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Role of Hormones in Pilosebaceous Unit Development

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ABSTRACT

Androgens are required for sexual hair and sebaceous gland development. However, pilosebaceous unit (PSU) growth and differentiation require the interaction of androgen with numerous other biological factors. The pattern of PSU responsiveness to androgen is determined in the embryo. Hair follicle growth involves close reciprocal epithelial-stromal interactions that recapitulate ontogeny; these interactions are necessary for optimal hair growth in culture. Peroxisome proliferator-activated receptors (PPARs) and retinoids have recently been found to specifically affect sebaceous cell growth and differentiation. Many other hormones such as GH, insulin-like growth factors, insulin, glucocorticoids, estrogen, and thyroid hormone play important roles in PSU growth and development. The biological and endocrinological basis of PSU development and the hormonal treatment of the PSU disorders hirsutism, acne vulgaris, and pattern alopecia are reviewed. Improved understanding of the multiplicity of factors involved in normal PSU growth and differentiation will be necessary to provide optimal treatment approaches for these disorders. (*Endocrine Reviews* **21:** 363–392, 2000)

I. Introduction

ANDROGENS are a prerequisite for sexual hair and sebaceous gland development (1, 1a). The importance of androgens in human hair growth was first established by Hamilton (2), who observed that castration before puberty prevented beard and axillary hair growth, while castration after puberty reduced hair growth in both areas. Furthermore, patients with androgen insensitivity typically have no pubic or axillary hair. Androgens have been shown to increase the size of the hair follicle, the diameter of the hair fiber, and the proportion of time that terminal hairs spend in anagen (3). Androgens are also important for sebaceous gland growth and differentiation as acne vulgaris, a disorder of the sebaceous gland, has been shown to be dependent upon the pubertal rise in androgen levels (4).

The pilosebaceous unit (PSU) consists of a piliary component and a sebaceous component. Each PSU has the capacity to differentiate into either a terminal hair follicle (in which a large medullated hair becomes the prominent structure) or a sebaceous follicle (in which the sebaceous gland becomes prominent and the hair remains vellus) (Fig. 1a). Androgens play a key role in the development of the PSU in most areas of the body. In androgen-sensitive areas before puberty, the hair is vellus and the sebaceous glands are small. In response to increasing levels of androgens, PSUs become large terminal hair follicles (sexual hairs) in sexual hair areas or they become sebaceous follicles (sebaceous glands) in sebaceous areas. Androgens appear to promote sexual hair growth by recruiting a population of PSUs to switch from producing vellus hairs to initiating terminal hair growth. PSU disorders, namely acne vulgaris, hirsutism, and pattern alopecia, do not occur until after the processes of puberty begin (5). However, it is clear that the pathogenesis of these disorders involves more than androgen (1, 3). For one thing, although the development of acne normally parallels the rise in androgen with pubertal progression, acne wanes in the late teenage years while blood androgen levels remain stable. For another, PSUs respond differently to androgen depending on their location; *e.g.*, sexual hairs grow only in certain areas of the body, while hairs on the scalp undergo regression from a terminal to a vellus type in genetically susceptible individuals. In addition, acne, hirsutism, and alopecia are variably expressed manifestations of androgen action, and the severity of acne or hirsutism is quite variable for a given degree of androgen excess. Furthermore, some women will develop acne or hirsutism at normal levels of androgen (id-

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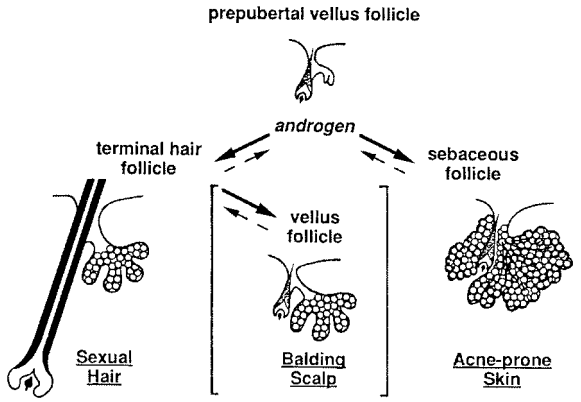

FIG. 1. Role of androgen in the development of the pilosebaceous unit. *Solid lines* indicate effects of androgens; *dotted lines* indicate effects of antiandrogens. Hairs are depicted only in the anagen (growing) phase of the growth cycle. In balding scalp (*bracketed area*), terminal hairs not previously dependent on androgen regress to vellus hairs under the influence of androgen. [Reprinted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:80S–88S, 1995 (1a) © Excerpta Medica Inc.]

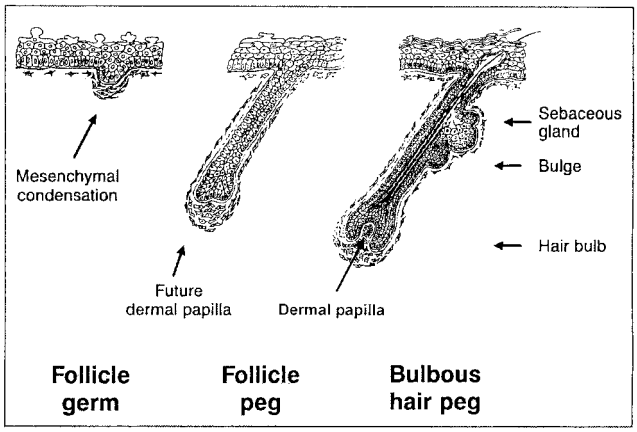

FIG. 2. Embryonic development of the pilosebaceous unit. The stages shown correspond to the respective stages at which (a) mesenchymal cells signal the overlying epithelium to initiate follicle differentiation; (b) the epithelium signals the mesenchyme to form a dermal papilla; and (c) the dermal papilla then signals for formation of the pilosebaceous unit. [Adapted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:805–885, 1995 (1a) © Excerpta Medica Inc.]

iopathic acne or hirsutism), while at the other extreme some women will have no manifestations of androgen excess (cryptic hyperandrogenemia). All of these considerations indicate that factors other than androgen play major roles in PSU development and in PSU disorders.

II. Embryology and Molecular Genetics of PSU Differentiation

PSU differentiation occurs in the embryo between 2 and 4 months gestation and requires precisely timed and localized interactions between the fetal epidermis and dermis (3, 6). Each PSU acquires an intrinsically determined morphology and pattern of behavior during its development, which may be modulated by hormones (7). The difference in the apparent density of sexual hair between men and women is due to a different density of terminal hairs rather than a difference in the number of PSUs, which is established before birth (8). Studies of glucose-6-phosphate dehydrogenase mosaicism have demonstrated that hairs originate as a clone from a pool of about five primitive epidermal cells (9). The respective roles of the epidermis and dermis in PSU formation have been elicited with tissue recombination experiments by which the epidermis and dermis are separated, and then the epidermis and dermis of different ages, locations, and species are combined and the formed appendages are studied (10).

PSU differentiation begins with formation of a dermal mesenchymal condensation that sends a signal to the overlying embryonic epithelium to "make an appendage here" (Fig. 2) (6, 11). This results in downward growth of an epidermal plug to form a skin appendage (6, 7). This initial message from the dermis to epidermis is common to all classes of vertebrates. The epidermis determines the type of appendage, directs its cephalo-caudal polarity, and determines species specificity of keratin composition. For example, mouse dermis can instruct the development of feather follicles in chick epidermis. Some authors postulate that the epidermis sends the first signal to the dermis and is responsible for the patterning of skin appendages (12). Lymphoid-enhancing factor-1 (LEF-1), a DNA binding molecule that acts by bringing together other DNA-bound transcription factors, is expressed in the epidermis just before the formation of the dermal mesenchymal condensations. Altering the expression of LEF-1 in transgenic mice results in abnormal hair follicle distribution and orientation (13, 14).

After the initial signal, the differentiating epithelium of the hair plug then sends a less well defined but species-specific signal back to the mesenchyme to "make a dermal papilla" (3). The dermal papilla subsequently sends a message back to the adjacent epidermal placode to "make a PSU." This message is species specific and cannot be interpreted by epithelial cells from other classes of vertebrates. In response, the PSU forms a hair bulb, a bulge region [site of attachment of the arrector pili muscle and presumptive location and source of stem cells that support regrowth of the follicle at the beginning of anagen (6)], and a sebaceous gland. Then the rapidly proliferating matrix cells at the base of the bulb grow rapidly downward, giving rise to all the inner layers of the hair.

The cells in the sebaceous anlagen are identical to those in the basal layer of the epidermis and the piliary canal. Most sebaceous glands arise in a cephalo-caudal sequence from hair follicles (15). The future common excretory duct, around which the acini of the sebaceous gland attach, begins as a solid cord. The cells composing the cord are filled with sebum, and eventually they lose their integrity, rupture, and form a channel that establishes the first pilosebaceous canal. Fetal sebaceous cells are quite large and functional and probably contribute to vernix caseosa.

Epithelial and mesenchymal cells appear to communicate during morphogenesis, and these interactions seem to involve molecules or "morphogens" that play a regulatory role in development. Likely morphogens include growth factors, cell adhesion molecules, extracellular matrix molecules, intracellular signaling molecules such as β-catenin and LEF-1,

hormones, cytokines, enzymes and retinoids, together with their receptors (16, 17). Growth factors such as epidermal growth factor (EGF), transforming growth factor α (TGFα), transforming growth factor β (TGFβ) and fibroblast growth factor (FGF) affect the proliferation and differentiation of the cells of the PSU during development (18). These growth factors appear to exert their effects via autocrine or paracrine pathways between cell types. EGF was the first growth factor to be implicated in hair development when it was shown that its administration to newborn mice delayed hair follicle development (19), and this effect occurred over the entire coat. Furthermore, growth of the first coat of hair in newborn mice is accelerated by the administration of antibodies to EGF (20). The EGF peptide has been found in the outer root sheath and sebaceous gland in later stages of follicular development in sheep skin (21). The EGF receptor has been found in embryonic skin by autoradiography and immunohistochemistry; however, it is present in the adjacent interfollicular epidermis rather than the placode and hair germ (22, 23). In later development, the EGF receptors are expressed in the outer root sheath and sebaceous epithelium, and in some species in the hair bulb, but no EGF receptors have been demonstrated in the dermal papilla (3). The specific distribution of EGF in skin and throughout follicle morphogenesis suggests that this growth factor has a more important role in differentiation than in proliferation (18). TGFα, which is in the EGF family and binds to the same receptor as EGF, has also been found to inhibit murine hair growth (24). Several members of the TGFβ family (TGFβ-1, β-2, β-3, bone morphogenetic protein-2, and bone morphogenetic protein-4) have also been localized to various regions of the developing PSU using *in situ* hybridization (25, 26). FGF was also found to affect hair follicle initiation and development, but the effects were confined to the area of treatment since FGF is not readily diffusible in the skin (18). The FGF receptor 2 is likely to be important in sebaceous gland development in humans, as a somatic activating mutation of this receptor has been associated with localized acne (27).

Cell adhesion molecules such as the cadherins, neural cell adhesion molecule (N-CAM), intercellular cell adhesion molecule (I-CAM), and tenascin are also thought to play a prominent role in PSU differentiation. Both E-cadherin and P-cadherin have been detected in developing follicles by immunohistochemistry (28). Whereas P-cadherin is expressed throughout the epithelium, E-cadherin is confined to cells in the presumptive matrix region. In studies of cultured lip skin, the addition of antibodies against E-cadherin and P-cadherin caused disruption of follicular development, and dispersal of the mesenchymal aggregate (3). Although both embryonic and fetal keratinocytes express E-cadherin, only embryonic keratinocytes express N-CAM, which is localized in the initial mesenchymal aggregate (6); N-CAM is probably important in cell adhesion and furthering cell aggregation. It is found in the dermal papilla and dermal sheath in the adult PSU (6). I-CAM is transiently expressed in the outer layer of the follicular cells, perhaps as a result of a signal from the condensing mesenchymal cells (6). Tenascin, an extracellular matrix protein, has been found to be expressed in the basement membrane underneath the hair germ, but not in the basement membrane between follicles (6). Tenascin is considered to be a marker for epithelial-mesenchymal interactions, but the exact function it plays in PSU development is not known.

Another molecule that may be important for PSU differentiation is epimorphin, which is a mesenchymal signal factor. Epimorphin is found in mesenchymal aggregates in embryonic rat skin and lung and may function in aggregative behavior of immature cells (29). Studies have shown that epimorphin can be detected in cell suspensions that have been aggregated by centrifugation, whereas it is not present in the same cells grown in monolayer. Other studies have shown that hair follicles fail to develop in embryonic skin cells cultured in the presence of antibodies to epimorphin (3). Other morphogens such as the wingless homolog Wnt and sonic hedgehog also seem important for the development and pattern of hair follicles (17, 30, 31).

Although more is being learned about the various molecules involved in cell-cell interaction within the PSU, these cells must be organized in a precise spatial and temporal order for proper function. The overall complexity of PSU morphogenesis indicates the involvement of multiple genes in a coordinated fashion, which suggests a role for homeobox (HOX) genes. HOX genes have been found to control the developmental fate of embryonic cells by encoding regulatory transcription factors that either induce or repress effector genes, which in turn are responsible for the position and development of each particular cell (32, 33).

The HOX genes are aligned in tandem as clusters arranged in a colinear fashion on four different chromosomes (Fig. 3). They are transcribed in "lock-step" with the first set of HOX genes being expressed anteriorly (34). In humans, 39 HOX genes have been identified (33). The HOX genes contain a homeobox, which is a highly conserved 180-bp DNA sequence. Point mutations within the homeobox were discovered to be the cause of "homeotic" malformations in fruit flies, *i.e.*, malformations in which one body part develops looking like another. The HOX genes encode monomer pro-

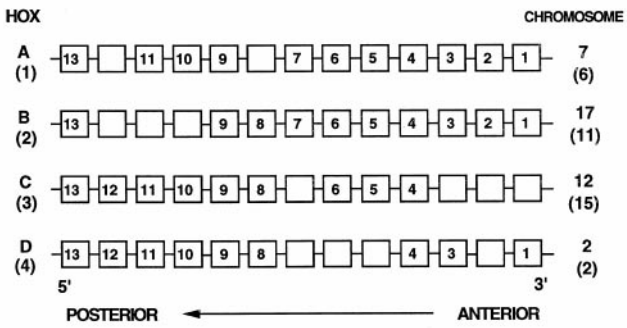

FIG. 3. Homeobox (HOX) gene clusters. The four sets of homeobox genes are organized in tandem on four different chromosomes. Previous nomenclature of the HOX genes is shown in *parentheses*, and the chromosomal location in mice is likewise shown in *parentheses*. The genes are numbered according to their anterior-posterior sequence and are expressed in a "lock-step" manner with genes in the 3′-end of the clusters being transcribed earlier in embryonic development than genes in the 5′-ends of the clusters. Anticipated HOX genes are represented by *unnumbered boxes*. HOX genes thought to be important for PSU morphogenesis include A4, A5, C4, C6, C8, and D4. [Adapted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:805–885, 1995 (1a) © Excerpta Medica Inc.]

teins with three α-helices, with the second and third helices being arranged in a helix-turn-helix configuration. The homeobox encodes the highly conserved homeodomain, which is thought to bind to specific areas of DNA of both HOX and non-HOX genes to regulate transcription.

HOX genes appear to be important in PSU morphogenesis. Chuong *et al.* (35) demonstrated HOX gene expression in the chick feather bud, which is an analog of the developing hair follicle. They showed that the HOX C6 and D4 genes are expressed in a pattern that is position specific (strongest expression in the anterior-proximal region of skin appendages) and that a homeoprotein gradient existed within the feather buds. Retinoic acid (RA) disrupted the normal pattern of the expression of these HOX genes. Bieberich *et al.* (36) studied the expression of HOX genes in murine hair development. They showed that the HOX C8 gene was expressed in skin in an ascending gradient from anterior to posterior. They also linked a HOX C8 clone to a β-galactosidase gene and demonstrated localization of this gene to the dermal papillae of anagen hair follicles. Recently, Stelnicki *et al.* (37) studied the expression of HOX genes during human fetal skin development. The HOX genes appeared to be expressed in a relatively conserved temporal and spatial pattern in developing skin and hair follicles. HOX A4 gene expression was found in both developing hair follicle (in the epidermal layer) and in sebaceous glands. In developing hair follicles, HOX C4 expression was also detected in the epidermal layer, while HOX A5 expression was limited to the inner root sheath.

Retinoic acid also plays an important role in PSU morphogenesis (see below), and the effects of retinoic acid on PSU development may be partly due to regulation of the pattern of expression of HOX genes by retinoic acid. Retinoic acid excess during a critical stage of mouse embryogenesis has been shown to cause abnormal development of hair follicles between the follicle peg and the bulbous follicle peg stage (38). Furthermore, retinoic acid stimulates sebaceous gland development and causes formation of a metaplastic branching tubular duct system from the developing follicle. Since retinoic acid alters the pattern of expression of HOX genes (34, 39), differential retinoic acid action on HOX gene expression within the PSU during embryogenesis may play a role in the subdivision of the PSU into its separate hair follicle and sebaceous gland components.

III. Postnatal Growth and Development of the PSU

A. Hair follicle

The hair follicle is composed of epithelial components (the matrix, medulla, inner root sheath, cortex, cuticle, and outer root sheath) and dermal components (the dermal papilla and connective tissue sheath) (40). During embryogenesis, the dermal papilla, upper outer root sheath [including the bulge area where hair follicle stem cells are thought to reside (3, 41)], and sebaceous gland are permanently established (11). Postnatally, the remainder of the follicle undergoes repetitive cycles of growth that recapitulate embryogenesis (1, 11, 42). Hair grows cyclically by passing from telogen (resting), to anagen (growth), and through the phase of catagen (shortening), back to the telogen phase to begin a new cycle. (Fig. 4). The PSU at the telogen-anagen transitional phase morphologically resembles the embryonic bulbous hair peg. The dynamics of the hair growth cycle vary between species, between different body sites in the same species, and between different follicle types in the same body site (3). It is likely that hair follicles have an intrinsic rhythmic behavior that is modulated by systemic factors (3, 16). In man, these follicle cycles occur independently, for the most part, with a superimposed modest summertime peak of sexual and scalp hair growth, which may reflect changes in androgen levels (42). The duration of anagen is the major determinant of the length to which a hair grows, and it varies with the location of the hair follicle. Mustache hairs grow for approximately 4 months and scalp hairs for 3 yr. In contrast, the percentage of time scalp and beard hairs spend in anagen only differs by about one-third. Other factors influencing the amount of hair growth in various areas of the body include the linear growth rate of the hair fiber, as well as the diameter and density of the terminal hairs. Whereas shaving does not induce hair growth, plucking a resting (telogen) hair causes an advancement in the onset of anagen and thus induces hair regrowth (3, 43).

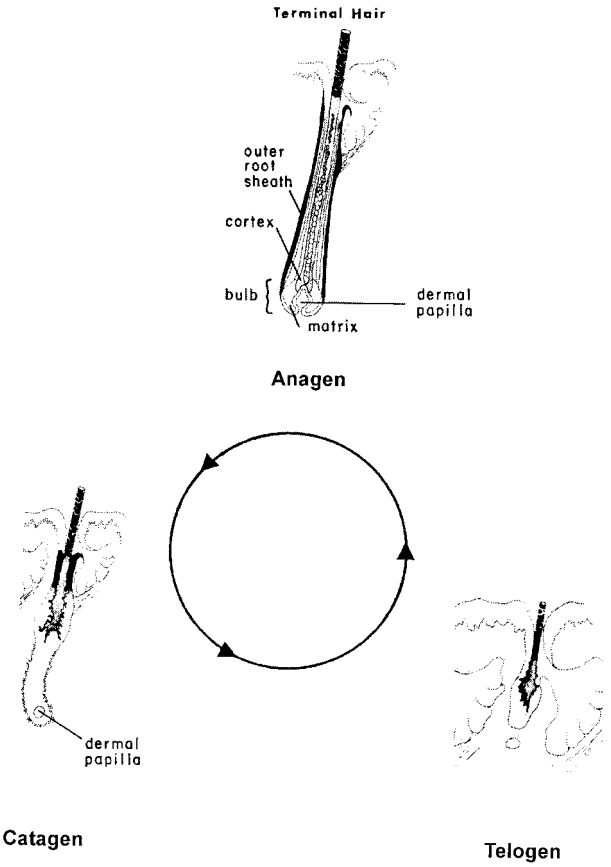

FIG. 4. The hair growth cycle. Hair follicles progress through repetitive cycles of growth, from anagen (active phase of growth which is the longest phase in the hair cycle), through catagen (shortening of the hair follicle), to telogen (resting phase of the hair cycle), after which the club hair is shed, and the follicle begins a new hair cycle again.

The postnatal hair follicle appears to retain the capability

for reciprocal interactions between hair epithelial cells and dermal papilla cells, similar to the embryonic hair follicle. The hair growth cycle is under the ultimate control of the dermal papilla. Studies by Oliver and his colleagues (7, 44) demonstrated that the dermal papilla must be present for regeneration of the hair follicle, and that cells from the dermal sheath serve as a source of a new papilla. In a classic series of experiments, he showed that removal of the dermal papilla from the base of a rat whisker (vibrissa) caused cessation of hair follicle growth, and reimplantation of dermal papilla stimulated the growth of generations of whiskers. Dermal papilla cells were also capable of inducing follicle formation when implanted elsewhere, with the hair type specificity being determined by the source of the dermal papilla. Cultured papilla cells from early passages retained the capacity to induce the differentiated growth of the hair follicle (45). Although the dermal papilla is a key factor in controlling the hair follicle growth, other components of the hair follicle also play a role. If only the dermal papilla is removed, after a lag time, a new dermal papilla will eventually regenerate and induce growth of normal whiskers. If the lower third of the hair follicle is removed (leaving the dermal sheath intact), the dermal papilla will regenerate, but the whiskers that grow will be proportional to the length of follicle used for the regeneration experiment. If more than the lower third of the follicle is removed including the portion of the follicle about the sebaceous gland and its outlet, the dermal papilla will not regenerate (44). Recently, Reynolds *et al.* (46) demonstrated that the transplantation of a few hundred cells from human dermal-sheath tissue from the scalp of an adult male into the skin of a genetically unrelated female induced the formation of a new dermal papilla and hair follicle.

There is evidence that PSU pluripotential stem cells reside in the bulge area of the outer root sheath, just beneath the sebaceous duct, and are capable of repopulating the hair matrix to the point where it will recapitulate ontogeny by reinducing the dermal papilla (47). This appears to be why the portion of the follicle about the sebaceous gland outlet is important for hair regeneration (48). Basal cells of the bulge form an outgrowth pointing away from the hair shaft and are therefore safeguarded against accidental loss due to plucking (41).

Changes take place in the dermal papilla during the hair growth cycle in terms of cell morphology, vascularization, and in composition and volume of the extracellular matrix (3). During late telogen the dermal papilla is pulled upward toward the bulge area. If the dermal papilla fails to ascend upward toward the bulge area during this phase, the follicle stops cycling and the hair is lost (49). This was deduced because mutations of the hairless gene, which encodes a transcription factor important for movement of the dermal papilla to the bulge area, results in permanent alopecia (50, 51). Stem cells of the bulge area are thought to be activated by dermal papilla cells, to which they respond by proliferating and growing down to push the dermal papilla away (41). Once the dermal papilla is pushed away, the bulge area becomes quiescent again. During anagen, the dermal papilla enlarges and develops an extensive extracellular matrix (3). At a given time, the anagen follicle receives a signal that terminates this phase and initiates catagen (see below). The catagen (regression) phase involves apoptosis (16, 52), which is associated with a decrease in volume of the extracellular matrix. In telogen the dermal papilla becomes a condensed ball of cells with almost nonexistent extracellular matrix located immediately below the lower pole of the follicular epithelium (3). There is a decline, and eventual cessation of mitotic activity in the extracellular matrix during telogen, and the matrix cells adjacent to the dermal papilla convert to lower outer root sheath cells. The hair becomes a club hair, which is eventually shed from the follicle to make room for new hair growth. The extracellular matrix becomes organized again around the papilla at the start of the next hair growth cycle (7). The dermal papilla has its own blood supply, and the capillary loops present in the dermal papilla in anagen are lost in telogen (3).

Multiple growth factors are ultimately involved with hair follicle growth and normal cycling including insulin-like growth factor-I (IGF-I), FGF-7 (also known as keratinocyte growth factor), FGF-5, and EGF. IGF expression is stimulated by androgen in dermal papilla cells, and IGF-I has been demonstrated to stimulate hair follicle growth *in vitro* (53). This suggests that some of the trophic effects of androgens on the hair follicle are mediated through growth factors such as IGF. IGF-I has also been shown to slow hair follicle entry into the catagen phase, which suggests that it is an important factor in control of the hair growth cycle (54). FGF-7 production has been found in the dermal papilla, and its receptor has been found in nearby matrix cells (55). When the FGF-7 receptor is disrupted in mice, the morphology of the hair follicles is abnormal and there are 60–80% fewer hair follicles present than in control mice (56). FGF-5 knock-out mice and mice with nonfunctional EGF receptors have long, fine angora-like hairs, due to an extended anagen phase, which suggests that these growth factors are potential signals that cause anagen to terminate and catagen to begin (43, 57, 58).

Hair grows through keratinocyte cell division, which takes place in the hair bulb close to the dermal papilla. The cells differentiate to form the various layers as they move up the follicle (59). Hair can thus be considered to be the holocrine secretion of the hair bulb. The mature hair consists of medulla, cortex, cuticle, inner root sheath (three layers), and outer root sheath. As matrix cells divide, they form keratin microfibrils, which mature in daughter cells in the upper bulb. At this point the keratin is 46–58 kDa in size. The hair shaft comes to consist essentially of solid packages of hard keratin fibrils embedded in an amorphous matrix. Accompanying this terminal differentiation of hair is the formation of larger keratins (53 and 63 kDa) in the shaft.

Keratins comprise more than 90% of hair proteins. Keratins are a group of water-insoluble, cystine-containing proteins. Each hair fibril consists of a bundle of coiled, low-sulfur keratins, which are in turn bundled in an α-helical pattern forming a coiled coil. The amorphous matrix into which these bundles are imbedded contains high-sulfur, lower molecular mass keratins. The molecular and biochemical basis for the ultrastructural differences among the layers of hair is unknown. Unlike scalp hairs, sexual hairs are curled around their axes. Racial differences also affect such diverse features of hair as shape and medullation. The concept of "donor

dominance" was elucidated in classic hair transplant studies (60), which indicated that these structural differences were inherent in the PSU, *i.e.* hairs retain the characteristics of their area of origin.

Before puberty, the androgen-dependent PSU consists of a prepubertal vellus follicle, which consists of a virtually invisible hair and a tiny sebaceous gland component (Fig. 1) (1a). Under the influence of pubertal amounts of androgens, PSUs in sexual hair areas differentiate in a distinctive pattern which depends on their location. Sexual hair development is normally not seen before age 9 in girls (average age of stage 3 sexual hair development, 12 yr) and age 10 in boys (average age of stage 3 sexual hair development, 13 yr) (61). In the sexual hair areas, a terminal hair follicle develops and the sebaceous gland develops only moderately. In the balding-prone area of scalp, PSUs respond to androgen in yet a different manner in individuals predisposed to pattern alopecia. Terminal hair follicles that previously grew without androgen gradually change with each growth cycle to an intermediate kind of follicle in which the hair component reverts to the vellus state, leaving an adult vellus follicle (Fig. 1). These phenomena are reversed by antiandrogens: both types of androgen-dependent PSUs revert toward the prepubertal state.

The most direct evidence that androgens are the principal hormones controlling sexual hair growth is that androgens stimulate hair growth in eunuchs and castration reduces it. The latter classic observation illustrates the plastic nature of the PSU response to androgens, *i.e.*, the reversion from terminal to vellus follicles. The sensitivity of sexual hair follicles to androgen is determined by their pattern of distribution (1a) and generally wanes from pubis to head (Fig. 5), or from posterior to anterior considering the embryogenesis of these PSUs. Thus, rising androgen levels (such as occur either normally during puberty or abnormally in hyperandrogenic states) recruit an increasing proportion of PSUs in a given area to initiate the growth of terminal hair follicles, each in accordance with its preset genetic sensitivity to androgen.

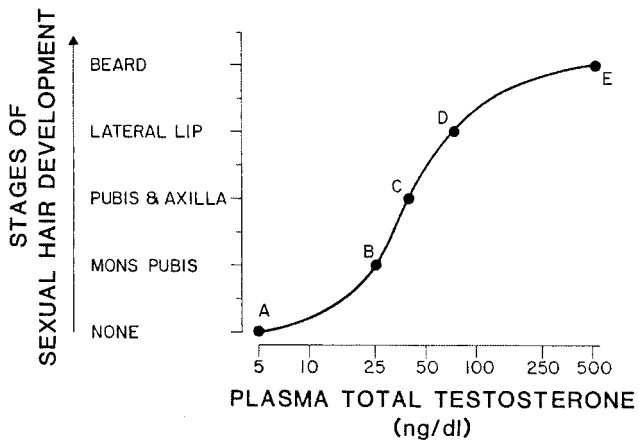

FIG. 5. Relationship of stages of sexual hair development to testosterone as a representative plasma androgen. Note logarithmic scale for testosterone. A, Prepubertal; B, stage 3 pubic hair; C, stage 5 pubic hair; D, moderate hirsutism; E adult male. [Reprinted with permission from R. L. Rosenfield: *Clin Endocrinol Metab* 15:341–362, 1986 (1) © W. B. Saunders Co.]

The apparent dose-response curve to androgen is fairly steep, with a mustache typically appearing at plasma testosterone levels just slightly above the upper limits of normal for women and the beard requiring 10-fold higher levels for full growth. There is considerable individual variability.

B. Sebaceous gland

The sebaceous gland is composed of acini, which are attached to a common excretory duct composed of cornifying, stratified squamous epithelium that is continuous with the wall of the piliary canal and, indirectly, with the surface of the epidermis (15). The life cycle of sebaceous cells (sebocytes) begins at the periphery of the gland in the highly mitotic basal layer. As sebaceous cells differentiate, they accumulate increasing amounts of lipid and migrate toward the central duct. Eventually, the most mature sebocytes burst and their lipid is extruded into the ducts of the sebaceous gland as the holocrine secretion sebum (1, 15). Sebaceous lipid is different from other skin surface lipid in that it is composed of 12% squalene and 26% wax esters in addition to the cholesterol, cholesterol esters, and triglyceride common to both kinds of epithelial secretions (1). The cells of sebaceous glands turn over more rapidly than those of hairs, as they are normally completely renewed every month (62). The sebaceous gland is thought to play an active role in processing of the sheath of terminal hair shafts. The shaft does not separate normally from the sheath in the absence of the sebaceous gland (63). The exact nature of this component is not known.

In acne-prone areas, androgen causes the prepubertal vellus follicle to develop into a sebaceous follicle in which the hair remains vellus and the sebaceous gland enlarges tremendously. The sensitivity of sebaceous glands to androgens seems to follow a different dose-response curve than the hair follicle, with most sebaceous glands being highly and similarly sensitive to testosterone. Sebum production is at its nadir at about 4 yr of age and begins to increase at about 8 yr of age. Microcomedones (1 mm or less in diameter), which form when desquamated cornified cells of the upper canal of the sebaceous follicle become exceptionally adherent and form a plug in the follicular canal, make their appearance in about 40% of 8–10 yr olds. Thus, sebaceous gland function begins before true puberty, at levels of testosterone below those ordinarily required for the initiation of pubic hair growth (Fig. 6). This development corresponds with adrenarche, the "adrenal puberty" marked by increasing production of the adrenal androgen dehydroepiandrosterone sulfate (DHEA-sulfate). Seventy-five percent of the normal male amount of sebaceous gland function is achieved at androgen levels normal for women. As for hair growth, there is considerable individual variability in the degree of sebum production to a given level of androgen. Although the apparent dose-response curves above are given in terms of the major circulating form of androgen, testosterone, other plasma androgens contribute to a greater or lesser extent, as will be discussed.

Retinoids antagonize the effects of androgen on the sebaceous gland. They appear to inhibit the proliferation and differentiation of sebocytes. This results in atrophy of seba-

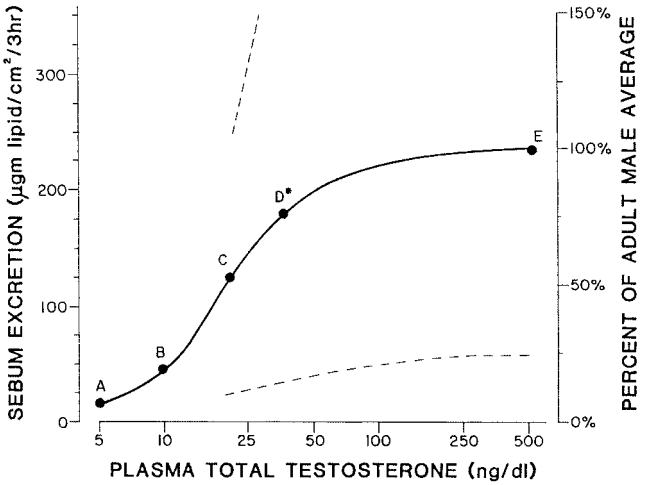

FIG. 6. Relationship between sebum output and testosterone as a representative plasma androgen. Note logarithmic scale for testosterone. *Dotted lines* show the normal range of sebum excretion. A, 4 yr-old children, computed from data on composition of sebum assuming epidermal lipid secretion rate of 10 $\mu g/cm^2/3$ h; B, 7- to 11-yr-old prepubertal children; C, castrated men; D, normal adult women, 20–40 yr of age; E, normal adult men, 20–40 yr of age; *, average sebum level of normal 15- to 19 yr-old boys and girls. [Reprinted with permission from R. L. Rosenfield: *Clin Endocrinol Metab* 15:341–362, 1986 (1) © W. B. Saunders Co.]

ceous glands and decreased sebum production in man (64–67) and animals (68–70).

IV. Growth and Development of the PSU *in Vitro*

Stromal-epithelial interaction is an important feature of the growth and differentiation of the epithelial cells of the skin and its PSU appendages *in vitro* just as it is *in vivo*. This is like the situation in other glands that are targets of sex steroid action (71).

A. Organ culture

Organ culture has permitted the short-term study of growth and development of hair follicles and sebaceous glands *in vitro* without disturbing the natural close relationship between the stromal and epithelial components of these structures (40, 67). Human hair follicles isolated by microdissection have less stringent requirements for maintenance and growth in culture than sebaceous glands (40). Cortisol and insulin are necessary for optimal growth (72). Hair follicles can be maintained in short-term organ culture while maintaining their *in situ* morphology and growth at a normal rate of about 0.3 mm/day (72–74). By day 14 in culture, the dermal papilla rounds up and the follicle no longer produces a keratinized hair fiber.

Human sebaceous glands isolated by microdissection can be maintained for up to 7 days in organ culture with full retention of their *in situ* morphology, rates of lipogenesis, and responses to steroid hormones (72, 75). Although they continue to form new cells at a normal rate until 14 days of culture, they do not differentiate normally after 7 days in culture unless phenol-red, an estrogen, is removed from the medium (67, 75). Insulin, cortisol, T_3, and bovine pituitary extract are required for optimal maintenance of the human sebaceous gland in organ culture (72).

B. Monolayer culture

Monolayer culture has been used to study the specific factors involved in the growth and development of hair and sebaceous epithelial cells. However, monolayer culture is known to be incompatible with the normal differentiation of skin epithelial cells (76). When epidermal cells are grown in monolayer, they progress almost directly from basal to a thin squamous cell layer without the intervening cell stages. On the other hand, when epidermal cells are grown on an artificial dermis (composed of 3T3 fibroblasts in a collagen lattice) lifted on a raft to the air-liquid interface, development is virtually normal. The normal balance between epidermal growth and differentiation in the lifted raft system has been attributed to a retinoic acid gradient being established at the epidermal-dermal junction (77). The nanomolar concentration of retinoic acid in FCS inhibits normal orderly cell maturation and the biochemical changes characteristic of terminal differentiation in a submerged raft system. When rafts are lifted to the surface of the medium, the level of retinoic acid falls in the suprabasal layers, so differentiation progresses normally. If, however, the retinoic acid concentration in the medium is raised in the lifted raft system, epidermal differentiation becomes disturbed like that in monolayer culture (78).

Hair and sebaceous epithelial cells are grown in epidermal type monolayer culture systems. Traditionally, this requires maintaining them in close contact with a stromal feeder layer consisting of 3T3-fibroblasts. For epidermal cells, the stromal growth factors have been identified and the requirements for growth in culture simplified: a collagen matrix or fibronectin are necessary for good plating efficiency, and insulin or IGF-I plus keratinocyte growth factor (FGF-7) are necessary for growth (79, 80). Hair and sebaceous epithelia have more stringent requirements for growth in primary monolayer culture. For sustained proliferation, most systems require a stromal support system and medium containing insulin, hydrocortisone, and cAMP-amplifying agent such as choleratoxin (72, 73, 81). Stromal support systems include 3T3 cells, nitrocellulose filters (72, 74), 3T3 cells mixed with collagen (76), and gelatin sponge supports (73). Although most previous studies have been done in the presence of serum, serum has been found to inhibit growth of hair follicles and to inhibit differentiation of sebaceous cells in culture (40, 73, 82). This may be due, in part, to inhibitory factors within serum such as TGFβ (73). Only recently have chemically defined serum-free media become available that support growth of hair and epithelial cells.

Hair and sebaceous epithelial cells form typical polyhedral epithelial cell colonies in culture, which resemble epidermal epithelial cell colonies by light microscopy. Nevertheless, they can be identified as unique epithelial cell populations by a variety of techniques. For example, early-passage cells cultured from plucked anagen hairs have the characteristics of outer root sheath cells according to ultrastructural analysis and the pattern of expression of hair proteins (83). They also

have a pattern of testosterone metabolism that favors androgen action (high ratio of 5α-reductase to 17β-hydroxysteroid dehydrogenase activities) compared with epidermal cells (84). Dermal papilla cells, which themselves have a distinctive profile in culture (85), are the only type of stroma known to support the growth and early differentiation of putative hair germ cells from epidermis (86). Fujie *et al.* (87) reported recently that, by using specific growth medium containing bovine pituitary extract, cells derived from human sebaceous glands could be maintained in primary culture and serially cultured under serum free conditions, without a biological feeder layer or specific matrices. This effect was demonstrated in both explant culture and dispersed cell culture. Sebocytes obtained from outgrowths (explants) from the periphery of the gland lobules (88, 89) can be passaged twice in monolayer culture without a stromal support system before rates of sebocyte proliferation fall (72). Proliferation of these cells *in vitro* has been found to depend inversely on the age of the donor and also on the specific body site where the skin was isolated (88). Recently, Zouboulis *et al.* (90) developed an aneuploid immortalized human sebaceous gland cell line that maintains the morphological and functional characteristics of normal differentiated human sebocytes in the absence of a stromal matrix.

Our studies of sebocyte growth and development have used rat preputial sebocytes. The preputial glands of the rat are located on either side of the penis in the male, and the clitoris in the female. The secretions of the preputial gland are thought to play a role in both territorial marking and mating behavior. The preputial gland has been an attractive source of cells for the study of sebaceous cell growth and differentiation as the paired glands are easy to isolate because they are large and encapsulated, they are available on demand, and single cell suspensions at all stages of differentiation can be prepared for study (91). Although the preputial gland may have physiological functions beyond that of the human sebaceous glands, the gland is a holocrine organ, and preputial sebocytes resemble human sebocytes in many ways (81, 92, 93). Single cell suspensions are prepared from isolated preputial glands and plated on a mitomycin-C treated 3T3-J2 feeder layer. After attachment, sebocytes are cultured in a serum-free, chemically defined cell culture medium that permits definition of the factors regulating sebocyte proliferation and differentiation (82).

These sebaceous cells have been shown to exhibit a number of differentiation characteristics in monolayer culture that are similar to those of human sebocytes and distinguish them from epidermal cells. Sebocytes form relatively slow-growing colonies (81, 94). They contain a variety of keratins, including cytokeratin K4, which is localized to suprabasal sebocytes and is constitutively expressed in culture (81, 88). Sebocytes also differ from epidermal cells by forming few cornified envelopes in culture, as *in vivo* (Fig. 7, *top panel*) (81, 94). They respond to β-adrenergic treatment *in vitro*, as *in vivo*, with a distinctive pattern of cAMP-regulatory subunit predominance (95, 96). A striking difference between the behavior of sebocytic and epidermal keratinocytes in culture is in their differential response to the administration of all-*trans*-retinoic acid (1a, 97). Retinoic acid causes dose-dependent inhibition of sebocyte proliferation but does not have an effect on epidermal cell growth (Fig. 7, *bottom panel*). In contrast to its inhibitory effect on sebocytes, however, retinoic acid appears to maintain the PSU duct (98). Sebocytes form sebum-specific lipids such as squalene and wax esters (88). Although fatty acid synthesis is greater in cultured sebocytes than in cultured epidermal cells (88, 94), the amount of lipid is not enough to clearly distinguish

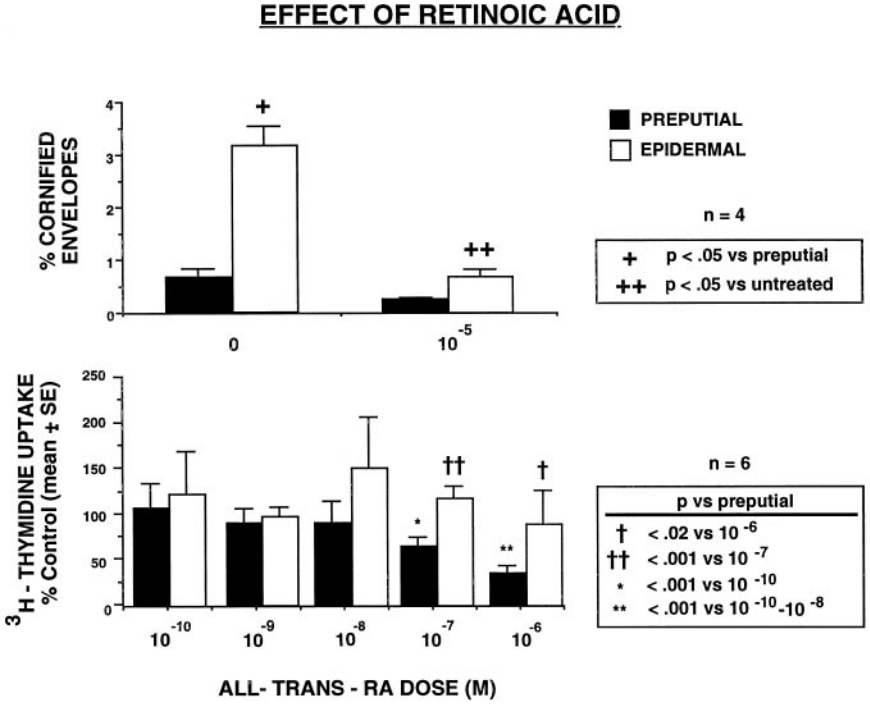

FIG. 7. Retinoic acid (RA) effects on preputial sebocytes grown in monolayer culture. *Top panel,* Epidermal cells develop more cornified envelopes in culture than sebocytes, and this development is inhibited by all-*trans*-RA. *Bottom panel,* All-*trans*-RA inhibits the proliferation of cultured sebocytes in a dose-related fashion but does not affect the growth of cultured epidermal cells. *P* values by Tukey's test after two-way ANOVA. [Reprinted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:80S–88S, 1995 (1a) © Excerpta Medica Inc.]

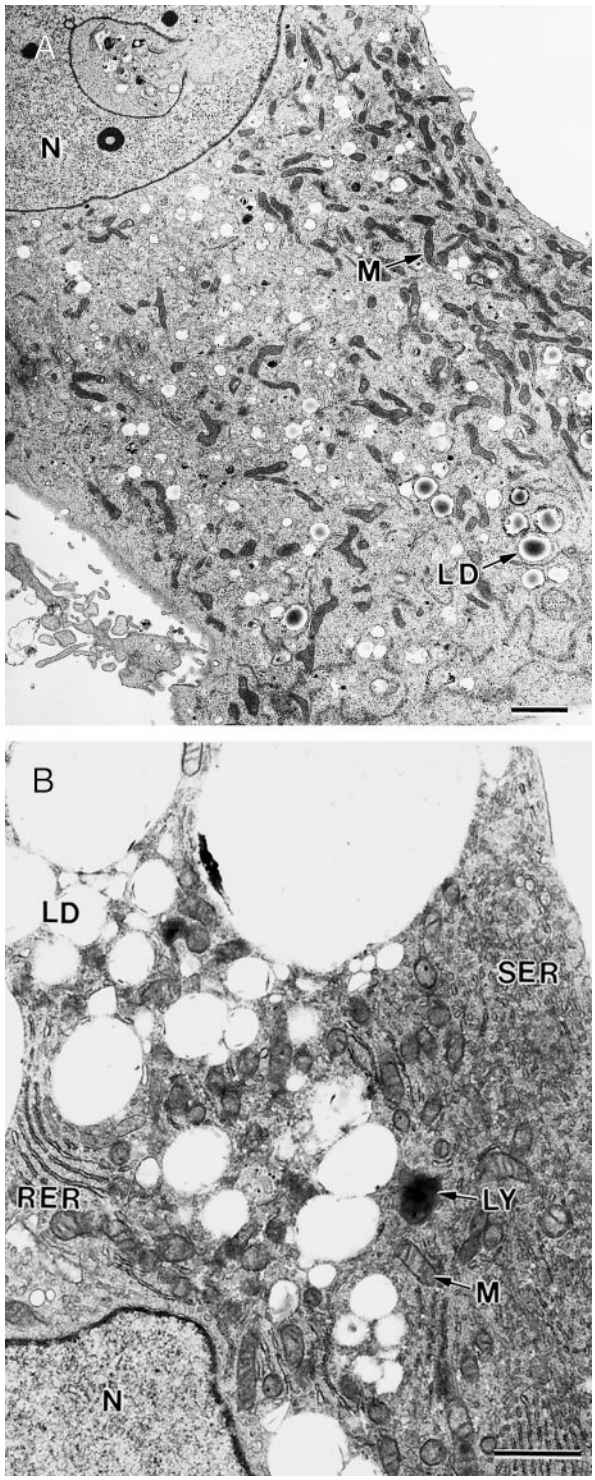

FIG. 8. Electron photomicrographs of preputial sebocytes in monolayer culture. *Top,* Typical early differentiated preputial cell. The cytoplasm is filled with much smooth endoplasmic reticulum, a few cisternae of rough endoplasmic reticulum, mitochondria (M), and small lipid droplets (LD). Several inclusions are present in the nucleus (N). *Scale bar,* 2 μm. *Bottom,* Maturing preputial cell near center of colony. The cytoplasm contains coalescing lipid droplets (LD), smooth (SER) and rough (RER) endoplasmic reticulum, mitochondria (M), and occasional lysosomes (LY). N, Nucleus. *Scale bar*, 1 μm. [Reprinted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:80S–88S, 1995 (1a) © Excerpta Medica Inc.]

them from epidermal cells by light microscopy (99). Electron microscopy reveals that sebocytes in monolayer culture form abundant tiny lipid droplets, but they undergo only abortive differentiation, with coalescence of these droplets in only a very few cells at the center of colonies (Fig. 8) (1a).

V. Androgen Mechanism of Action in the PSU

Skin is a major site of testosterone formation in women, in whom half of testosterone production is derived from peripheral conversion of secreted 17-ketosteroids such as DHEA, DHEA-sulfate, and androstenedione (5). Although the skin is not capable of *de novo* synthesis of androgens from cholesterol, it contains all the enzymes necessary to convert the prohormones DHEA and androstenedione into testosterone and the most potent androgen, dihydrotestosterone (DHT) (1, 100–102). Serum levels of DHEA-sulfate have been found to correlate with sebum production in early puberty (103) and with the presence of acne vulgaris in prepubertal girls (4, 104). The metabolic pathway involved in forming active androgens from DHEA-sulfate is illustrated in Fig. 9.

The androgen-sensitive skin appendages (sweat gland, hair follicle, and sebaceous gland) each metabolize androgens in a characteristic pattern; however, sweat glands and sebaceous glands account for the vast majority of androgen metabolism in skin (Table 1) (105–108). 3β-Hydroxysteroid dehydrogenase (HSD) is particularly prominent in sebaceous glands (109, 110). Type 2 17β-HSD mRNA expression has been reported in outer root sheath cells of cultured human hair, and type 3 17β-HSD expression in beard and axillary dermal papilla cells from both sexes (111). The former favors inactivation of estrogen; the latter favors formation of testosterone. The predominant 17β-HSD isozyme expressed in human sebaceous glands at both the mRNA and protein level is the type 2 form (112). Furthermore, the oxidative activity (conversion of estrogen and testosterone to less active precursors) of 17β-HSD is greater in sebaceous glands from non-acne-prone skin as compared with acne-prone regions. A predominance of 5α-reductase over 17β-HSD activity appears to favor DHT formation in sweat glands and in the outer root sheath cells of pubic, as compared with scalp, hairs. In addition, 5α-reductase is 2 to 4 times more active than 17β-HSD in sebaceous glands from facial skin (113). A summary of the localization of the mediators of androgen signal transduction in the PSU is provided in Table 2.

The biological activity of testosterone on target tissues is effected in large part by its conversion to DHT by 5α-reductase, which is a microsomal NADPH-dependent enzyme (114–117). 5α-Reductase was first suspected to play a key role in androgen action when DHT was found to be the predominant form of steroid bound to the androgen receptor in prostate glands after the administration of testosterone (118, 119). Testosterone and DHT stimulate 5α-reductase mRNA and 5α-reductase activity, an effect mediated through the androgen receptor (116, 120–122). Two forms of 5α-reductase exist, which are differentially expressed in various tissues, likely as a result of their respective promoters. They have different pH optima and sensitivity to inhibitors. The

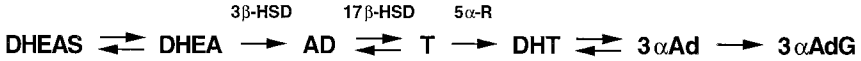

FIG. 9. A schematic representation of androgen metabolism in the skin. The skin metabolizes weak androgens such as DHEA-sulfate (DHEAS) to the more potent ones, such as DHT. The enzymes 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and 5α-reductase (5α-R) are discussed further in the text. AD, Androstenedione; T, testosterone; 3αAd, 3α-androstanediol; 3αAdG, 3α-androstanediol glucuronide.

TABLE 1. Pattern of androgen metabolism within skin organelles of axilla and scalp

	Relative enzyme activity (% of total)		
	5α-Reductase (n = 8–10)	17β-HSD (n = 6–16)	3β-HSD (n = 2)
Sebaceous gland	17	21	} 50
Hair follicle	8	15	
Sweat gland	60	47	40
Dermis	9	6	8
Epidermis	6	11	2
Total	100%	100%	100%

Regional differences between axilla and scalp in enzyme activity may exist. [Modified from Ref. 108.]

two isozymes are approximately 46% identical in sequence, have similar gene structures, are both hydrophobic, and share similar substrate preferences (115, 116). The type 2 isozyme is important for most androgen actions in sexual organs (123), and a deficiency of 5α-reductase type 2 in humans is a cause of male pseudohermaphroditism (115, 124). However, the type 1 isozyme is the major form of 5α-reductase in skin.

Both 5α-reductase isozymes are expressed at variable times in development. Thigpen *et al.* (123) used immunoblotting to demonstrate two waves of expression of the type 1 isozyme in human skin, the first appearing at birth and lasting through age 2–3 yr and the second beginning during puberty and continuing throughout life. This suggested induction by androgens secreted perinatally and at puberty. In contrast, there was just a single wave of expression of the type 2 isozyme in skin, beginning at or just before birth and ending around age 2–3 yr. Since they did not detect 5α-reductase type 2 expression in adult skin, they postulated that the tendency to balding may be programmed by the expression of the 5α-reductase type 2 in early life. However, the 5α-reductase type 2 isozyme has been localized by immunohistochemistry to hair follicles of human scalp; specifically to the innermost portion of the outer root sheath and the proximal inner root sheath (125). In addition, the 5α-reductase activity in the dermal papilla from beard resembles that of the type 2 isozyme in having an acidic pH optimum and a lower Michealis-Menten constant than that of nonsexual hairs (101); this suggests that dermal papillae of sexual hairs form more DHT than those of nonsexual hairs.

5α-Reductase activity has been found in cultured fibroblasts from sexual and nonsexual skin sites, in a distribution compatible with regional specialization of mesenchymal cells with respect to this important determinant of androgen action (1). 5α-Reductase activity is successively greater in fibroblasts cultured from nonsexual, pubic, and genital skin (Table 3) (108). Further studies found evidence for regional specialization of androgen metabolism in sexual and nonsexual epithelial cell types. DHT formation from testosterone in cells cultured from skin organelles increased in the following order (%/mg DNA/min): epidermal (0.8%) < scalp hair (2.8%) < pubic hair (8.1%) < foreskin fibroblasts (71%) (84).

The activity of 5α-reductase has been found to be higher in sebaceous glands of the scalp and facial skin than in other skin areas (106). The type 1 isozyme is the major form of 5α-reductase in the scalp, and there are no obvious differences in type 1 isozyme expression between balding *vs.* nonbalding areas of adult scalp according to immunohistochemical studies (117, 123, 125). Within the scalp, the type 1 isozyme is localized primarily to the sebaceous glands, with lower levels present in the hair follicle and dermis. Imperato-McGinley *et al.* (126) found sebum production in patients with 5α-reductase type 2 deficiency to equal that of normal males. This suggested that either the male level of testosterone compensated for the decreased DHT and was capable of sustaining sebum production or that sebum production was under the control of the type 1 isozyme. The type 1 isozyme is also the predominant isozyme in rat preputial sebocytes (127).

Androgens act after binding to the androgen receptor, which is a member of the subfamily of steroid hormone receptors that includes the progesterone, mineralocorticoid, and glucocorticoid receptors (128). At low concentrations, potent agonists of the androgen receptor facilitate interactions between the amino-terminal and carboxy-terminal regions of the androgen receptor, which stabilizes the receptor and likely causes a slowing of ligand dissociation from the receptor (129). Both testosterone and DHT bind to the same high-affinity androgen receptor, but they bind with different affinities and dissociation rate constants, have different efficacy in stabilizing the androgen receptor, and have different physiological roles (130). Once testosterone or DHT is bound to the androgen receptor, the substrate-receptor complex binds to the androgen receptor response element and regulates gene expression by acting as a transcription factor. The DHT-receptor complex appears to be the more effective complex at activating gene transcription (115) and may also be capable of activating genes that the testosterone-receptor complex cannot activate.

Androgen receptors in skin are primarily localized to dermal papilla, sebaceous epithelium, and eccrine sweat epithelium according to immunohistochemical analysis (131–133). They are also present in lesser amounts in basal epidermal cells and scattered reticular dermal fibroblasts. Successively greater numbers of androgen receptors have been found in fibroblasts cultured from nonsexual, pubic, and genital skin (Table 3) (108). It is unclear whether there is specific androgen receptor immunoreactivity in the hair bulb or outer root sheath. In rat preputial sebocytes, androgen receptor expression has been found to increase with sebocyte differentiation (93). Androgen receptor mRNA abundance

TABLE 2. Parameters of androgen action in the pilosebaceous unit

Parameter	Sebaceous gland		Sexual hair	
	Stroma	Sebocytes	Dermal papilla	Hair epithelium
3β-HSD	?	+++	?	±
17β-HSD	?	Type 2	Type 3	Type 2
5α-Reductase	+	Type 1	Type 2	Type 1
Androgen receptor	+	++++	++++	±

TABLE 3. Relative 5α-reductase activity and androgen receptor content of fibroblasts cultured from sexual and nonsexual skin sites

Site	5α-Reductase activity (relative %)	Androgen receptor sites (relative %)
Genital	100	100
Pubic	54	51
Nonsexual	4	34

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seems to approach its maximum at the stage at which sebocytes achieve competence for their specific pattern of lipid accumulation.

The dermal papilla cells are thought to be the primary target cells within the hair follicle that mediate the growth-stimulating signals of androgens, by releasing growth factors that act in a paracrine fashion on the other cells of the hair follicle (16, 134). Studies by Itami *et al.* (135) support this concept. These workers reported a stimulatory effect of androgen on the growth of beard hair epithelium in monolayer culture. However, as with outer root sheath cells grown from other sexual hairs (84), androgen did not directly stimulate the growth of outer root sheath cells, nor did androgen affect the growth of beard dermal papilla cells. However, when beard outer root sheath cells and beard dermal papilla cells were cocultured, androgen stimulated the growth of the beard epithelial cells, and antiandrogen countered this effect. Furthermore, dermal papilla cells cultured from androgen-sensitive (beard) hair follicles not only have more androgen receptor binding sites than do those from less androgen-sensitive (scalp) sites (134), but the dermal papilla are also larger (42). Saturation analysis revealed more androgen receptors in dermal papilla cells cultured from balding scalp, as compared with nonbalding scalp, which supports the hypothesis that androgens work via the dermal papilla cells (136).

The mode of androgen action on sebocyte proliferation is unclear. Akamatsu *et al.* (137, 138) reported a direct stimulatory effect of androgen on the growth of passaged human sebocytes in monolayer culture. In addition, there is evidence that the effect of testosterone and DHT on human sebaceous cell proliferation depends on the area of skin from which the glands are obtained. In one system, proliferation of sebaceous cells obtained from facial skin was stimulated up to 50% in a dose-dependent manner by both testosterone and DHT, whereas in sebaceous cells isolated from the extremity, testosterone had no effect at all, while DHT had only a small effect (137, 139). Furthermore, spironolactone, which exhibits antagonistic activity to androgens at a cellular level, inhibited sebocyte proliferation, thus supporting a receptor-mediated effect (139). In another system, the androgen effect on proliferation of facial sebaceous cells was maximized (50% increase) at about 10^{-9} M and lost at 10^{-7} M (87). On the other hand, androgen has an inhibitory effect on preputial sebocyte proliferation in primary monolayer culture (140). It is unclear whether these different effects are due to variances in culture technique or species differences.

Androgens have been shown to stimulate the differentiation of sebocytes, although this effect is modest *in vitro* (75, 141). However, androgen augments the differentiative effect of peroxisome proliferator activated receptor-γ (PPARγ). Recent research also suggests important postreceptor interactions of androgen with retinoic acid derivatives and GH.

VI. Role of Peroxisome Proliferator-Activated Receptors in Sebocyte Development

Despite the fact that sebaceous gland growth is dependent on androgen *in vivo*, androgens have not had a clear effect on sebocyte differentiation in a variety of *in vitro* culture systems (75, 137, 142, 143). Since androgen receptors are present in sebaceous cells (93), we postulated that a downstream signal transduction pathway involved in the regulation of lipid metabolism was not being expressed in cultured sebocytes. We tested the hypothesis that mechanisms involved in lipogenesis during adipocyte differentiation may be similarly used in sebocyte differentiation.

We found that PPAR activators induced lipogenesis in rat sebocytes *in vitro* (141), although *in vivo* studies had not shown the systemic administration of the PPAR activators, clofibric acid (144) or eicosatetraynoic acid (145), to stimulate sebum activity. PPARs have been shown to regulate multiple lipid metabolic genes in peroxisomes, microsomes, and mitochondria by acting on PPAR response elements (146, 147). PPARs were originally identified as part of a subfamily of "orphan receptors" within the nonsteroid receptor family of nuclear hormone receptors (148). There are three PPAR subtypes: α, δ, and γ. Activation of PPARγ and α by their respective specific ligands, the thiazolidinedione rosiglitazone and the fibrate WY-14643, induced lipid droplet formation in sebocytes but not in epidermal cells. Linoleic acid and carbaprostacyclin, both PPARδ and α ligand-activators, were more effective but less specific, stimulating lipid formation in both types of cells. Either was more effective than the combination of PPARγ and α activation, suggesting that PPARδ is involved in this lipid formation. Linoleic acid 0.1 mM stimulated significantly more advanced sebocyte maturation than any other treatment, including carbaprostacyclin, which was compatible with a distinct role of long chain fatty acids in the final, terminally differentiated stage of sebocyte maturation. When DHT was added with the PPAR activators, an additive effect on lipid droplet formation in sebocytes was seen only with the combination of DHT and a PPARγ activator (Fig. 10). This suggests that PPARγ influences a step in sebocyte differentiation which is related but distinct from that influenced by androgen.

PPAR subtype mRNA expression was also detected in rat sebocytes (141). PPARγ1 mRNA was demonstrated in sebocytes, but not in epidermal cells; it was more strongly expressed in freshly dispersed than in cultured sebocytes. In

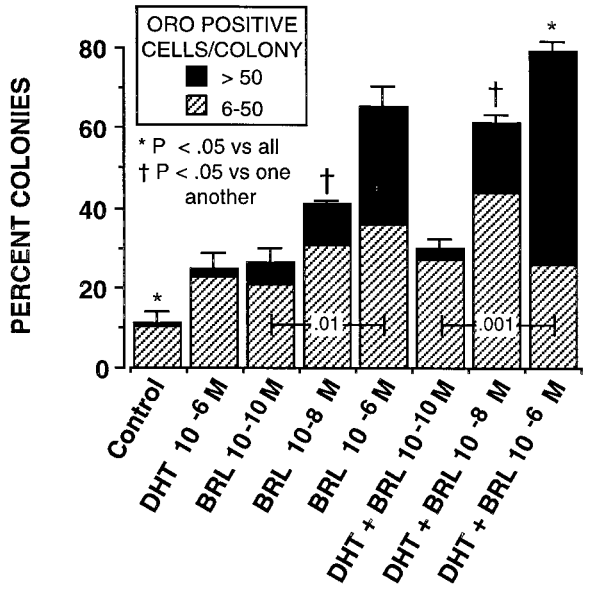

FIG. 10. Differentiation of preputial sebocytes in primary culture after treatment with dihydrotestosterone (DHT) 10^{-6} M and/or the thiazolidinedione rosiglitazone (BRL-49653) in serum free medium in the presence of insulin 10^{-6} M (n = 5). Lipid is stained with Oil Red O (ORO). Means ± SEMs are shown. DHT 10^{-6} M has a small but significant effect ($P < 0.05$). BRL has a dose-response effect over a broad range commencing at 10^{-10} M ($P < 0.01$ *vs.* control, with 10^{-8} M BRL differing from the higher and lower doses at the P level shown). DHT is additive in its effect with BRL = 10^{-8} M, and the effect of DHT + BRL 10^{-6} M is the greatest of all. [Adapted with permission from R. L. Rosenfield *et al.*: *J Invest Dermatol* 112:226–232, 1999 (141).]

contrast, PPARδ mRNA was expressed to a similarly high extent before and after culture in both sebocytes and epidermal cells. These findings are compatible with the concept that androgen-enhanced PPARγ1 gene expression plays a unique role in initiating the differentiation of sebocytes, while activation of constitutively expressed PPARδ by long-chain fatty acids finalizes sebocyte maturation.

Since increased sebum production is an important element in the pathogenesis of acne vulgaris (149, 150), the finding that PPARs appear to mediate sebocyte cytoplasmic lipid accumulation may have implications for the treatment of acne. It may be feasible to develop PPAR antagonists that can interfere selectively with sebum formation without invoking the side-effects of currently available treatment modalities.

VII. Retinoid Effects on the PSU

Retinoic acid derivatives (retinoids), which are analogs of vitamin A, have an effect on growth and differentiation of diverse tissues. Retinoids likely play a role in the hair follicle, since, like androgens, they are involved in epithelial-mesenchymal interactions in morphogenesis and embryological development, and the hair growth cycle partially recapitulates the embryogenesis of the hair follicle (42). Furthermore, retinoids alter the expression of HOX genes, which are likely to be involved in PSU morphogenesis. Retinoids have also been shown to affect the hair follicle growth-cycle in mice (151–153), with topical application increasing the length of the anagen phase, and decreasing time in telogen.

Retinoids have profound effects on sebaceous gland activity. Whereas trace amounts promote sebocyte growth and differentiation, larger doses cause atrophy of sebaceous glands and a decrease in sebum secretion in both animals and humans (64, 65, 69, 154). Retinoids have been postulated to inhibit lipid synthesis in sebocytes either directly, through an inhibition of lipogenic enzymes, or indirectly, by decreased cell proliferation (155). Retinoids have been used for the treatment of acne vulgaris for a long time although the precise mechanism for their efficacy has not been completely elucidated.

Retinoids act via specific nuclear receptors that belong to the superfamily of nuclear receptors (which include steroid receptors and PPARs) and act as ligand-dependent transcriptional regulators (128, 148). There are two classes of retinoid receptors, the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) (156, 157). Each class of receptor contains three subtypes: α, β, and γ (128, 158). The expression of retinoid receptors is tissue-specific. Whereas sebaceous glands express predominantly RXRα and RARγ in mice and humans (159–161), RARβ is highly expressed in cultured dermal papilla cells from human scalp hair follicles (162).

Two distinct cellular retinoic acid binding proteins (cRABPs) have been found, which may serve to regulate the intracellular level of retinoic acid and thus further regulate retinoid action (152). cRABP-I is postulated to enhance the metabolism of retinoic acid to inactive derivatives and thus limit the retinoic acid-specific action that hinders cell differentiation, and cRABP-II may facilitate retinoic acid signal transduction (152).

All-*trans*-retinoic acid, the natural ligand of RAR, acts after binding to RAR and also acts via its metabolism to 9-*cis*-retinoic acid, which is the natural ligand for RXR (128, 163–165). Activation of these selective receptors shows distinct biological effects on different cell types. However, the mechanisms underlying the divergent effects of RAR and RXR activation are unclear (166, 167). It has been suggested that they activate different signaling pathways, regulate the expression of distinct target genes, and/or have opposing effects on the same target gene (147, 168–170). Unlike RAR, which functions only when heterodimerized with RXR, RXR functions by forming either homodimers or heterodimers with other ligand-regulated receptors including PPARs, thyroid hormone receptor, and vitamin D receptor (171–175). RXR thus has been termed a master regulator since it functions as a key regulator of the activity of several nuclear receptors. The heterodimerization of RXR with one nuclear receptor may limit its ability to heterodimerize with other receptors if the quantity of RXR is limited. Homo- or heterodimers bind to retinoic acid response elements on DNA, typically in the promoter regions of susceptible genes, and thus control the transcription of specific genes. The characterization of retinoic acid response elements has revealed a complex pattern of retinoid recognition and activation (128, 148). Potential retinoid receptor-dependent signaling pathways that mediate cell proliferation and differentiation include those pathways involved in the induction of apoptosis and regulation of multiple growth factors and metabolic enzymes (147, 166–170, 176–178).

Previous studies using all-*trans*-retinoic acid have not been

able to discern the individual actions of each receptor as all-*trans*-retinoic acid acts on both RAR and RXR receptors. It has not been entirely clear which of the retinoid receptor pathways is involved in the specific processes of sebocyte growth and development. Recently, however, we tested selective RAR and RXR ligand-activators and their antagonists for their effects on preputial sebocyte growth and development (179). RARs (especially the β - or γ-subtypes) mediate both the antiproliferative and antidifferentiative effects of retinoids. However, RXRs had prominent differentiative and weak proliferative effects. Therefore, the antiproliferative and antidifferentiative effects of all-*trans*-retinoic acid are probably mediated by RARs, whereas its differentiative effect at high dose may be mediated by RXRs via all-*trans*-retinoic acid metabolism to 9-*cis*-retinoic acid. The stimulatory effects of the specific RXR ligand on sebaceous cells suggested that the RXR effect may be related to the ability of RXR to heterodimerize with PPARs (141). Indeed, our preliminary data suggest that a low dose of RXR ligand augments the sebocyte response to activating ligands of PPARs (180).

VIII. Roles of Nonandrogenic Hormones in PSU Development

GH, IGF, insulin, glucocorticoids, and estrogen are well recognized to play roles in PSU growth and development in lower animals (3). Clinical evidence indicates that they also play a role in man.

Several observations suggest that GH and IGF play a role in PSU growth and development. GH replacement augments the amount of sexual hair growth in response to testosterone in panhypopituitary children (Fig. 11) (181). In addition, GH substitution in adult GH-deficient men has been found to enhance androgen effects on hair growth (182). Furthermore, there may be a positive association between high IGF-I levels and the likelihood of vertex baldness in men (183). Acne vulgaris, a sebaceous gland disorder, increases at puberty when GH as well as androgen levels are rising (1). However, acne peaks in midadolescence and then normally wanes while androgen levels remain high. This course corresponds less closely to plasma androgen levels than it does to GH and IGF-I levels (184), suggesting an effect of GH and IGFs on sebaceous gland development. In addition, the GH excess of acromegaly is known to be associated with excess output of sebum (seborrhea) (185).

Animal studies support the concept that GH is important for sebocyte growth and development. Ebling *et al.* (186) demonstrated that the atrophic preputial glands of hypopituitary rats could not be restored to normal size with testosterone alone, but were fully restored when GH was added. Sebum production in response to testosterone was similarly found to depend upon GH (187). Ozegovic and Milkovic (188) examined the effects of GH on female preputial glands and found that preputial gland growth could be stimulated by GH. However, in view of the impurity of the pituitary GH available at the time these studies were performed, these results were not necessarily indicative of a GH effect.

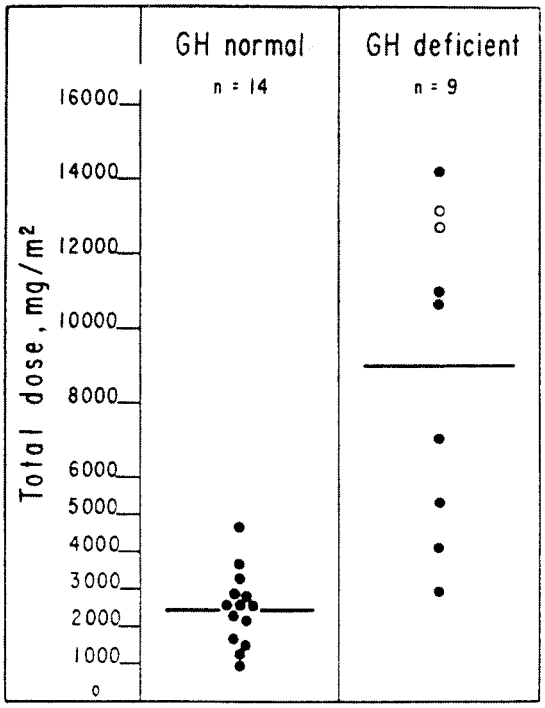

FIG. 11. Comparison of the total cumulative testosterone dose needed to induce axillary hair growth in 23 boys with and without GH deficiency. The mean total dose of testosterone (*lines* in figures) needed to induce axillary hair growth was significantly higher in the GH-deficient group as compared with the non-GH-deficient group. The *open circles* represent two patients who did not develop axillary hair despite 2.2 and 3.7 yr of testosterone treatment. [Reprinted with permission from M. Zachmann and A. Prader: *J Clin Endocrinol Metab* 30:85–95, 1970 (181). © The Endocrine Society.]

GH is an important factor for organ growth and cell differentiation (189). After binding to the GH receptor, it has both direct effects as well as indirect effects through IGF production (190–192). The IGFs make up a family of peptides that are partly GH dependent and mediate many of the mitogenic and anabolic actions of GH (190, 192).

The GH receptor has been found in hair follicles and the acini of sebaceous glands by immunohistochemistry (193–196). IGF-I has also been detected in hair follicles (197), with the IGF-I receptor being localized to the outer root sheath and matrix cells of the hair bulb (198). IGF-I has been localized to the peripheral cells of the sebaceous gland in normal rat skin by immunohistochemistry (197). This location of IGF-I corresponds with the position of the basal, highly mitotic cells of the gland. These observations support the possibility that GH and IGFs are trophic factors acting directly on hair follicles and sebaceous epithelium.

IGF-I at physiological doses is essential for hair follicle growth *in vitro* (54, 73). IGF-I prevents hair follicles from entering catagen and thus may be an important physiological regulator of hair follicle growth and the hair growth cycle (73). During the rat hair growth cycle, a marked decrease in IGF-I receptor expression is found during late anagen and early catagen (199), suggesting that a potential signal for hair follicles to enter catagen is a decrease in IGF-I receptor expression. Furthermore, IGF-I may mediate some of the an-

drogen effects on PSUs, by inducing the up-regulation of 5α-reductase by DHT in genital skin fibroblasts (200). IGF-I is 10 times more potent than IGF-II in stimulating hair follicle growth (54).

Insulin may be directly involved in hair growth as hair growth can be retarded in diabetes mellitus and accelerated by insulin treatment (201). Insulin is one of many additives required for optimal growth of many epithelial cell types in culture (72, 81, 202); in high doses it has been found to be necessary for fat cell differentiation, where it likely serves as a key regulator of lipid biosynthetic enzymes (203, 204). Insulin may act as an IGF-I surrogate as it has approximately 50% amino acid homology to the IGFs (205–207), and it binds to the IGF-I receptor at high concentrations (208). Insulin at supraphysiological doses is essential for hair follicle growth *in vitro* (54, 73), and in the absence of insulin, hair follicles prematurely enter into a catagen-like state *in vitro* (54, 209). Insulin in high dosage stimulates sebocyte proliferation in culture (140, 210).

Our data in preputial sebocytes indicate that GH, IGFs, and insulin have distinct effects on sebaceous cell growth and differentiation *in vitro* (140). GH stimulates differentiation of sebocytes yet surprisingly has no effect on DNA synthesis (Fig. 12). GH also augments the effect of DHT on sebocyte differentiation, an effect that is beyond that found with IGFs or insulin (Fig. 13). In contrast, IGF-I exerts its major effect on proliferation, while having an effect similar to insulin on differentiation (Fig. 12). Insulin in supraphysiological doses is an important factor in sebocyte differentiation, and dose-response considerations suggest that its effect of potentiating the GH induction of differentiation exceeds that expected from its action as an IGF-I surrogate (Fig. 14). These data indicate that GH may in part exert its metabolic effects on sebocytes directly rather than indirectly through IGF production. These data are consistent with the concept that increases in GH and IGF production contribute in complementary ways to the increase in sebum production during puberty and in acromegaly. The exact mechanism by which GH affects sebocyte differentiation is not completely understood, and much remains to be clarified in the chain of events after GH stimulation.

Glucocorticoids also have effects on PSUs. Hypertrichosis is present in Cushing's syndrome and acne is aggravated by glucocorticoid pharmacotherapy (211, 212). These observations suggest a role of cortisol in PSU growth and differentiation. *In vitro* studies of human sebocytes have shown that hydrocortisone stimulates sebocyte proliferation in a dose-dependent manner (210). Furthermore, our preliminary studies in rat preputial sebocytes demonstrate that cortisol is essential for sebocyte differentiation and necessary for the optimal differentiative response to GH and IGF-I (213). At low doses, cortisol also augments the proliferative effects of IGF-I. Thus, the aggravation of acne by cortisol may be related to its stimulatory effects on sebocyte differentiation and proliferation in the presence of other growth-promoting factors.

Estrogen prolongs the growth period of scalp hair by increasing cell proliferation rates and postponing the anagen-telogen transition (214). Estrogen in low dosage stimulates pubic and axillary hair growth slightly. This is clear from

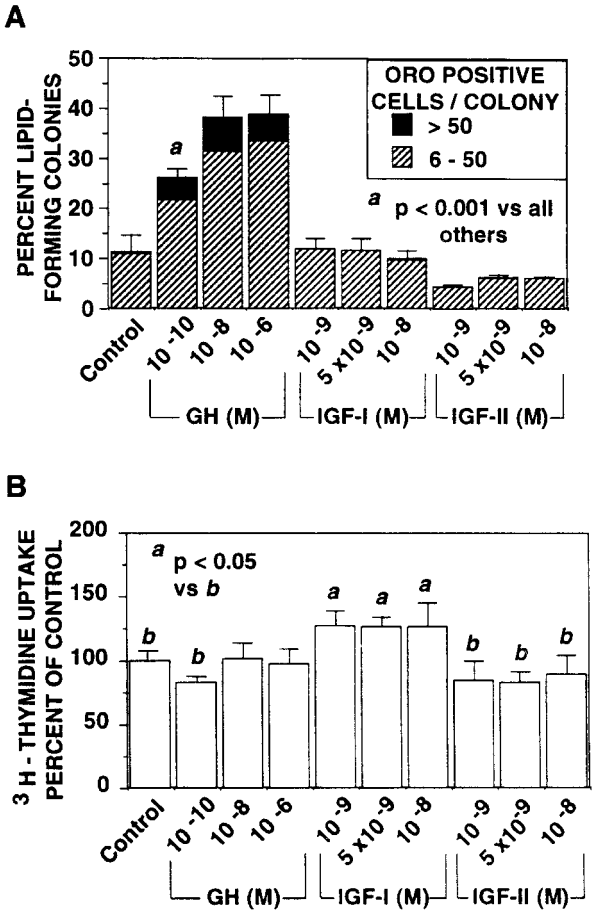

FIG. 12. Differentiation (A), and proliferation (B) of preputial sebocytes in primary culture after treatment with GH, insulin-like growth factor-I (IGF-I), or insulin-like growth factor II (IGF-II) in serum free medium in the presence of insulin 10^{-6} M (n = 4). Means ± SEMs are shown. A, The effects of these treatments on lipid-forming colonies. ORO, Oil Red O. At 10^{-8} M, GH was 4 times more potent than IGF-I, and 6 times more potent than IGF-II ($P < 0.001$). IGFs (10^{-9} to 10^{-8} M) were no better than insulin 10^{-6} M alone (control) in stimulating differentiation. B, The effect of these treatments on ^{3}H-thymidine incorporation in comparison to the control group. GH had no effect on DNA synthesis. IGF-I increased DNA synthesis significantly more than IGF-II. [Reprinted with permission from D. Deplewski and R. L. Rosenfield: *Endocrinology* 140:4089–4094, 1999 (140) © The Endocrine Society.]

observations that pubic hair increases upon inducing puberty in hypogonadal patients with physiological doses of estradiol alone (215). This occurs without a detectable increase in plasma androgens. It is possible that this effect of estrogen on hair growth is mediated in part by induction of androgen receptors (216), or by increase in IGF-I (217). In late pregnancy, when estrogen levels are high, a high proportion of scalp hair follicles remain in anagen (218). Postpartum, a large number of hair follicles simultaneously advance into telogen, causing loss of a large number of hairs. This postpartum telogen effluvium has been postulated to be caused by the rapid decrease of estrogen at the time of delivery (219). On the other hand, estrogen directly suppresses sebaceous gland function (1, 3, 75, 220, 221). An estrogen effect is clear at ethinyl estradiol doses of 35 μg/day or more (222).

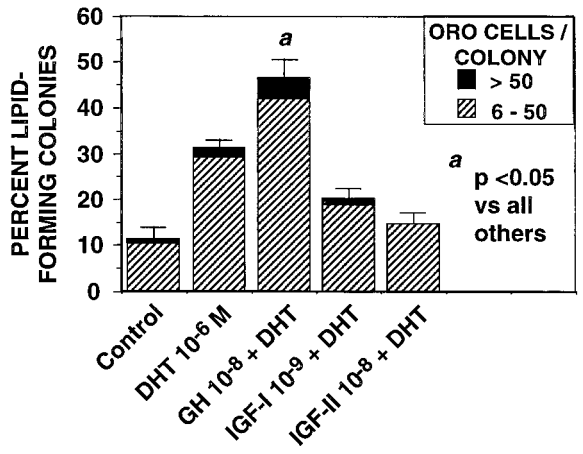

FIG. 13. Sebocyte differentiation in monolayer culture in response to maximum effective doses of GH, IGF-I, or IGF-II with DHT 10^{-6} M in the presence of insulin 10^{-6} M. Control group represents insulin 10^{-6} M without DHT. Means ± SEMs of lipid-forming colonies are shown. GH 10^{-8} M significantly augmented the effect of DHT on sebocyte differentiation, while IGF-I and IGF-II tended to have the opposite effect.

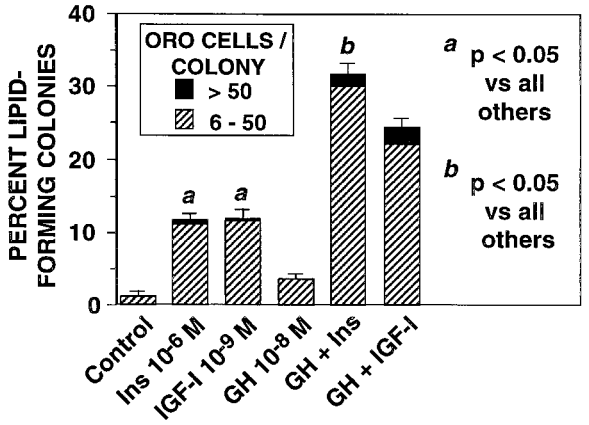

FIG. 14. Sebocyte differentiation in monolayer culture in response to maximum effective doses of insulin (Ins) and IGF-I with and without GH 10^{-8} M. Control group is without Ins, IGF-I, or GH. Means ± SEMs of lipid-forming colonies are shown. Insulin 10^{-6} M and IGF-I 10^{-9} M each stimulate approximately 12% of sebocyte colonies to differentiate. However, when these same doses of insulin and IGF-I are added with GH, the insulin/GH combination has a significantly greater effect on sebocyte differentiation (32% of colonies differentiate) than the IGF-I/GH combination (24% of colonies differentiate). [Adapted with permission from D. Deplewski and R. L. Rosenfield: *Clin Endocrinol Metab* 15:341–362, 1986 (1) © W. B. Saunders Co.]

Prolactin plays a role in PSU function as indicated by the development of hirsutism and seborrhea in hyperprolactinemia in women. To a great extent this PRL effect is mediated by its stimulation of adrenal androgen production (223). However, Wielgosz and Armstrong (224) reported in 1977 that PRL caused a significant increase in preputial gland weight in hypophysectomized, ovariectomized immature rats, suggesting that PRL acts directly as a sebotropic hormone. This may be a somatotrophic effect. However, PRL receptors have been localized to the dermal papilla and sebaceous glands in sheep skin (225).

Other hormones thought to be important in PSU growth and development include thyroid hormone and catecholamines. Thyroid disturbances lead to changes in hair character and growth. Hypothyroidism causes scalp hair to become dull and brittle. A diffuse alopecia occurs with a greater proportion of hair follicles in telogen (226, 227). Replacement treatment typically reestablishes the normal anagen/telogen ratio (227). Hyperthyroidism can also lead to diffuse hair loss (201). Thyroid hormone has also been shown to stimulate sebum secretion in hypophysectomized/castrated rats (228). Thyroid hormone receptors have recently been localized to the outer root sheath, dermal papilla, and sebaceous gland in human PSUs by immunohistochemistry (229, 230). Studies using RT-PCR have indicated that the β1 isoform is the major isoform expressed in the adult human PSU (230). Furthermore, the addition of T_3 to culture media causes the proliferation of outer root sheath and dermal papilla cells (229). Catechols are leading candidates as important natural activators of cAMP pathways. Epinephrine has been reported to rapidly stimulate sebocyte lipogenesis (96), so it may be involved in the aggravation of acne by stress.

The PTH-related protein (PTHrP), the vitamin D receptor, and melanocortin-5 receptor also play roles in PSU development. PTHrP is produced by cells of the inner root sheath of the hair follicle, and treatment of mice with an antagonist of this protein increases the number of hair follicles in the anagen phase (231). Furthermore, PTHrP knockout mice have shaggy hair and hypoplastic sebaceous glands (232), while mice with PTHrP overexpression in skin fail to initiate or have a delay in hair follicle development and have hyperplastic sebaceous glands (232, 233). The vitamin D receptor has been found to be expressed in the outer root sheath and dermal papilla of hair follicles and in sebaceous glands (234, 235). Mutations of the vitamin D receptor have been associated with alopecia in humans (236). Furthermore, vitamin D receptor knock-out mice also develop alopecia starting at 1 month of age (237). Histology of the skin of these knock-out mice reveals dilatation of the hair follicles and development of dermoid cysts. When the mineral status of these mice is normalized by dietary supplements, alopecia still occurred, which suggests that the abnormality in the vitamin D receptor mediates the alopecia (238). The melanocortin-5 receptor is a widely distributed receptor for ACTH and melanocortin peptides. Targeted disruption of this receptor leads to a decrease in sebaceous lipid production and a defect in water repulsion in mice, indicating the importance of the melanocortin-5 receptor in sebaceous gland function (239).

IX. PSU Pathophysiology in Hirsutism, Acne Vulgaris, and Pattern Alopecia

Hirsutism, acne, and pattern alopecia are variably expressed manifestations of androgen excess and may exist singly or in combination in different women (1). Since the sebaceous gland and hair follicle form a single morphological entity, such differences in expression of androgen action seem to reflect genetic variations in the diverse factors that modulate the effects of androgens on these distinct organelles. At one end of the normal spectrum are women

whose PSUs seem hypersensitive to normal blood androgen levels; this seems to account for idiopathic hirsutism and acne. At the other end of the spectrum are women whose PSUs are relatively insensitive to androgen; this seems to account for cryptic hyperandrogenism (hyperandrogenemia without skin manifestations).

A. Hirsutism

Hirsutism is typically defined as excessive male-pattern hair growth in women. This definition distinguishes hirsutism from hypertrichosis, which is the term reserved to describe the androgen-independent growth of body hair which is vellus, prominent in nonsexual areas and most commonly familial or caused by metabolic disorders (*e.g.*, thyroid disturbances, anorexia nervosa, porphyria) or medications (*e.g.*, phenytoin, minoxidil, or cyclosporine). The degree of hirsutism can be graded by the method of Ferriman and Gallwey (Fig. 15), whereby each of the nine body areas that are most hormonally sensitive are assigned a score from 0 (no hair) to 4 (frankly virile) and the scores are summed. A total score of 8 or more is abnormal for adult Caucasian women. Thus, it is normal for women to have a few terminal hairs in most of the "male" areas. Although the Ferriman-Gallwey scale is a useful clinical scoring systems, it does have its limitations. It does not take into account the fact that clearly abnormal amounts of hair growth may be confined to only one or two areas without raising the total hirsutism score, it does not weight the face appropriately to its cosmetic importance, and it does not allow one to grade the sideburn or neck areas, although these are the areas that are usually worrisome cosmetically. Furthermore, it omits grading of hair on the perineal and buttock area, which can be a source of embarrassment to affected women.

Hirsutism in women corresponds better to the plasma free testosterone than to the plasma total testosterone concentration (240, 241). Disparities between plasma levels of total and free androgens are due to the relatively low fractional binding of 17β-hydroxysteroids to plasma proteins, which in turn results from the low testosterone-estradiol binding globulin (sex hormone binding globulin) level characteristic of such women.

To better delineate the relationship between hirsutism and androgen levels, we have related hirsutism score to plasma free testosterone (Fig. 16) (242). Influences of race and age were minimized by confining the study to Caucasian females 18–21 yr of age. There was a striking variability of the relationship between hirsutism score and the plasma free testosterone concentration. Although plasma free testosterone was significantly elevated in those with mild hirsutism (hirsutism score 8–16), it was normal in half the subjects. To look at it another way, among women with modest elevations of plasma free testosterone (up to 2-fold), 22% had moderate hirsutism (hirsutism score 17–25), 43% had mild hirsutism, and 35% had none. All four moderately hirsute women in this study had elevated plasma free testosterone. The variation in the plasma free testosterone concentration within the mildly hirsute group was twice as great as could be accounted for by the normal episodic, diurnal, and cyclic variations in plasma free testosterone. This suggested that more than half of the variability in the response of the population to androgen was independent of the plasma free testosterone

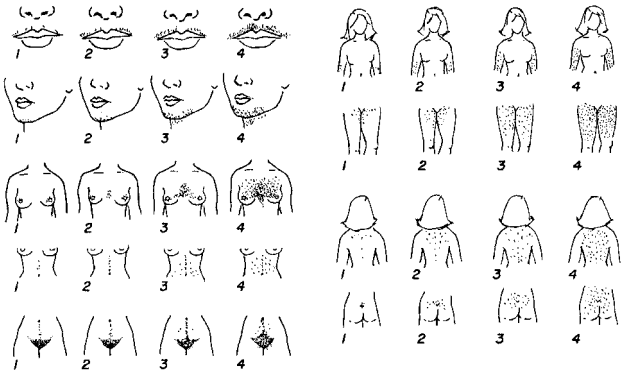

FIG. 15. Hirsutism scoring system of Ferriman and Gallwey. The nine body areas possessing androgen-sensitive pilosebaceous units are graded from 0 (no terminal hair) to 4 (frankly virile). [Reprinted with permission from R. L. Rosenfield: *Clin Endocrinol Metab* 15: 341–362, 1986 (1) © W. B. Saunders Co.]

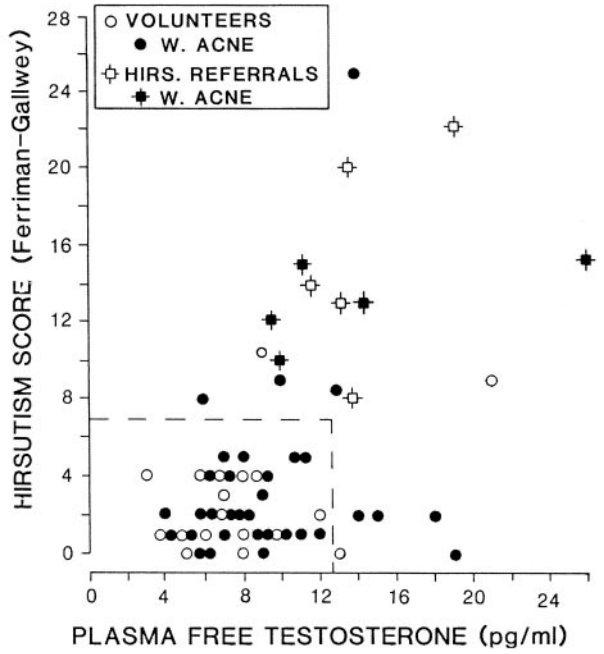

FIG. 16. Relationship of the plasma free testosterone concentration to hirsutism score and acne. *Dotted lines* show upper limits of normal (upper 5% population limit) for plasma free testosterone and hirsutism score. The study population consisted of unselected volunteers attending a student health clinic and a group, designated by *crosses*, who were referred for evaluation of hirsutism. *Solid symbols* indicate patients with acne. Acne was minor or mild in all but one case whose free testosterone level was 11 pg/ml. Only half of the women with mild hirsutism (hirsutism score 8–16) had significantly elevated plasma free testosterone levels, while all four women with moderate hirsutism (hirsutism score 17–25) had elevated free testosterone levels. Acne was a variably expressed manifestation of elevated free testosterone levels, since in four patients, acne was the only manifestation of the increased free testosterone, and acne was only present in half of the hyperandrogenic patients with hirsutism. [Reprinted with permission from R. L. Rosenfield: *Clin Endocrinol Metab* 15:341–362, 1986 (1). © W. B. Saunders Co.]

concentration. It is possible that differences among individuals with respect to other plasma androgens could make the difference. However, only one of the seven hirsute women with a normal plasma free testosterone had an elevated plasma DHT level and that was minimally abnormal. This supports the theoretical consideration which suggested that the contribution of other plasma androgenic steroids to hirsutism appears to be relatively low (108). These data illustrate the marked variability in the relationship of hirsutism to plasma free testosterone. We concluded that mild hirsutism is associated with hyperandrogenemia in half of cases, and that the great majority of women with moderately severe hirsutism are hyperandrogenic.

B. Acne vulgaris

Sebum, the holocrine secretion of sebaceous glands, plays a central role in the pathogenesis of acne vulgaris. Acne occurs at the onset of puberty, peaks at midpuberty, and usually resolves by the mid-20s (104, 243). Virtually all adolescents have at least a few open and closed comedones, which are noninflammatory enlarged sebaceous follicular ducts known as blackheads and whiteheads, respectively. Androgens are an incitant of acne vulgaris since they are necessary for the growth and differentiation of sebaceous glands (1). Furthermore, acne does not occur until plasma androgen levels rise at puberty. However, there is more to acne than sebaceous gland growth and sebum production: abnormal sebaceous duct keratinization, bacterial colonization with *Propionibacterium acnes*, and host immune response factors are also important (1).

The pathogenesis of acne is thought by most to commence with plugging of the outlet of the sebaceous gland with desquamated cornified cells of the upper canal of the follicle. These cells become abnormally adherent, thus interrupting the normal process of shedding and discharge through the follicular orifice, and a hyperkeratotic plug is formed. The more severe stages of acne are the consequences of obstruction and impaction, with bacterial secondary infection of static sebum occurring in an anaerobic environment. *P. acnes* is an anaerobic diphtheroid that is a normal constituent of the cutaneous flora and populates the androgen-stimulated sebaceous follicle. It is not present on the skin in an appreciable amount until the onset of puberty (244), and skin of acne-prone patients has a greater population of this bacteria than those without acne (245). *P. acnes* causes hydrolysis of triglycerides to liberate FFA as irritants, as well as releasing chemotactic factors that attract neutrophils, which cause further damage and eventual rupture of the follicular wall (246).

A closed comedone takes 2 months to form from its precursor lesion, the microcomedone (149). Inflammatory acne, consisting of papules, pustules, nodules, and cysts, is a later phenomenon that develops from comedonal acne. Although inflammatory lesions may be fewer in number compared with comedones, these are what usually lead patients to seek treatment (247). Inflammatory acne may leave deep physical and psychological scars. A clinically useful acne grading system is shown in Table 4.

Similar to hirsutism, acne vulgaris in adult women corresponds better to the plasma free testosterone than to the total testosterone. Lucky *et al.* (248) found that women with acne alone had plasma free testosterone levels as high as did those with hirsutism with or without acne. Schiavone *et al.* (249) obtained similar results; in addition, they found only a weak correlation between the severity of acne and the plasma free testosterone level. In our study of 18- to 21-yr-old females, as described above for hirsutism, acne was a variably expressed manifestation of hyperandrogenemia: in four patients, minor acne was the only manifestation of an elevated plasma free testosterone level, and only half the hyperandrogenemic patients with hirsutism had acne (Fig. 16) (242). Furthermore, acne severity did not correlate with free testosterone levels.

DHEA-sulfate also plays a role in acne through its conversion to more potent androgens that stimulate sebum production. Prominence of 3β-hydroxysteroid dehydrogenase in sebocytes (109, 110) permits DHEA to be a prominent prohormone for DHT formation within skin. Plasma DHEA-sulfate is likely the most important androgen for the initiation of comedonal acne in early puberty, as it rises first (4). Marynick *et al.* (250) found that DHEA-sulfate plasma concentrations correlated specifically with cystic acne. Excessive DHT formation in skin has also been implicated in the pathogenesis of acne vulgaris (100, 251), suggesting that activity of 5α-reductase may also play an important role.

When the onset of inflammatory acne is early (before age 8) or late (in the third decade), persists beyond the teen years, is resistant to appropriate therapy, flares severely with the menstrual cycle, or is accompanied by hirsutism or oligomenorrhea, underlying hyperandrogenism should be suspected (242, 252). However, acne alone, even comedonal acne alone, may be due to androgen excess (242, 248). Thus, minor or mild acne can be the sole manifestation of hyperandrogenemia.

C. Pattern alopecia

Pattern alopecia is the androgen-dependent thinning of hair that occurs progressively with advancing age in genet-

TABLE 4. Grading of acne lesions

Severity	Pustules/papules	Nodules (>5 mm)
Microcomedonal (<1 mm)	None	None
Comedonal (≥1 mm)	None	None
Mild inflammation	Few to several (<10)	None
Moderate inflammation	Several to many (10–25)	Few to several
Severe inflammation	Numerous and/or excessive[a] (>25)	Many

Face, chest, shoulders and back may be scored separately. [Adapted with permission from R. L. Rosenfield and A. W. Lucky: *Endocrinol Metab Clin North Am* 22:507–532, 1993 (5) © W. B. Saunders Co.]

[a] Persistent draining, sinus tracks, ongoing scarring.

ically susceptible men and women. However, it can begin as soon as the early teenage years. The process is mainly the result of miniaturization of terminal to vellus hair follicles (Fig. 1). There is also moderate loss of PSUs with time and often moderate inflammatory changes with perifollicular lymphocytic infiltration (253). The androgen dependency of pattern alopecia was initially deduced on the basis of eunuchs not suffering from male pattern hair loss unless they are given replacement testosterone (254). Pattern alopecia is generally thought to be distinct from the diffuse thinning of scalp hair associated with aging. However, Whiting found a 10% loss of hair follicles in the transitional zone of balding in men with pattern alopecia (personal communication). Thus, it remains possible that pattern alopecia may partially be due to an accentuation of the normal process of hair loss associated with aging. In men, pattern alopecia typically presents as temporo-occipital pattern (male-pattern) balding (Fig. 17) (255). In female pattern alopecia, the thinning typically begins with involvement of the crown of the scalp (rather than the vertex and bifrontal areas as in men) and may become fairly diffuse (Fig. 18) (256). Women can have isolated pattern alopecia or seborrhea with neither hirsutism nor acne, with a clear elevation of plasma free testosterone (257). Pattern alopecia can be psychologically devastating in both sexes.

The genetic predisposition to pattern alopecia is still poorly understood. However, the pattern of inheritance is considered to be polygenic with variable penetrance (258–260). It is likely that the penetrance is greatly determined by the height of the plasma androgen level. A common genetic defect has been suggested to cause male-pattern baldness in men and polycystic ovaries in women (261–263). The exact nature of this component is currently unclear.

D. PSU sensitivity to androgen

We have devised a model for the interaction between intrinsic PSU sensitivity to androgen and plasma androgen levels in the pathogenesis of hirsutism, acne, or pattern alopecia (Fig. 19) (1). In this model, the apparent sensitivity of the skin to androgens is as great a factor, if not greater, than the plasma androgen level in determining the skin manifestations of androgen excess. The variability in the response to androgen may be both quantitative (severity) or qualitative (hirsutism and/or acne and/or alopecia). Indeed, in some women, hyperhidrosis (*i.e.*, excessive sweat gland function) may be the only skin manifestation of androgen excess (264). At a normal plasma free testosterone concentration, only a small percentage of women will have hirsutism or acne, those with a high apparent skin sensitivity to androgen being

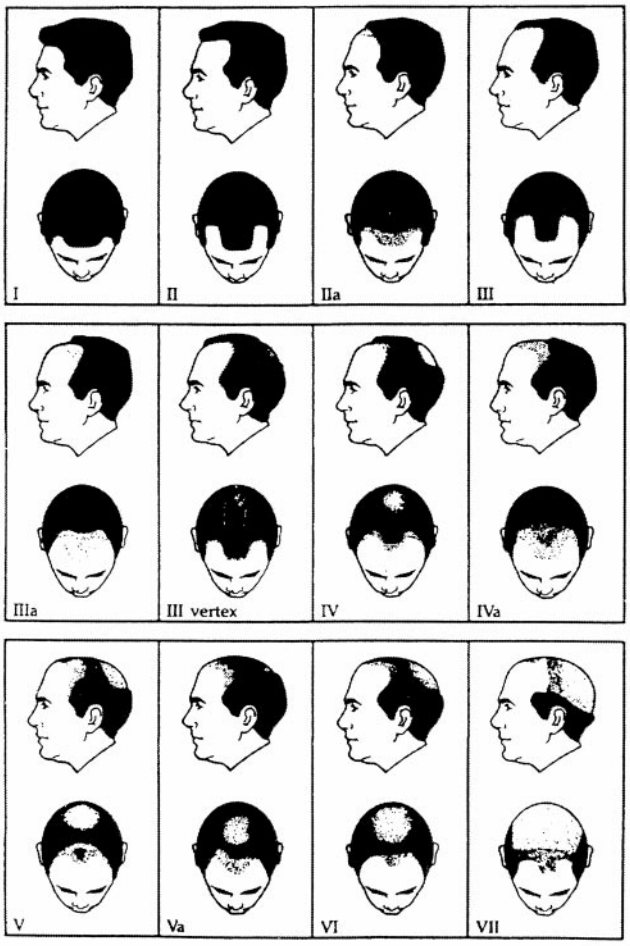

FIG. 17. Male pattern baldness. The Hamilton Baldness Scale as modified by Norwood. [Reprinted with permission from O. T. Norwood: *South Med J* 68:1359–1365, 1975 (255).]

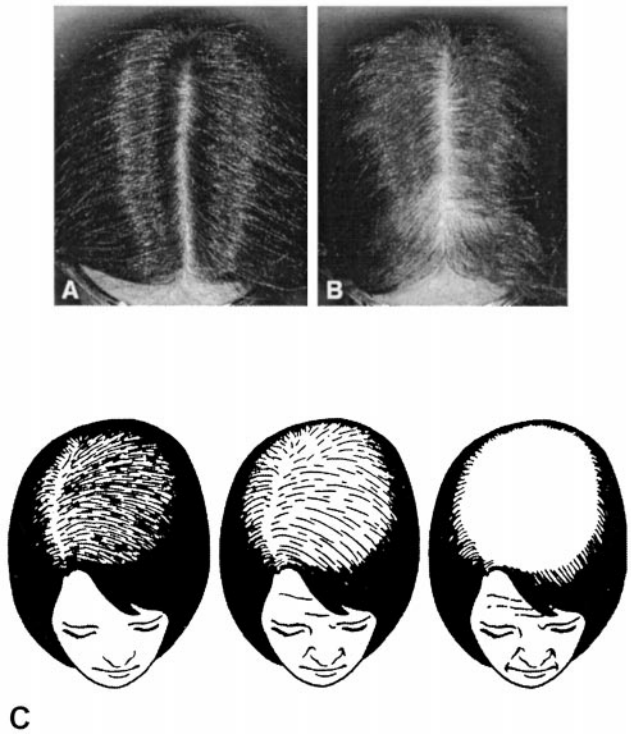

FIG. 18. Female pattern baldness. A, Midline part in an unaffected female. B, Midline part in a women with mild-pattern alopecia. Note the "Christmas tree" like pattern of hair thinning. An early finding in female-pattern alopecia is irregularity of the borders of the part. C, Ludwig patterns of alopecia in women from mild (pattern I on left), to more extensive alopecia (pattern III on right). [Reprinted with permission from E. A. Olsen: *J Am Acad Dermatol* 40:106–109, 1999 (256). © Mosby, Inc.]

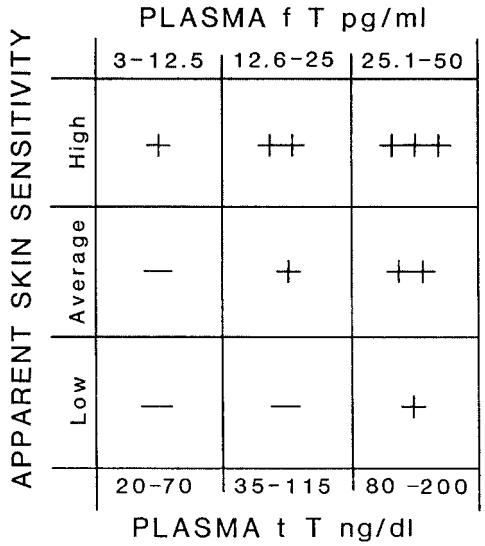

FIG. 19. Model of the apparent interaction between pilosebaceous sensitivity (hirsutism and/or acne) and plasma androgens (exemplified by plasma free testosterone (ft) concentrations). The plasma total testosterone (tt) concentrations corresponding to the plasma free testosterone categories are approximations that overlap because the fraction of plasma testosterone that is free varies widely with the concentration of testosterone binding globulin. Reprinted with permission from R. L. Rosenfield and D. Deplewski: *Am J Med* 98:80S–88S, 1995 (1a) © Excerpta Medica, Inc.]

identified clinically as having idiopathic hirsutism or acne. We advocate reserving the term "idiopathic hirsutism" for those patients in whom excessive growth of terminal hair is not explained by androgen excess. In this sense, we believe that hirsutism is either hyperandrogenic or idiopathic. (Parenthetically, this terminology distinguishes idiopathic hirsutism from "idiopathic hyperandrogenism," in which the source of androgen excess can not be localized to the ovaries or adrenal glands (265), an entity that is often confused with idiopathic hirsutism). At a modestly elevated plasma free testosterone concentration (up to 2-fold), most women will have hirsutism or acne, but in a few cases there will be no skin manifestations. This variability would seem to be the basis of the cryptic hyperandrogenemia reported to occur with mild hyperandrogenic states, such as polycystic ovary syndrome and nonclassic congenital adrenal hyperplasia (266–268). At moderate elevations of free testosterone (over 2-fold), virtually all women will have some degree of hirsutism and/or acne.

It is not yet clear what controls the nature of the response of a PSU to androgen (*e.g.*, whether the response will be hirsutism alone, acne alone, or both). The variability in PSU responsiveness to androgens may be related in part to variations in androgen metabolism. This concept is supported by the finding that skin 5α-reductase activity and 5α-reduced testosterone metabolites are related to hair growth (269), and increased 5α-reductase activity has been demonstrated in skin of hirsute women (as compared with nonhirsute women) (270), as well as in sebaceous glands of acne-prone skin (271). In addition, greater 3β-hydroxysteroid dehydrogenase activity has been found in sebaceous glands from balding scalp than nonbalding scalp (102). Furthermore, many authors have postulated that androstanediol glucuronide and other androgen conjugates are biochemical markers of cutaneous androgen metabolism and action (272, 273), and these levels are elevated in hirsute women (273, 274). Toscano *et al.* (274) found variations in androgen metabolism in patients with hirsutism as compared to those with acne. Hirsute women had higher levels of 3α-androstanediol and its glucuronide, whereas women with acne alone had levels similar to the control group. This suggested that the presentation of hirsutism or acne was dependent on differential skin metabolism of androgens. However, Rittmaster (275, 276) has reviewed evidence that counters this hypothesis and strongly suggests that androgen conjugates are more likely to be markers of adrenal steroid production and metabolism than of skin metabolism. This controversy is still under debate. In summary, the expression of two separate clinical manifestations of PSU disorders may be due to different metabolic fates of DHT itself. Alternatively, these findings may simply be explained by the fact that sebaceous and sweat glands are the skin organelles with the highest 5α-reductase activity and that they undergo varying degrees of hypertrophy in hyperandrogenic states.

There are associations of hirsutism and acne with hyperprolactinemia (223), acromegaly (277), and insulin-resistant states (278–281). The hirsutism in these states has been generally thought to be mediated by hyperandrogenism. Two considerations suggest that insulin-like growth factors also likely play a direct role. First, GH and IGF-I directly promote PSU growth and/or differentiation. Second, hirsute patients with adrenal hyperandrogenism reportedly have an increase in IGF-I levels, while those with ovarian hyperandrogenism have decreased levels of IGF binding protein-3, which would seem to enhance IGF-I bioavailability (282).

The possibility that variations in androgen receptor expression are related to PSU responsiveness seemed unlikely from early studies (283). However, recent investigations have reopened the question by examining the length of the polymorphic CAG repeat in exon 1 of the androgen receptor (which can affect receptor activity) and the imprinting status of the androgen receptor (as the androgen receptor is subject to X chromosome inactivation) in hirsute and nonhirsute females (284–286). No correlation was found between the number of CAG repeats and the presence or absence of hirsutism (284–286). Whereas Vottero *et al.* (285) reported that hirsute patients had skewing of X chromosome inactivation with the shorter of the two alleles of the androgen receptor (the most active) being significantly less methylated, a recent larger study by Calvo *et al.* (286) showed no evidence of skewed X chromosome inactivation in hirsute women. Thus, neither the number of CAG repeats in the androgen receptor gene nor skewed X chromosome inactivation is likely to play a role in variable PSU responsiveness to androgens.

X. The Role of Hormonal Treatment in PSU Disorders

Hirsutism can be controlled by cosmetic measures such as shaving, plucking, waxing, and electrolysis. Topical agents

and retinoids play an important role in the treatment of acne, and minoxidil is the first line treatment for pattern alopecia. However, mechanical hair removal does not alter the underlying tendency of hirsutism to worsen with time, and topical treatments often yield incomplete control of acne, particularly when there is a hyperandrogenic basis. Hormonal treatment of the PSU manifestations of hyperandrogenism is used to either suppress ovarian or adrenal androgen production or to block the action of androgens within the skin (Table 5). Typically, 9–12 months of treatment are needed to judge the efficacy of a given treatment on hair growth, because of the long duration of the hair growth cycle, and 2–3 months may be needed to see the full effect of treatment on acne.

Combination estrogen-progestin therapy, in the form of an oral contraceptive, is first-line endocrine therapy for PSU disorders in women with ovarian hyperandrogenism. The estrogenic component, in particular, is responsible for the suppression of LH and thus serum androgen levels (287) and also results in a dose-related increase in sex hormone binding globulin (SHBG) (288), which lowers the fraction of plasma testosterone that is unbound. Combination therapy has also been demonstrated to decrease DHEA-sulfate levels, perhaps by reducing ACTH levels (289, 290). The adequacy of androgen suppression can be assessed at the end of the third week of treatment.

The estrogenic component of most oral contraceptives currently in use is either ethinyl estradiol or mestranol. The choice of a specific oral contraceptive should be predicated on the progestational component as many progestins have some androgenic potential. Ethynodiol diacetate has a relatively low androgenic potential, while progestins such as norethindrone, norgestrel, and levonorgestrel are particularly androgenic (291), as can be judged from their attenuation of the estrogen-induced increase in SHBG level (292). Norgestimate exemplifies the newer generation of progestins that are virtually nonandrogenic (293) and seems to be the progestin of choice for treatment of hyperandrogenic women (222).

In clinical trials of estrogen-progestin therapy, the extent of hair growth, based on shaving frequency, is improved in half of women (294). This treatment can be expected to arrest progression of hair growth and reduce the need for depilation procedures.

TABLE 5. Hormonal modalities for treatment of hirsutism, acne, and pattern alopecia

Androgen suppression
Oral contraceptive pills
GnRH agonists
Ketoconazole
Glucocorticoids
Antiandrogens
Cyproterone acetate
Spironolactone
Flutamide
5α-Reductase inhibitors
Finasteride
Insulin-lowering agents
Metformin
Thiazolidinediones
D-Chiro-inositol

Estrogens also have a direct, dose-dependent suppressive effect on sebaceous cell function, with a uniform effect on acne at a dose of 100 μg/day. Recent studies have demonstrated that treatment of women with moderate acne with oral contraceptives containing 35 μg of ethinyl estradiol in combination with norgestimate improves the extent of acne by 50–70% (222, 295). Indeed, an oral contraceptive pill combining these agents (Ortho-Tri-Cyclen, Ortho Pharmaceutical Corp., Raritan, NJ) has recently been approved for the treatment of acne in women by the Food and Drug Administration.

Chronic administration of GnRH agonists suppresses pituitary-ovarian function, thus inhibiting both ovarian androgen and estrogen secretion. These agonists have been reported to be effective in the treatment of hirsutism (296, 297). However, because of the concomitant reduction of serum estrogen levels and reductions in bone mineral density observed when GnRH agonists are used alone (298, 299), it is unwise to use these agents for longer than 6 months. It has been suggested that "add back" therapy in which estrogen and progestin replacement is prescribed in conjunction with a GnRH agonist may be effective in treating androgen excess without the side effects of hypoestrogenemia (300). This therapy would seem to be useful as an alternative to oral contraceptives in women who cannot tolerate the high estrogen dose of a contraceptive pill.

Ketoconazole, a synthetic imidazole antifungal agent, inhibits multiple steps in the biosynthesis of testosterone (301, 302). In doses of 400 mg per day for 6 months it has been demonstrated to have a moderate salutary effect on acne and hirsutism. However, side effects are relatively frequent and include nausea, dry skin, pruritis, and transaminase elevation (303).

Glucocorticoid therapy can be helpful in those women with hyperandrogenism from an adrenal source, as adrenal androgens are more sensitive than cortisol to the suppressive effects of glucocorticoids (304). Glucocorticoids are the mainstay of treatment of the adrenal androgen excess of CAH, but appear to be less effective in other forms of functional adrenal androgen excess (305, 306). Prednisone in doses of 5–10 mg at bedtime is usually effective in suppressing adrenal androgens while posing minimal risk of the sequelae of glucocorticoid excess such as adrenal atrophy, weight gain, and decreased bone mineral density. We do not advocate the use of dexamethasone because it is difficult to prevent long-lasting Cushingoid striae even with doses as low as 0.5 mg daily. DHEA-sulfate levels are used to indicate the degree of adrenal suppression; the target is a level of approximately 70 μg/dl. It has been suggested by some that antiandrogen therapy in the form of cyproterone acetate (307) or spironolactone (306) is at least as effective as glucocorticoid for the treatment of hirsutism due to adrenal androgen excess (see below).

A number of therapies are available to interfere with the action of androgens at the target organ level. This is accomplished by either inhibiting the binding of testosterone or DHT to the androgen receptor or by inhibiting the conversion of testosterone to DHT by 5α-reductase. Antiandrogens currently available include cyproterone acetate, spironolactone, and flutamide, while finasteride is available as a 5α-reduc-

tase inhibitor. These agents can be expected to reduce the Ferriman-Gallwey score by approximately 15–40% in 6 months with considerable variations between studies and between individuals (308, 309). All of these agents must be used with adequate contraception in women to prevent the possibility of genital ambiguity in a male fetus.

Antiandrogens act by competitively inhibiting binding of androgen to the androgen receptor. Ebling *et al.* (310) examined several hair parameters to ascertain which might best shed light on the effect of antiandrogen given in combination with ethinyl estradiol. Shaved thigh hairs were examined in one hirsute woman. Their diameter decreased by 33%, length by 50%, and medullation by 90%. In contrast, the linear growth rate decreased by only 10%. Subsequent studies confirmed a modest decrease in the diameter of sexual hairs (311, 312) and indicated that antiandrogens act primarily by decreasing the density of anagen hairs (312). In response to antiandrogen treatment a marked (65–75%) decrease in the density of anagen hairs accounted for a decrease in the overall density of plucked hairs by 24%. Thus, these data indicate that antiandrogens reverse hirsutism primarily by inhibiting the initiation of the growth of sexual hair follicles, with the result that the remaining PSUs revert toward the vellus type.

Cyproterone acetate is the prototypic antiandrogen. It was developed as a potent progestin and was found to be a moderately potent antiandrogen and a weak glucocorticoid. It acts mainly by competitive inhibition of the binding of testosterone and DHT to the androgen receptor (313). It has the added benefit of suppressing ovarian androgen secretion and subsequently lowering serum testosterone, and, in addition, it may act to enhance the metabolic clearance of testosterone by inducing hepatic enzymes. It is an effective treatment for hirsutism and acne (314–316). Although not available for use in the United States, it is widely used throughout Canada, Mexico, and Europe. Because of its potent progestational activity and its prolonged half-time, it is administered in a "reverse sequential" manner: cyproterone acetate, 50–100 mg per day, from day 5 to 15 of the cycle with ethinyl estradiol in a dose of 35–50 μg per day from day 5 to 26 of the cycle (315). The dose of cyproterone acetate may be reduced incrementally at 6- month intervals. Diane (2 mg of cyproterone acetate with 50 μg of ethinyl estradiol) is effective in maintaining improvement in milder cases of hirsutism, and Dianette (2 mg of cyproterone acetate with 35 μg of ethinyl estradiol) (Schering, Berlin, Germany) is a leading therapy for the treatment of acne in women. Side effects of cyproterone acetate include irregular uterine bleeding, nausea, headache, fatigue, weight gain, and decreased libido.

The spironolactone metabolite canrenone binds competitively to the androgen receptor with 67% the affinity of DHT (317). It also works as a weak inhibitor of testosterone biosynthesis and a weak progestin, especially at higher doses. The antiandrogen properties are seen when the drug is given at high doses of (100–200 mg daily, in two divided doses). Several studies have demonstrated the efficacy of spironolactone in the treatment of hirsutism and acne (318–322) and a potential benefit in pattern alopecia (323). It may be possible to reduce the maintenance dose after the maximal effect has been achieved. The side effects of spironolactone tend to be dose-related (308), possibly because of its structural relationship to progesterone (324). The most common side effect is menstrual irregularity; thus, it is often helpful to use an oral contraceptive along with spironolactone to regulate the menstrual cycles. Other less common side effects of spironolactone include nausea, dyspepsia, fatigue, and breast tenderness. Patients should be monitored for hyperkalemia, hypotension, and liver dysfunction.

Flutamide is a potent nonsteroidal antiandrogen marketed for prostate cancer. It has no progestational, estrogenic, corticoid, antigonadotropic, or androgenic activity (325). Flutamide is typically used at a dose of 125–250 mg twice daily with or without the addition of an oral contraceptive. Its clinical efficacy has been shown to be similar to spironolactone (320). Trials using flutamide in women with hirsutism and acne have demonstrated a marked improvement in hirsutism and complete clearing of acne (326, 327). Flutamide is not extensively used for the treatment of hirsutism because of its expense and the possible side effect of hepatocellular toxicity (328). However, it must be noted that a recent prospective, randomized trial comparing low-dose flutamide, finasteride, ketoconazole, and combination cyproterone acetate-ethinyl estradiol demonstrated relative superiority of flutamide and cyproterone acetate-ethinyl estradiol in the treatment of hirsutism (329).

Finasteride, a 4-aza-steroid, is an inhibitor of the type 2 5α-reductase that converts testosterone to DHT. Many studies have demonstrated some degree of efficacy of finasteride in treating hirsutism (309, 321, 322, 330, 331). It has recently received Food and Drug Administration approval for the treatment of pattern alopecia in young men. Kaufman *et al.* (332) showed that an oral dose of 1 mg daily leads to a gradual 16% increase in scalp hair count and slowing of the progression of hair loss over 2 yr in most men with male-pattern hair loss. However, 14% of treated men had no response. Finasteride at this dosage has been shown to have no effect on spermatogenesis or semen production in young men (333). This low-dose finasteride treatment has been shown to decrease both scalp skin and serum DHT levels (334). Preliminary studies in postmenopausal women show little benefit of low-dose finasteride treatment on pattern alopecia (335).

There is considerable current interest in the possible role of insulin-lowering agents in the therapy of hyperandrogenism because of the evidence that hyperinsulinemia may play a critical role in the pathogenesis of the hyperandrogenism of polycystic ovary syndrome (PCOS), the most common cause of female hyperandrogenism. The insulin excess produced by resistance to the glucose-metabolic effects of insulin seems to amplify the androgen response to trophic hormones in the ovary and adrenal cortex and to cause acanthosis nigricans (336, 337). It is also possible that the insulin-IGF-I system acts in concert with androgen to stimulate PSU development, as reviewed above.

Several different modalities have been used to lower insulin levels in PCOS. These include weight loss (338), metformin (339–348), thiazolidinediones (349–351), and D-chiroinositol (352). To a greater or lesser degree, all of these insulin-lowering maneuvers lower plasma androgen levels. The extent to which these effects are translated into improvement in hirsutism or acne remains to be determined.

Metformin is a disubstituted biguanide that improves glucose tolerance usually in association with moderate reductions of serum insulin levels (353, 354). Although there is modest improvement in glucose disposal rate with metformin, the primary mechanism of action appears to be in its effect on reducing hepatic glucose output (355). A number of studies that examine the effects of metformin in women with PCOS have been published (339–348). These studies vary widely in design (dose of metformin, duration of treatment, methods of assessment of insulin resistance, etc.). While there is an inconsistent effect of metformin on carbohydrate metabolism and androgen secretion across these studies, on balance there does appear to be a modest benefit from metformin treatment in PCOS, particularly when weight loss can be achieved.

Thiazolidinediones are a class of antidiabetic drugs that improve the action of insulin in the liver, skeletal muscle, and adipose tissue (356). The first of these to become available for clinical use was troglitazone (which has recently been replaced in the market by rosiglitazone and pioglitazone). In contrast to the effects observed with metformin, troglitazone has a major impact on glucose disposal rate, with a modest effect on hepatic glucose output (355). As such, thiazolidinediones are most appropriately viewed as a true insulin sensitizing agents. There is a high degree of concordance in the findings of the published studies in which troglitazone was administered to women with PCOS (349–351). Importantly, the metabolic profile was improved in such a way as to lower cardiovascular risk factors. Furthermore, troglitazone has also been recently shown to enhance ovulatory function in PCOS (350). The attenuation of hyperinsulinemia is associated with improvement of hyperandrogenemia in obese women with PCOS. The effect on acne will be of particular interest since this class of agents would seem to have potentially counterbalancing effects on sebaceous cell function, with suppression of insulin and androgen levels tending to lower sebum output and direct PPARγ activation tending to increase it.

XI. Conclusions

Androgens are prerequisites for the growth and differentiation of sexual hairs and sebaceous glands. However, the mode by which they interact with other factors to bring about PSU development and PSU disorders is incompletely understood. During embryogenesis, PSUs acquire the ability to respond to androgens in distinct ways according to their pattern of distribution. Homeobox genes are likely involved in coordinating the interaction of the multiple genes involved in this differentiation process. The postnatal hair cycle, cycling back and forth from the growth to the resting phase, recapitulates the embryonic development of the hair follicle repetitively throughout life. Hair follicle growth, and possibly sebaceous follicle growth, involve a close reciprocal interaction between the epithelial and stromal components. The dermal papilla is likely the source of the growth signals that regulate the hair cycle. The nature of the growth factors that it elaborates is unknown. Cell culture methods have begun to yield insight into some of the specific factors involved in PSU growth and development, but it has not yet been possible to entirely replicate the androgen effects *in vitro*. However, androgens have been found to interact with PPARγ, and less directly with many other factors, to stimulate sebocyte differentiation (Fig. 20). Retinoids are important factors in PSU development. They not only regulate aspects of embryonic development through regulation of homeobox genes, but they exert ongoing effects on the PSU. In sebocytes, RARs appear to mediate the suppression of growth, while the RXRs appear to stimulate differentiation via their interaction with PPARs. IGFs and insulin play important roles in PSU development. IGF-I is essential for hair follicle growth and sebocyte growth *in vitro*. Insulin in high doses substitutes for IGF-I and seems to exert effects on sebocyte differentiation beyond its effect as an IGF-I surrogate. GH promotes both sexual hair growth and sebocyte differentiation in response to androgen, and its effect in the latter regard seems to be direct, not mediated by IGF-I. Many other hormones, such as glucocorticoids, estrogen, and thyroid hormone, play roles in PSU growth and development, but their exact roles remain to be elucidated.

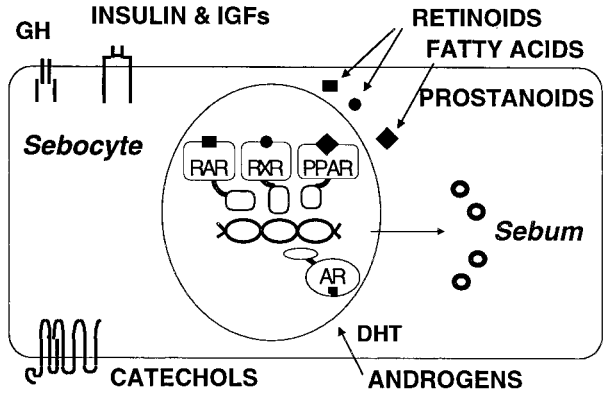

FIG. 20. Model of the interaction of androgen with other factors important for the growth and differentiation of sebocytes.

PSU disorders such as hirsutism, acne, and pattern baldness can be psychologically devastating, and current available treatments with agents that interfere with androgen action are less than optimal. Improved understanding of the role of the multiple factors involved in normal PSU growth and development is necessary to enhance our comprehension of PSU disorders and to provide new treatment approaches for these disorders.

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