Human Meibomian Glands: A Histochemical Study for Androgen Metabolic Enzymes

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Human meibomian glands were treated for the histochemical demonstration of several enzymatic activities. The 3α-hydroxysteroid dehydrogenase (3α-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) appeared intensely reactive in the differentiating excretory cells, and weakly reactive in the basal cells and in the epithelial cells of the proximal portion of the ducts. The results indicate that meibomian glands can metabolize androgens by the reductive pathway, characteristic of target tissues. The finding of an intense reactivity for the enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) is also discussed. Invest Ophthalmol Vis Sci 31:771-775, 1990

It is well known that androgens regulate the activity of the human sebaceous glands and that an increase in their concentration in the plasma causes an increase in sebum excretion; androgens therefore could be a cause of some dermatological disorders of the skin, such as acne, hirsutism and androgenetic alopecia.1-4 Androgen-sensitive organs, such as skin, can convert circulating androgens into biologically active reduced metabolites, such as dihydrotestosterone, 5α-androstane-3α, 17β-diol, and 5α-androstane-3β, 17β-diol, which play important roles in the regulation of cellular activities. The principal enzymes involved in androgen metabolism are Δ^2-3-ketosteroid-5α-reductase, 3α-hydroxysteroid oxidoreductase, 3β-hydroxysteroid oxidoreductase, and 17β-hydroxy-C19-steroid oxidoreductase, all utilizing NAD or NADP as coenzymes. These enzymatic activities have been biochemically demonstrated in most male accessory sex organs and other androgen-sensitive structures of various mammals, including man,5-14 although their cytochemical localization has been determined only in some organs.15-22

Several studies have illustrated the morphology, the lipid composition, and the mechanisms of excretion of the meibomian glands,23-26 but no data are available on androgen influence in these glands. If these enzymes are in fact present in meibomian glands, they may be implicated in certain afflictions of the margin of the eyelid, such as blepharitis, ocular seborrhea and chalazion. The cytochemical localization of the principal steroid-metabolizing enzymes in human meibomian glands was studied in order to verify their presence in these particular sebaceous glands. The investigation has been extended to the enzymes glucose-6-phosphate dehydrogenase, and 6-phospho-gluconic dehydrogenase because these enzymes have been related to the metabolism of androgen hormones.27-29

Materials and Methods

Upper lid specimens were obtained by surgery from four female and five male patients ranging in age from 18 to 60 years at the time of operation for palpebral ptosis. All tissues were normal at histological examination. The samples were rapidly frozen and, after a few hours, cryostat sections (10 μm) were cut.

Groups of slides were then incubated for the demonstration and localization of steroid-dehydrogenases: the method of Wattenberg13 (1958) was followed for the demonstration of 3β-hydroxysteroid dehydrogenase (3β-HSD; E.C. 1.1.1.51), that of Pearson and Grose16 (1959) for 17β-hydroxy-dehydrogenase (17β-HSD; E.C. 1.1.1.63) and that of Balogh19 (1966) for 3α-hydroxy-dehydrogenase (3α-HSD; E.C. 1.1.1.50). The substrates used were dehydroepiandrosterone, testosterone and androsterone, respectively. Other sections were treated for the demonstration of glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGD; E.C. 1.1.1.43) with glucose-6-phosphate disodium salt and 6-phosphogluconate Ba salt, respectively, as substrates, according to the

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methods of Teutsch and Rieder\textsuperscript{29} (1979). In each experiment, control sections were incubated with a substrate-free medium. All substrates were purchased from the Sigma Chemical Company (St. Louis, MO).

**Results**

In the human meibomian glands three types of acinar cells were discernible. In order of increasing holocrine maturation, they were: (1) basal cells; (2) differentiating cells; and (3) degenerating cells. Differentiating cells overlaid basal cells and occasionally were located with the basal layer. Degenerating cells were observed in the center of the clustered excretory cells adjacent to the lumen (Fig. 1).

The steroid-dehydrogenases displayed identical reactivity in the sections of meibomian glands of both sexes. The 3\(\beta\)-HSD, 17\(\beta\)-HSD and 3\(\alpha\)-HSD appeared strongly reactive in the differentiating and degenerating cells, whereas basal cells were weakly reactive (Figs. 2–4). Moreover, the sections showed a weak reactivity for these enzymes in the epithelial cells of the proximal portion of the ducts. 3\(\alpha\)-HSD, 3\(\beta\)-HSD and 17\(\beta\)-HSD activities appeared in the form of small dark granules of formazan deposits, and were distributed in variable amounts throughout the glands and their proximal ducts.

G6PD and 6PGD activities showed the same distribution of 3\(\alpha\), 3\(\beta\), and 17\(\beta\)-HSD, being strongly reactive in the differentiating excretory cells and weakly reactive in the basal excretory cells and in the ductal cells (Figs. 5, 6).

**Discussion**

The glands of the ocular adnexa excrete different products and together they function as a unit to produce the precorneal tear film. This film is composed of three layers: a superficial oily layer, a fluid layer and a mucoid layer. The superficial oily layer is produced primarily by the meibomian glands. These glands, located within the upper and lower tarsal plates of the eyelids, are generally considered as enormously enlarged sebaceous glands arranged in a single row, running perpendicular to the palpebral margin. Although there are similarities, there are also differences between meibomian glands and other sebaceous glands. The meibomian gland in fact does not have a structure comparable to the pilosebaceous canal. The multiple lobules of this gland drain into a common duct whose orifice opens into the lid margin\textsuperscript{23,24,26}. This distinguishes the meibomian glands from many other sebaceous glands which open into the hair follicle. Comparing the excreted end-product of the holocrine meibomian gland with the end-product of the sebaceous gland, Andrews\textsuperscript{30} (1970) and Nicolaides et al\textsuperscript{25} (1981) have demonstrated differences between their lipid composition; for this reason the term "meibocyte" has been coined as the meibomian gland equivalent to the sebaceous gland sebocyte\textsuperscript{25}.

Our results show that human meibomian glands display a marked reactivity for the steroid-dehydrogenases and therefore are sites of steroid metabolism. The histochemical demonstration of 17\(\beta\)-HSD and 3\(\beta\)-HSD activities does not denote the occurrence of androgen activation, since these enzymes are involved not only in the reductive, but also in the oxidative process, which leads to the formation of inactive steroids. They have been in fact detected in both androgen-target and non-target tissues\textsuperscript{5,8,13–16, 20–22,31,32}. Androgen reduction, which yields biologically active metabolites, requires the presence of 5\(\alpha\)-reductase and 3\(\alpha\)-HSD. The 5\(\alpha\)-reduction in fact rep-
Fig. 2. 3β-HSD. Fig. 3. 17β-HSD. Fig. 4. 3α-HSD. Original magnifications ×203. The 3β-HSD, 17β-HSD and 3α-HSD appear strongly reactive in the differentiating excretory cells and more weakly reactive in the basal and ductal cells.

Fig. 5. G6PD. Fig. 6. 6PGD. Magnifications ×203. G6PD and 6PGD activities show the same distribution of the 3α-, 3β-, and 17β-HSD.
resents the key step in androgen activation, but the 5α-reductase is not histochemically detectable. The 3α-HSD takes part only in the activating mechanism in target organs, since it requires 5α-reduced steroids as optimal substrates. In our recent histochemical studies, we have demonstrated the presence of 3β-HSD and 17β-HSD, but not 3α-HSD, in the salivary and ceruminous glands of human beings, while we have also found 3α-HSD in the human male accessory sex glands. The lack of histochemically detectable 3α-HSD could mean that the ceruminous glands, like the salivary glands, normally are not sites of significant androgen activation, because a considerable level of 3α-HSD seems to be required for this process, typical of androgen-controlled tissues. Thus, the presence of 3β-HSD, 17β-HSD and especially 3α-HSD in the human meibomian glands may indicate that these glands also can activate androgens like other androgen-dependent organs such as the sebaceous glands.

The literature concerning steroid metabolism by the human skin indicates that the major sites of testosterone activation reside in the sebaceous glands and large apocrine glands, whose growth and function are strictly controlled by the sex hormones, but other structures, such as hair follicles, are primarily involved as well. Recent studies have suggested that not only endogenous androgens, but also exogenous ones cause an increase in sebaceous gland size and sebum excretion, as well as acne vulgaris in healthy young adult males. The presence of an intense glucose-6-phosphate dehydrogenase and 6-phospho-gluconic dehydrogenase activities suggests that the pentose shunt may be functioning. G6PD and 6PGD catalyze the first two steps of the pentose phosphate shunt, which represents an important source of NADPH. This metabolic process seems to be controlled by the sex hormones in some nongenital tissues such as salivary glands and liver. In all androgen target tissues, a possible role of the pentose shunt would be the production of NADPH required for androgen activation. However, the complex interaction between androgen hormones and pentose metabolism in target organs needs to be clarified.

The present study has shown that there is a basic similarity between meibomian and sebaceous glands in the reductive pathway of androgen metabolism. This metabolic similarity may be the basis for certain dysfunctional states and disorders of the lid margin, such as ocular seborrhea, blepharitis and chalazion.

Key words: meibomian glands, human, enzymatic activities, histochemical study

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References


