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SEM demonstration of elastic fibres in the integument of small and densely-haired mammals

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The combination of SEM and autoclave methods gave a clear three-dimensional demonstration of integumental elastic fibres in small densely-haired mammals. The specific organisation of a fine and spongy elastic network was characterised by uniformly thin elastic fibres which were homogeneously distributed between both hair follicle types throughout the whole dermis. All the hair follicles were connected with each other by elastic fibres along their complete intradermal length. The advantage of such a specific elastic dermis construction is that all hair follicles can be moved together and simultaneously along the entire body, so that a better and rapid insulation is achieved after erection of the hair follicles during very low temperatures.

key words: elastic fibres, integument, small densely-haired mammals, SEM, autoclaving, skin biology

INTRODUCTION

The dermis of the mammalian integument is specifically constructed to resist mechanical strain, whereby the elastic fibres with their high capacity of expansion and relaxation are responsible for the physiological elasticity of this skin layer. This implies that the dermis permits remarkable elastic deformations by requiring only low forces, considering that such abilities may also be directly correlated with the architectural arrangement of the fibrous tissue in the dermis [1,8,11–13]. Relevant methods to visualise the specific three-dimensional distribution of elastic fibres have been developed, however, relatively lately and, in particular, by using autoclave technique together with scanning electron microscopy [6,8,16]. Based on the improvements obtained by such a comprehensive methodical approach, and with regard to the fact that the number and arrangement of elastic fibres in the integument varies as related to the animal species and groups investigated [8,10], the present study was designed to provide first information about the specific conditions of the elastic element in small and densely-haired mammals. In this way preliminary results [8] are corroborated to some extent from a comparative point of view.

MATERIAL AND METHODS

For this study the following small and densely-haired domesticated mammalian species were used: 1. Rodentia — laboratory rat (Han/DA, Han/Wist; 8 females, 2 males), laboratory mouse (Han/Wist, 6 females), guinea pig (several mixed breeds; 8 females, 2 males); 2. Lagomorpha — rabbit (several mixed breeds, German Giant; 6 females, 2 males). Skin specimens were generally taken from the hairy skin (integumentum commune) of adult animals of medium age and three different body regions (back, flank, abdomen), and processed as described below. The skin material was obtained with the help of several institutes and clinics of the Hannover School of Vet-

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erinary Medicine. Sections of the dermis, approximately 1 mm thick, were placed in Aqua dest. and autoclaved at 110° C and 103 kPa for 6–10 hrs in a high pressure autoclave system (Keller, type S-ECZ) [8,16]. After autoclaving, the skin specimens were immediately immersed in Karnovsky's fluid for 4–6 hrs at room temperature, and afterwards rinsed in 0.1 M cacodylate buffer (pH 7.4) for 1 hr at +4°C. After careful serial dehydration in graded ethanol (20–100%, +4°C), the tissue was placed in 100% xylene, and then dried very slowly for several weeks in a xylene saturated atmosphere in small, partly perforated glass jars [5]. After dehydration, several specimens were also critical-point-dried through CO_2 (Polaron E 3100, Ser. I). Finally, all specimens were sputtered with gold (Balzer SCD 040), and viewed in the Zeiss DSM 940 scanning electron microscope.

The efficiency of the autoclaving procedure, i. e. the removal of collagen, was controlled by standard light microscopical methods, i.e. after fixation in Bouin's fluid, paraffin embedding and orcein staining [2,6]. The possible influence of shrinkage artefacts on structure demonstration by SEM methods was evaluated with the help of a fluorescence staining (basic fuchsin) of formalin-fixed frozen sections [8].

RESULTS

From the methodical point of view; it has to be emphasised that xylene vapour drying of the specimens was more helpful than critical-point-drying. After the latter procedure, often more or less fine crumbly debris could be found within the specimens (see Fig. 5), which sometimes became detached and moved uncontrollably between the structures present so that, for example, the taking of photographs was disturbed.

Elastic fibres were very numerous and branching in the relatively thin dermis (200–500 μm) of the small fur-bearing mammals studied. The rather fine elastic meshwork formed was structurally continuous in the upper and mid-dermis. This specific close-meshed elastic net very distinctly anchored and connected the numerous primary and secondary hair follicles present. The uniformly thin elastic fibres were homogeneously distributed between both hair follicle types throughout the whole dermis, and all hair follicles were connected with each other by elastic fibres along their complete length within the skin (Figs. 1, 2A). In addition, the hair follicle groups were completely surrounded by a fine elastic net anchoring in the connective tissue sheath of the hair follicles (Fig. 1C). The normally inconspicuous deeper dermis in small densely-haired species was characterised by a clearly more wide-meshed and very loosely structured elastic system. This dermis part was integrated into the connective tissue of the hypodermis, with no distinct border zone between the two skin layers. In both mammalian groups studied, the elastic fibres then were always closely interwoven with the elastic sheath of the cutaneous muscle.

When the different species studied were compared, it became obvious that the rodent species, especially the Myomorpha (mouse, rat) had the finest elastic system in the dermis, whereas the rabbit, as a typical lagomorph species with a lower hair density, showed rather coarse elastic fibres. Within the rodent group, the arrangement of elastic fibres in the dermis seemed to be more strictly horizontally in the very densely-haired murid species than in the comparatively more sparsely-haired guinea pig.

Regional differences in the arrangement and content of elastic fibres could be detected because the amounts of elastic tissue were related to hair density, i.e. in body regions with very high numbers of hair follicles, such as the abdomen, elastic fibres were most abundant and formed broader sheet-like structures (Fig. 2B).

DISCUSSION

The study was based on a methodical approach that combined SEM and autoclave methods to give a better three-dimensional representation of the elastic fibre component of the skin, including rather realistic spatial relationships of all the skin structures present [8]. In connection with the fact that the autoclaving had to be applied to a very thin integument, as followed by fixation in Karnovsky's fluid and dehydration, xylene vapour drying of the specimens [5] was superior to the critical point drying procedure, because in this way fine crumbly debris within the specimens could be avoided.

The specific organisation of the integumental elastic network as observed in the small and densely-haired mammals studied (rat, mouse, guinea pig, rabbit; hair density 3000–25000 H/cm^2; [9,15,17]), was characteried by uniformly thin elastic fibres which were homogeneously distributed between both hair follicle types throughout the whole dermis. This finding was in contrast to observations from the elastic meshwork in medium sized densely-haired species with a somewhat thicker integument (cat, dog, sheep, goat; hair density 2000–15000 H/cm^2; [7,14]), where the elastic fibres form a typical elastic mat with horizontal fibres that anchors and connects

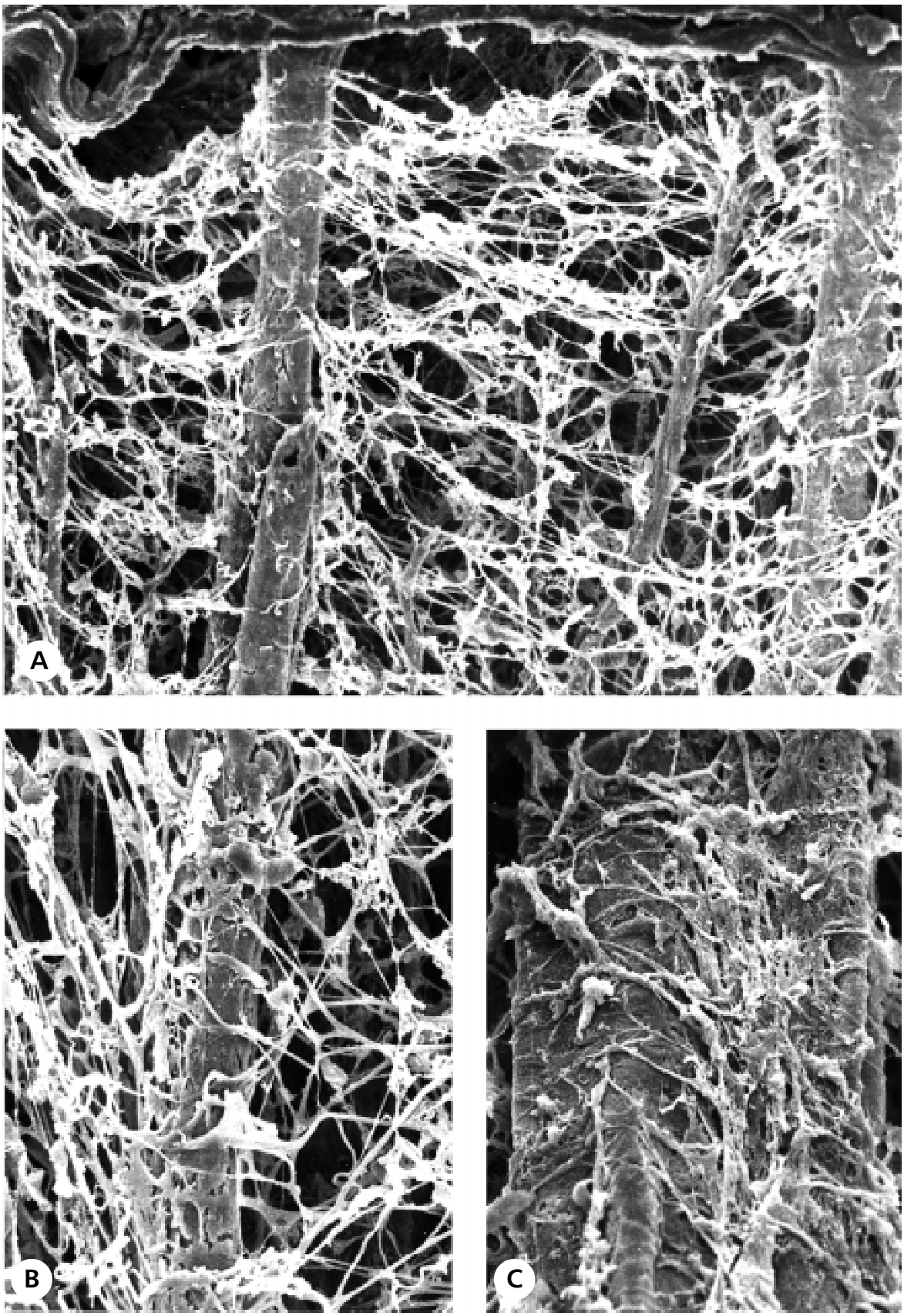
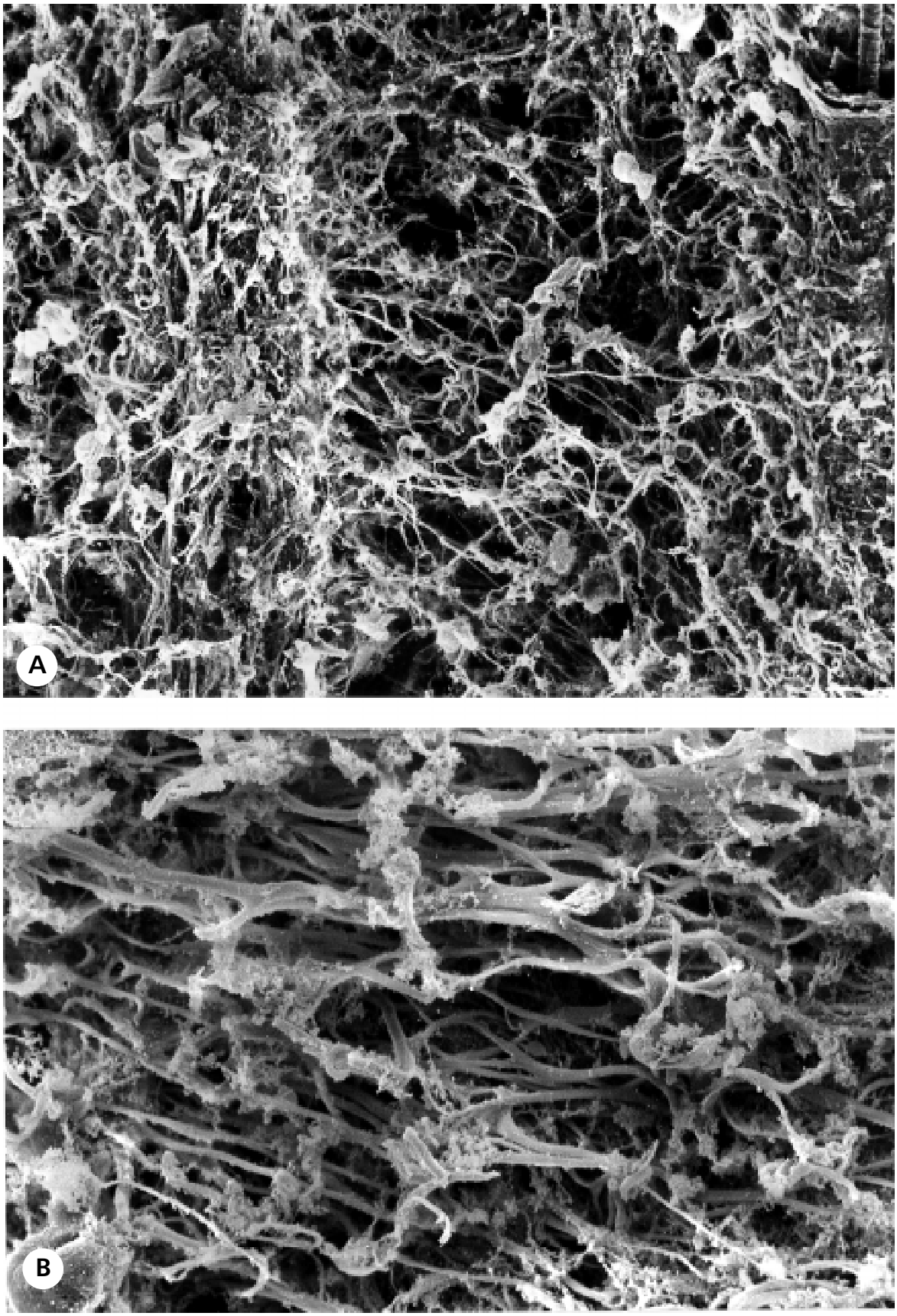


Figure 1. Elastic fibres in the dermis of the rat; A) homogeneous distribution of horizontally arranged elastic fibre in the upper dermis of the dorsal body region, × 425; B) anchoring of a secondary, hair follicle in the fine elastic net of the mid-dermis, × 850; C) closely surrounding elastic net of a hair follicle group; × 850.



Figures 2. Elastic fibres in the dermis of the guinea pig; A) fine but only weakly horizontally arranged elastic fibre in the upper dermis of the dorsal body region, x 360; B) sheet-like structure of elastic fibres in the mid-dermis of the abdominal body region, note fine crumbly debris after critical point drying, x 860.

the hair follicles especially in the upper part of the mid dermis [8]. In large and sparsely-haired mammals (pig, cow, horse; hair density 250–1300 H/cm^2; [3,4,7], additionally, sheet-like elastic elements are found at the border zone of dermis and hypodermis [4,6,8].

The advantage of such a specific elastic interweaving of hair follicles in the dermis as found in small densely-haired mammals is that all primary as well as secondary (wool) hair follicles can be moved together and simultaneously along the entire body, so that a better and rapid insulation by the hair coat is achieved, considering that the primary hair follicles have to be erected by their arrector pili muscle during very low temperatures. Thus, the secondary hair follicles without such muscle could easily follow this movement. When the arrector pili muscles relax, all hair follicles are brought back to their former position by the contraction abilities proper of the elastic fibre system. This functional aspect of normal skin biology seems important, in particular, for small mammals, because they have a comparatively larger body surface area than medium-sized and large mammals where heat loss is concerned. Especially the latter and normally sparsely-haired animals, thus, can use their often rather massive elastic fibre network of the dermis in a completely different way from the small mammals. This pertains, particularly, to the laterial and abdominal integument in the horse and the cow, which has to rely on high extensibility because of the varying intestinal volumina during feeding [8].

In conclusion, it becomes obvious that it is not the content but the arrangement of elastic fibres in the skin of mammals which is one of the dominant factors for the specific skin biology of the different mammalian groups.

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