HISTOCHEMICAL DEMONSTRATION OF MONOAMINE OXIDASE IN HUMAN SKIN*

WALTER B. SHELLEY, M.D., PH.D., SHELBY BRENNER COHEN, B.S. AND GEORGE B. KOELLE, M.D., PH.D.

In an excellent review of the enzymes in skin, Lerner (1) has recently pointed out that these protein metabolic catalysts fall into two groups: (A) enzymes affecting energy release from carbohydrates, fats and protein, and (B) enzymes concerned with specialized activities. Monoamine oxidase (MAO), the subject of this communication, is an enzyme which comes under the second group. It oxidizes mono-amines [primary ($R\text{-}NH_2$), secondary (R_2NH), or tertiary (R_3N)] to the corresponding aldehydes, but does not affect diamines such as histamine.

Monoamine oxidase was first discovered in 1928 in liver (2). Here it was postulated to play an important role in the oxidative detoxification of amines produced in the intestine by the bacterial decarboxylation of certain amino acids. It has subsequently been shown to occur in many other tissues, including the brain, kidney, uterus, placenta, thyroid, and smooth muscles (3). In addition, it is found in plants, e.g., in Salvia, where it appears to be concerned with alkaloid synthesis. Its concentration can be determined in tissue homogenates or acetone powers by using tyramine as a substrate and measuring either NH_3-formation or O_2-uptake. More recently, histochemical procedures have been developed for localizing its presence in tissue sections (4, 5). Monoamine oxidase has never been purified sufficiently to permit its chemical characterization. It apparently is a constituent of the cell mitochondria and does not appear in the nuclei. It is inhibited by antihistaminics, local anesthetics, amphetamine, ephedrine and imidazolines, whereas atropine, carbon monoxide and cyanide are without effect (3, 6).

Physiologically, the role of MAO is still to be defined in detail. In addition to oxidative detoxification of amines in the liver, it has been considered to take part in the metabolism of the naturally occurring sympathomimetic amines: epinephrine, norepinephrine and 5-OH-tryptamine (3, 7).

The present study was undertaken to determine the histochemical localization of MAO in normal human skin. Using another recent histochemical method (4), Hellmann (8) found evidence of high MAO activity in the sweat glands of the horse, low activity in the cat, but none in the glands of human skin.

METHOD

Five axillary biopsies were taken from normal healthy young male adults. Three were taken from areas which had been locally anesthetized with procaine,

* From the Department of Dermatology, School of Medicine, and the Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

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and two from unanesthetized skin. The latter was achieved using a high speed rotary punch (9). The specimens were immediately placed on ice or dry ice, and kept frozen until sectioning. The histochemical technic employed was the one recently devised by Koelle and Valk (5). Fresh frozen sections were incubated at 37° C for 2 hours in an oxygenated medium containing tryptamine, 2-OH-3-naphthoic acid hydrazine, Na_2SO_4, and phosphate buffer. The condensation product of the hydrazine with the aldehyde resulting from the action of the MAO on tryptamine was coupled with tetrazotized o-dianisidine, forming a deep purple precipitate. Controls to distinguish non-enzymatic staining artifacts were obtained by (1) selective inhibition of the enzyme by exposure to 1-isonicotinyl-2-isopropylhydrazine, and (2) incubation in the absence of the substrate (tryptamine). Details of the method are given in the original paper (5). Any staining in either type of control slide was considered to be an artifact, and the corresponding areas were discounted in evaluating the distribution of the enzyme.

RESULTS

The most intense staining for MAO activity was seen in the sweat glands. In the eccrine glands, the tubules revealed heavy punctate purplish deposits which readily distinguished them from the ducts, which showed diffuse light staining. The apocrine sweat glands showed similar evidence of MAO activity. Here the secretory cells showed numerous pigmented granules. The myoepithelium did not stain. The sebaceous glands also revealed the presence of appreciable amounts of MAO. (Figures 1–4).

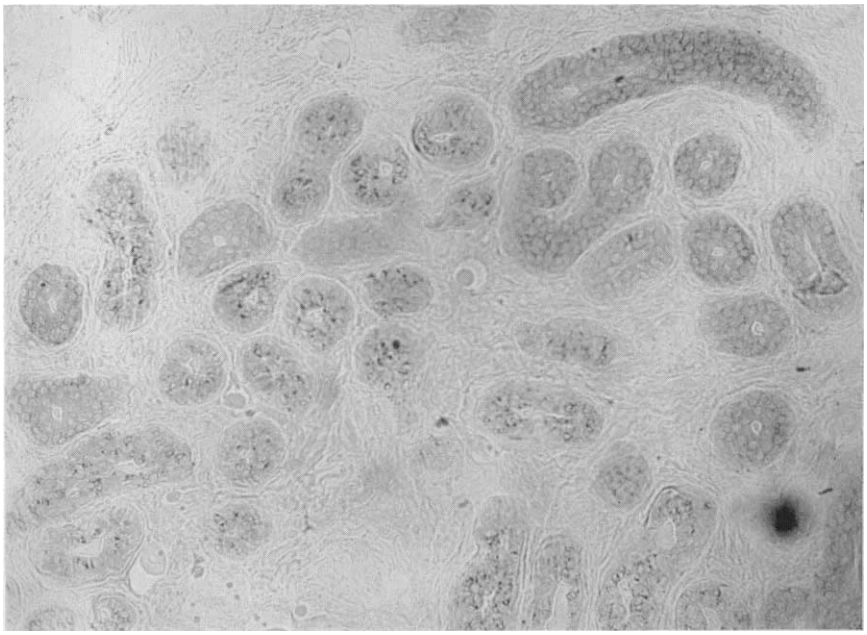

FIG. 1. MAO stain: Ducts and secretory coils of eccrine sweat glands. ×160

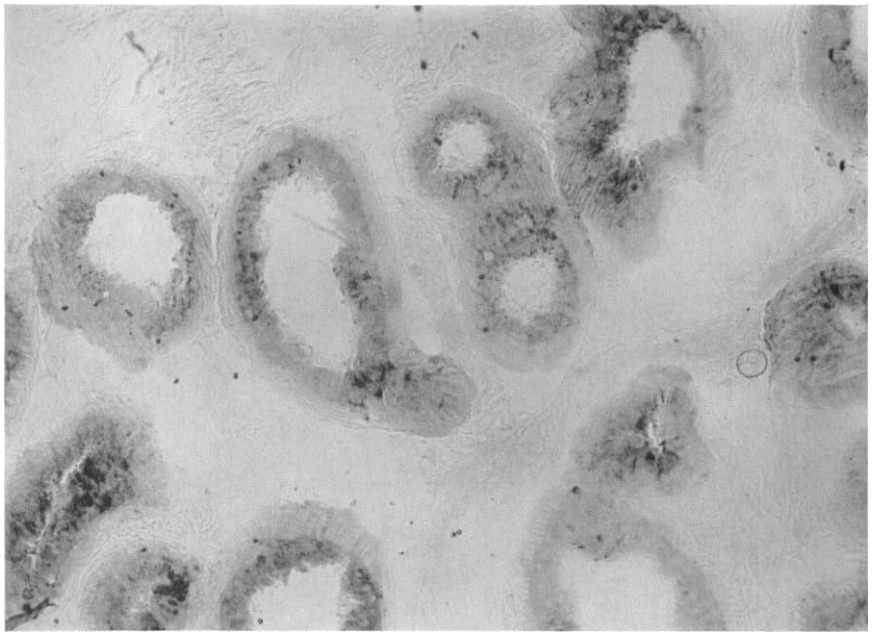

FIG. 2. MAO stain: Apocrine sweat gland secretory tubules. ×160

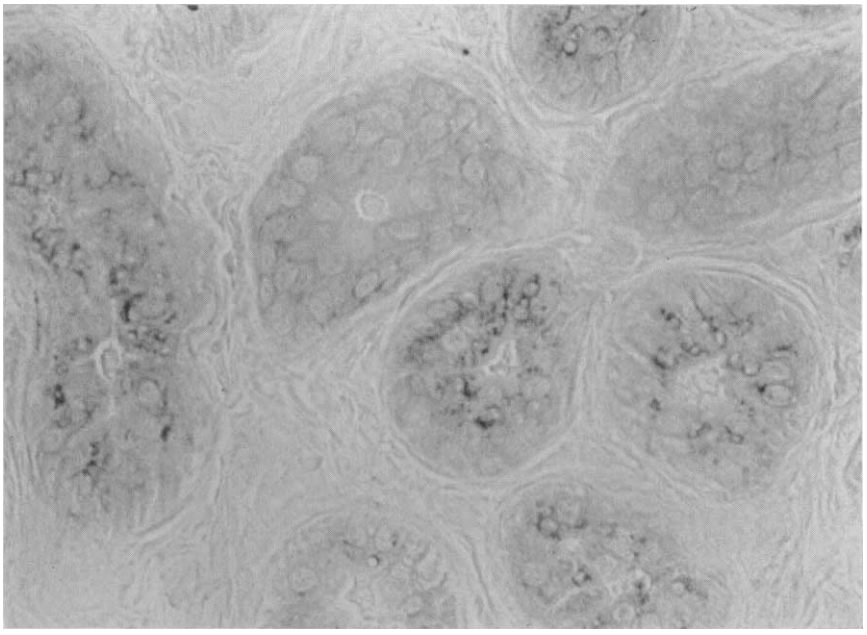

FIG. 3. MAO stain: Secretory coil and ducts of eccrine sweat gland. This high power view shows dark granular deposits of pigment in the secretory cells in striking contrast to the diffuse staining of the ductal cells. ×500

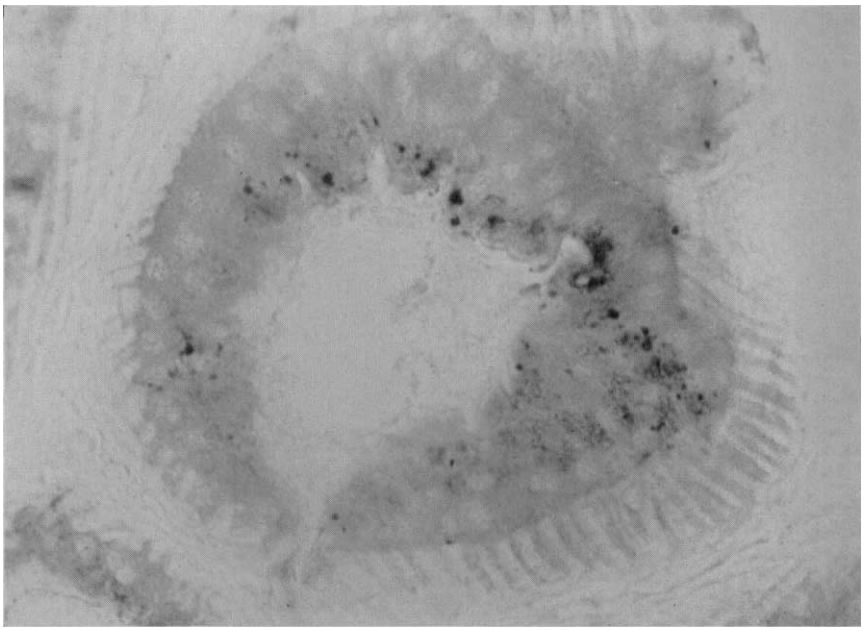

FIG. 4. MAO stain: Secretory cells of apocrine sweat gland acinus. Note granular pigment and also absence of stain in nuclei and myoepithelium. Some of the larger granules were also seen in control specimens, and represent normal pigment of apocrine secretory cells. ×500

The epidermal cells showed faint diffuse staining. The keratin, however, was not stained nor did the melanocytes show any darkening. The wall of the hair follicle shared in the faint epidermal staining. The collagen was not stained and it appeared probable that elastic fibers had but little, if any, color. Arrectores pilorum showed slight staining. No nerve tissue could be visualized as having been stained by the reaction.

DISCUSSION

These observations indicate the wide-spread occurrence of yet another enzyme in the skin. Monoamine oxidase appears to be associated chiefly with the epidermal cells and their derivatives, sebaceous and sweat glands. In the secretory cells of the sweat glands the enzyme appeared in granular form, which in the eccrine gland may represent the mitochondria. Interestingly, this enzyme may be partly responsible for the ammonia which appears in eccrine sweat since it is liberated by oxidative deamination of primary amines. It is also possible to speculate that this enzyme may be related to the appearance of colored apocrine sweat, i.e. chromidrosis. The oxidation of tryptamine or 5-OH-tryptamine, can lead to the formation of a dark pigment (4). Thus, in the presence of tryptamine, or other chromogenic amines, the apocrine secretory cells may form such a pigment which then could color the small volume of apocrine sweat produced. In the eccrine gland a similar pigment formation might also take place, but no color would be expected because of the factor of dilution.

Initially it was hoped that localization of MAO activity might provide a means of distinguishing adrenergic from cholinergic nerve fibers. In the rabbit, evidence was found of its selective localization in the former but this distinction did not hold in the cat (5). In the present work, however, we were unable to identify any staining of nerve tissue. No nerve fibrillae were seen around either the eccrine or apocrine gland. Presumably, the level of activity in nerves of human skin is below the limit of sensitivity of the method.

Despite the use of procaine in the biopsy procedure, no evidence of enzyme inhibition could be seen. It is likely that the anesthetic was washed out of the slides by pretreatment rinses.

CONCLUSION

Monoamine oxidase has been shown histochemically to exist in normal human skin. It was found in the epidermis, sebaceous gland, eccrine and apocrine glands.

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