネットワークを形成した。

真皮・表皮接合部およびコラーゲン線維と皮膚付属器官の間には, 細い弾性線維のネットワークが存在した。