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Lysozyme Distribution in Healthy Human Skin

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Lysozyme is present in human skin as well as in other tissues and secretions [2, 4]. Previous personal investigations [1] demonstrated lysozyme activity of between 85 and 195 μg/g wet weight (average 142 μg/g wet weight) in the skin of clinically healthy subjects.

Indirect immunofluorescence studies [3] revealed that lysozyme is mainly located in the cytoplasm of epidermal cells and that only small quantities are present along the dermal collagen bundles.

Forty hospitalized volunteers of both sexes, aged between 19 and 71 years, were studied. All were free from clinical infections and had only limited traumatic or proliferative skin lesions.

Lysozyme activity was determined in both serum and 0.5 mm thick samples of skin taken from the lumbar region and treated according to Binazzi et al. [1].

Histochemical studies were carried out on fine frozen sections of skin removed from the same area and/or from the internal surface of the forearm in an identical condition. Immunofluorescence was performed by the routine technique using anti-human milk muramidase rabbit IgG (Dakopatts, Denmark) and ITCF-labeled anti-rabbit IgG swine serum (Dakopatts, Denmark). The peroxidase conjugate method (Table 1) was used for the immunoperoxidase studies. Controls were performed by absorbing the primary anti-human muramidase antiserum on purified human milk lysozyme before immunostaining, thus blocking the reactivity for muramidase.

Lysozyme activity was found to be between 1.25 and 18 μg/ml (average 5.15 μg/ml) in serum and between 75 and 198 μg/g wet weigth (average 119.5 μg/g wet weight) in skin samples. These results did not show significant differences with respect to those previously reported [1].

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Table 1. Peroxidase conjugate method

1. 5 μm frozen sectons;
2. fixed in 2% paraformaldehyde for 5–10 min;
3. washed in PBS at pH 7.2 for 20 min;
4. incubated with 1/20 normal swine serum for 10 min;
5. incubated with 1/10 antimuramidase rabbit IgG (Dakopatts, Denmark) for 4 h at 37°C;
6. washed in Tris-HCl-buffer at pH 7.6 for 1 h;
7. incubated with 1/20 antirabbit IgG swine serum conjugated with horseradish peroxidase (Dakopatts, Denmark) for 30 min;
8. washed in Tris-HCl-buffer at pH 7.6 for 30 min;
9. 3-3′ Diaminobenzidine (Sigma, USA) reaction for 5 min;
10. counterstained with hematoxylin and mounted in buffered glycerine

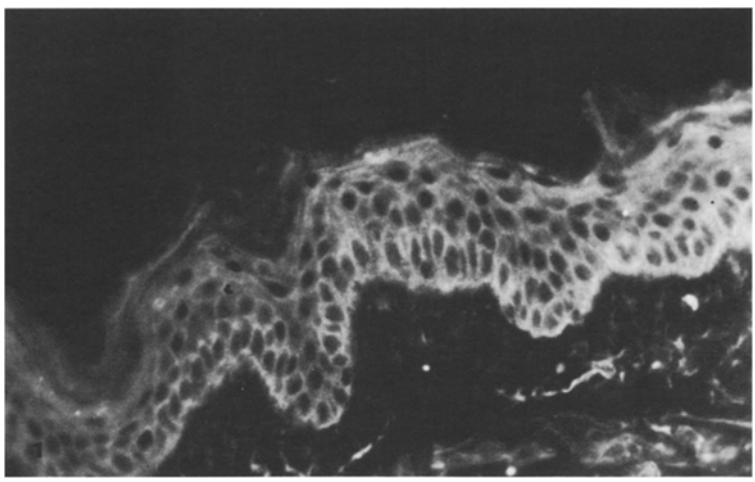

Fig. 1. Lysozyme as revealed by immunofluorescence in epidermal cells. Indirect immunofluorescence, ×80

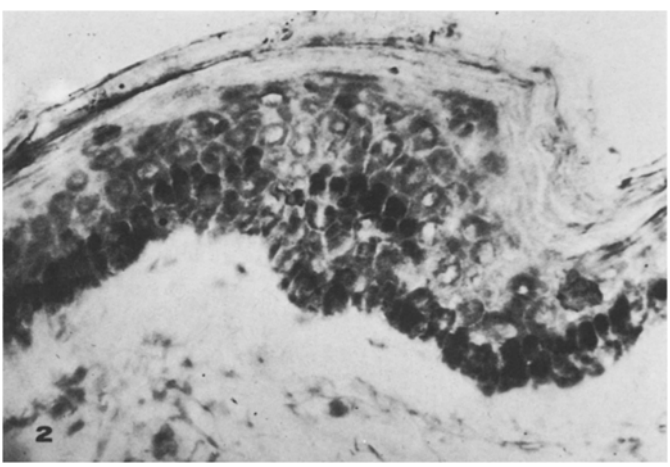

Fig. 2. Lysozyme in epidermal cells demontrated by the immunoperoxidase technique. Immunoperoxidase with hematoxylin counterstain, ×80

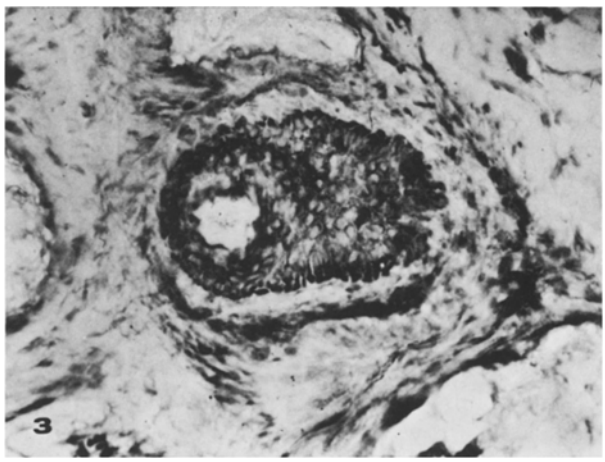

Fig. 3. Lysozyme is present in the bulb cells. Immunoperoxidase with hematoxylin counterstain, × 80

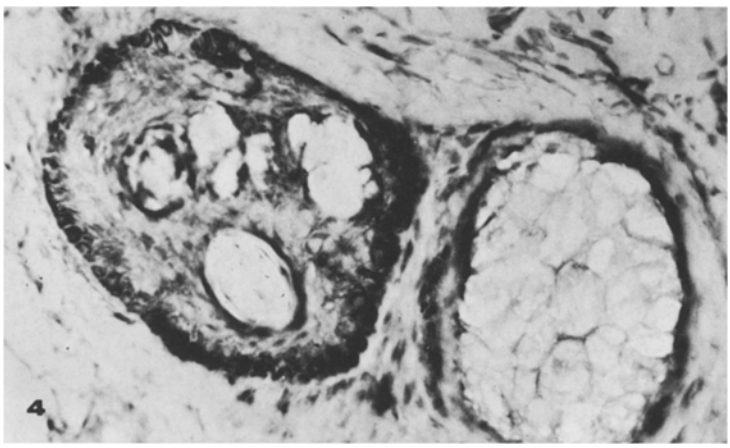

Fig. 4. Lysozyme distribution in pilo-sebaceous follicle and sebaceous acinum. Immunoperoxidase with hematoxylin counterstain, × 80

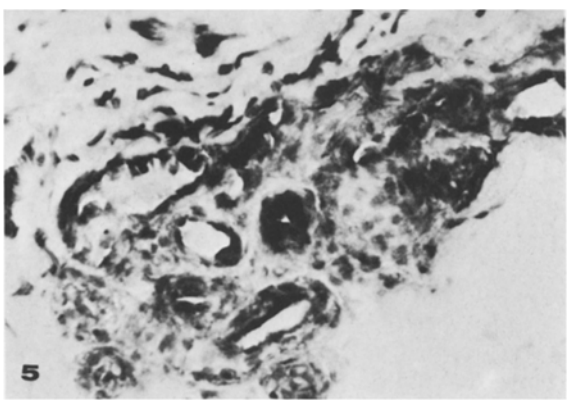

Fig. 5. Lysozyme in eccrine sweat gland. Immunoperoxidase with hematoxylin counterstain, × 80

The results of the immunofluoresence studies were almost superimposable on those of the immunoperoxidase studies. The horny layer was lysozyme-negative. Lysozyme was detected in the cytoplasm of granular layer cells and Malpighian cells, but not in the nuclei (Figs. 1, 2). The intensely positive staining seen in the basal layer was probably in part due to melanin. Lysozyme was also present in pilo-sebaceous follicle cells, in the follicle sheath and the bulb (Fig. 3), but not in the hair-shaft or in the acini of the sebaceous glands (Fig. 4). Lysozyme was also observed in all parts of the eccrine sweat glands (Fig. 5).

Brownish bands, indicative of slightly specific staining, were seen along some of the collagen fiber bundles in the dermis, while the fibroblasts were negative, as were the small and medium blood vessels.

There were no evident differences between forearm and lumbar skin samples.

The results of the present research confirm previous findings [3] of lysozyme in human epidermal cells and establish its distribution in the pilo-sebaceous adnexa, eccrine sweat glands, and dermis, even though they do not clearly indicate the capacity of individual cells to produce this enzyme.

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