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# AN ALPHA-D-GLUCOSIDASE IN MAMMALIAN SKIN: AN HISTOCHEMICAL STUDY\*

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Alpha-glucosidase catalyzes the hydrolysis of the glucosidic linkages (1, 2). These enzymes have been found in some plants, including the bacteriaceae and micetaceae, and in the small intestine and kidneys of rats, guinea pigs, rabbits, dogs, and mice (3). This is a report of their presence and distribution in the skin of several species of mammals.

# MATERIAL AND METHODS

The post-coupling technic of Rutenburg (3), slightly modified, was applied to specimens of the skin of man (4), cat, rat, mouse, rabbit, guinea pig, sheep, ox, pig and mole.

The material was fixed in 10% neutral formalin for one hour, and frozen sections were incubated at 26° C. in the substrate solution for 24 hours. The solution was prepared by dissolving 6-bromo-2-naphtyl- $\alpha$ -D-glucopyranoside in a 2-methoxyethanol (1 mg/ml) buffered at pH 6.3 with 0.1 M phosphate buffer so that the final substrate concentration was 0.1 mg/ml. As a control, a similar substrate without the 6-bromo-2-naphthyl- $\alpha$ -D-glucopyranoside was used.

After incubation, the sections were washed in distilled water and placed in 5 ml of Diazo blue B (10 mg Diazo Blue B dissolved in 5 ml of sodium bicarbonate, 1 mg/ml at pH 8.0) for 10 minutes, washed again in distilled water, and mounted in glycerine jelly. The color reaction varied from orange to purple to violet, the last color being typical of the regions of maximal enzyme concentration (3).

#### RESULTS

In the epidermis, the basal cells stained weakly. The reaction became increasingly darker in the upper parts of the malpighian layer; in some of the animals, the granular layer stained a dark orange. The stratum corneum always remained unstained, as did also the stratum lucidum in the friction surfaces. In the growing terminal hair follicles, the glassy membrane was unstained. The outer root sheath behaved like the surface epidermis. In the inner root sheath, Huxley's layer was moderately colored; in the rest of the follicle the cuticle layer, the cortex, medulla, matrix, and papilla showed

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\* From the Clinica Dermatologica dell'Universita di Genova-Genova, Italy. no reaction. The sebaceous glands, especially those of the ox and the mole (Figs. 1 and 2), had the strongest concentration of enzymatic activity. The stainability was strongest in the undifferentiated peripheral cells, which were violet; the mature cells and sebum stained a purple-violet.

In the eccrine sweat glands, the secretory cells stained an orange color; the dermal part of the duct had a slight coloration and the intraepidermal portion appeared altogether indistinguishable from the surrounding epidermis. The apocrine sweat glands stained like the eccrine glands, particularly where the secretory cells bulged into the lumen.

Collagen, elastic and reticular fibers, the smooth muscle of arteries, and the arrectores pilorum muscles were negative. None of the cells in the dermis were stained.

In contrast, the myelinated nerve fibers (Fig. 3) were very reactive, staining a strong purple especially in the ox; the fat cells in the adipose layer colored an intense blue-violet in all animals.

## DISCUSSION

This appears to be the first report (3) of the presence of  $\alpha$ -D-glucosidase in the skin of mammals.

The topographic distribution of the enzyme is similar to that of  $\beta$ -glucuronidase (5–6). The differences encountered in reactivity in different species are common occurrences in enzyme histochemistry.

It is not certain that this enzyme should be considered as a transglucosidase (7). These observations support the presence of one more enzyme in the skin, presumed to be involved in carbohydrate metabolism. However, we found the strongest reactivity in those structures which have a high lipid content, *i.e.* the sebaceous glands, the myelinated nerves, and fat cells. It is therefore suggested that  $\alpha$ -D-glucosidase may have some influence upon the metabolism of lipids.

## SUMMARY

Alpha-D-glucosidase has been histochemically demonstrated in the skin of a number of mammals. Its pattern of reactivity is similar to that of  $\beta$ glucuronidase. The reaction is strongest in fatcontaining cells and suggests the possibility that the enzyme may be involved in lipid metabolism.



Fig. 1. Note the intense reactivity of the sebaceous glands as counterposed to the considerably lesser staining of the epidermal structures (ox). FIG. 2. Another example of strong reactivity of the sebaceous glands (mole)

FIG. 3. The intense reaction of myelinated nerves (ox)

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