

# Exclusive androgenic effect of dehydroepiandrosterone in sebaceous glands of rat skin

A Sourla, V Richard, F Labrie and C Labrie

Oncology and Molecular Endocrinology Research Center, Centre Hospitalier Universitaire de Québec (CHUQ), CHUL Pavilion, Department of Medicine and Laval University, Québec, Québec G1V 4G2, Canada

(Requests for offprints should be addressed to C Labrie, Oncology and Molecular Endocrinology Research Center, CHUL Research Center, 2705 Laurier Boulevard, Québec, Québec G1V 4G2, Canada; Email: [claudelabrie@crchul.ulaval.ca](mailto:claudelabrie@crchul.ulaval.ca))

## Abstract

In order to analyze the hormonal effects of dehydroepiandrosterone (DHEA) in skin sebaceous glands, the precursor steroid was administered to ovariectomized (OVX) female Sprague–Dawley rats at a dose of 30 mg applied on the dorsal skin, twice daily, for 3, 6 and 12 months. In a parallel experiment, female OVX rats were treated with DHEA at the same daily percutaneous dose of 30 mg, alone or in combination with the antiandrogen Flutamide or the pure antiestrogen EM-800, for 12 months, in order to determine the androgenic and/or estrogenic components of DHEA action. Treatment of female OVX rats with DHEA resulted in a similar mild to moderate hyperplasia of the sebaceous glands of both dorsal (site of application) and ventral skin, as illustrated by an increase in the number and size of the acini. The above-indicated

effects were observed at all time intervals studied, beginning at 3 months of treatment, and they were not further increased after longer term administration of DHEA (for 6 and 12 months). The addition of Flutamide to DHEA treatment completely prevented the DHEA-induced changes in the sebaceous glands, whereas the antiestrogen EM-800 had no effect. The present data indicate an exclusive androgenic stimulatory action of DHEA on the sebaceous glands, thus pointing out the importance of local intracrine DHEA transformation into androgens for skin anatomical integrity and function, while showing that estrogens, if active in rat skin, do not originate from DHEA.

*Journal of Endocrinology* (2000) **166**, 455–462

## Introduction

Skin, the largest organ in the human body, is composed of a series of androgen-responsive tissues, namely the hair follicles (Hamilton 1951, Hamilton *et al.* 1969), sebaceous glands (Strauss & Pochi 1963), sweat glands (Shelley & Hurley 1953, Papa & Kligman 1965), epidermis (Papa & Kligman 1965) and dermis (Black *et al.* 1970). Furthermore, all the above-mentioned skin appendages and components contain  $17\beta$ - and  $3\alpha$ -hydroxysteroid dehydrogenase (HSD) activities, as well as  $3\beta$ -hydroxysteroid dehydrogenase- $\Delta^4$ - $\Delta^5$  isomerase ( $3\beta$ -HSD) and  $5\alpha$ -reductase activities (Hay & Hodgins 1978, Dumont *et al.* 1992, Martel *et al.* 1992, Luu-The *et al.* 1994, Martel *et al.* 1994, Chen *et al.* 1996b, Puy *et al.* 1996). The presence in skin of the main steroidogenic enzymes (Ellis 1958, Baillie *et al.* 1966, Cameron *et al.* 1966, Pochi & Strauss 1969, Hay & Hodgins 1978, Dumont *et al.* 1992, Labrie *et al.* 1992, 1994) makes it likely that this tissue, in the human, synthesizes a significant proportion of total sex steroids including the potent androgen dihydrotestosterone (DHT) from dehydroepiandrosterone (DHEA) (Labrie 1991, Labrie *et al.* 1994, 1995, 1996b, 1997). Moreover,

receptors for androgens and estrogens have been described in squamous cells, sweat and sebaceous gland cells as well as in hair-follicle cells (Hasselquist *et al.* 1980, Mowszowicz *et al.* 1981, Choudry *et al.* 1992, Randall *et al.* 1992, Kimura *et al.* 1993, Hibberts *et al.* 1998).

There is already convincing evidence that DHEA of adrenal origin may have an important androgenic influence on sebaceous gland activity. In fact, transformation of DHEA into testosterone and other metabolites has been observed in the skin (Cameron *et al.* 1966, Gallegos & Berliner 1967, Faredin *et al.* 1969, Chrousos *et al.* 1982, Voigt *et al.* 1984, Martel *et al.* 1992, 1994). Nevertheless, although the steroids synthesized in various skin compartments could possibly have some systemic effects, it is more likely that the steroids synthesized locally in each appropriate cell type from adrenal precursors exert their effects in the same cells in which they are produced, without being released outside the cells of origin; this new area of endocrinology is called intracrinology (Labrie *et al.* 1988, Labrie 1991).

Abnormalities of androgen action are frequent and cosmetically important findings. These abnormalities include acne, seborrhea, hirsutism and androgenic

alopecia. Such androgen excess results from either increased androgen production in the ovary, overproduction of DHEA or androstenedione by the adrenal gland, excess local formation of androgens or a combination of these types of mechanisms (Lookingbill *et al.* 1985). In fact, increased skin 5 $\alpha$ -reductase (Sansone & Reisner 1971, Kuttann *et al.* 1977, 1979) and 3 $\beta$ -HSD (Thomas & Oake 1974) activities have been reported in hirsutism and acne. On the other hand, several studies describe elevations in serum testosterone and/or dehydroepiandrosterone sulfate (DHEA-S) in women with acne (Lucky *et al.* 1983, Marynick *et al.* 1983, Schiavone *et al.* 1983, Held *et al.* 1984). Moreover, changes in androgen receptor levels could be involved (Choudry *et al.* 1992, Randall *et al.* 1992, Hibberts *et al.* 1998).

In the present study, we used the ovariectomized female Sprague–Dawley rat as a model to evaluate the effect of DHEA (given percutaneously for 3, 6 or 12 months) on the histomorphology of the skin and its appendages and on the sebaceous glands in particular. In addition, we administered a combination of DHEA and the pure antiandrogen Flutamide (Neri *et al.* 1967, Simard *et al.* 1986) or the pure antiestrogen EM-800 (Gauthier *et al.* 1997, Luo *et al.* 1997a,c, Simard *et al.* 1997, Luo *et al.* 1998) for 12 months in order to analyze the androgenic and/or estrogenic component(s) of the action of DHEA in rat skin.

## Materials and Methods

### Animals

Adult female Sprague–Dawley rats (CrI:CD(SD)Br) (Charles River Laboratory, St-Constant, Canada), at 3 months of age and weighing 230–310 g at the start of the study, were used. The animals were acclimated to the environmental conditions (temperature at 22  $\pm$  2 °C, 14 h light, 10 h dark cycles, lights on at 0715 h) for at least one week before the start of the experiment. The animals were housed two per cage and were allowed free access to tap water and a commercial pellet diet (Agway ProLab R-M-H 4018, Syracuse, NY, USA). The experiment was conducted in a facility approved by the Canadian Council on Animal Care and in accordance with the CCAC Guide for Care and Use of Experimental Animals.

### Treatment

In the first experiment, the animals were randomly divided into three groups each containing eight rats as follows: a) intact; b) ovariectomized (OVX) control; and c) OVX+DHEA. In the second experiment, the animals were divided into five groups each containing eight animals, as follows: 1) intact control; 2) OVX control; 3) OVX+DHEA; 4) OVX+DHEA+Flutamide (FLU); 5) OVX+DHEA+EM-800.

On the first day of the experiment, the animals of the appropriate groups were bilaterally OVX under isoflurane-induced anesthesia. DHEA was administered percutaneously on an area 2  $\times$  2 cm in 0.050 ml 50% ethanol/50% propylene glycol (v/v) on shaved dorsal skin at a dose of 30 mg, twice daily, for 3, 6 and 12 months; in the second experiment, the duration of treatment was 12 months. The dose of DHEA chosen was based on our previous study, in which different doses and routes of administration were compared (Labrie *et al.* 1996a).

The antiandrogen Flutamide (FLU, 4'-nitro-3'-trifluoromethylisobutyranilide; 7.5 mg) was administered by s.c. injection, twice daily, while the antiestrogen EM-800 ((+)-7-pivaloyloxy-3-(4'-pivaloyloxyphenyl)-4-methyl-2-(4'-(2''-piperidinoethoxy)phenyl)-2H-benzopyran) (Gauthier *et al.* 1997, Luo *et al.* 1997b, Simard *et al.* 1997) was administered orally at a dose of 250  $\mu$ g, once daily. Treatment was initiated on the morning of day 1 of the experiment. FLU and EM-800 were administered in 4% ethanol, 4% polyethylene glycol-600, 1% gelatin and 0.9% NaCl. Flutamide was generously supplied by Dr Rudi Neri (Schering-Plough Research Institute, Kenilworth, NJ, USA) while DHEA was purchased from Diosynth Inc. (Chicago, IL, USA). EM-800 was synthesized in the medicinal chemistry division of our laboratory as described elsewhere (Gauthier *et al.* 1997).

### Histological procedures

At the end of the experiment, the animals were killed (by decapitation) and sections of dorsal skin (from the site of application and the adjacent area 1–3 cm away from the application site) as well as ventral skin from each animal were carefully excised, after shaving, flattened and then fixed in 10% buffered formalin solution. Tissue sections were routinely processed in a tissue processor and 5  $\mu$ m-thick sections were mounted and stained with hematoxylin–eosin. Four longitudinal sections from both dorsal- and ventral skin specimens were examined. Histopathological examination was performed using light microscopy.

### Sebaceous gland histomorphometry

Measurements of dermal sebaceous glands were obtained from four longitudinal sections. Images were captured with a DC-330 3 CCD color camera (Dage-MTI, Michigan City, IN, USA) and quantified using IMAGE-PRO PLUS 3.0 software (Media Cybernetics, Silver Spring, MD, USA). Using a  $\times$  5 objective (Leica Microsystems, Willowdale, Ont., Canada), both the number and area of all sebaceous gland acini were collected on five luminal fields from each of the four sections of dorsal or ventral skin, for a total of 20 luminal fields analyzed per animal.

Additionally, the total length of each luminal field analyzed was measured at the internal limit of dermis reached by the sebaceous glands.

## Results

In control, OVX, untreated animals 3, 6 or 12 months after ovariectomy, the sebaceous glands of both the dorsal and ventral skin are composed of small cells each showing poor staining of the nucleus and cytoplasm, a small number of acidophilic granules and an absence of lipid droplets. The acini have a small lumen in the immediate vicinity of the duct of the acinus (Fig. 1). The appearance was not different from that of intact female rats of the same age.

After 3, 6 or 12 months of DHEA administration to OVX animals, a mild to moderate increase in the number and size of the sebaceous glands is seen, to a similar degree, in both dorsal and ventral skin. This change is due to the enlargement and budding of the acini as well as an increase in the size of the individual sebaceous cells (Fig. 1). Interestingly, no differences were observed in the degree of the above-indicated histological changes of the sebaceous glands after either 3, 6 or 12 months of DHEA administration.

The quantitative analysis performed after 6 months of treatment demonstrates that DHEA caused increases of 170 and 175% in the number of glands in the dorsal and ventral skin, respectively (Fig. 2A and B). The total surface area of the sebaceous glands was similarly stimulated by DHEA at both sites, showing increases of 225 and 260% in the dorsal and ventral skin, respectively (Fig. 3A and B).

In order to differentiate between androgenic and/or estrogenic effects of DHEA, the pure antiandrogen Flutamide and the pure antiestrogen EM-800 were administered concomitantly with DHEA. Concomitant administration of DHEA and Flutamide abolished the effects of DHEA on the number and surface of sebaceous glands in both dorsal and ventral skin (Figs 2–4). In the group of animals that received DHEA and Flutamide, the histological pattern was similar to that seen in OVX control animals (Fig. 4). It is noteworthy that the effect of Flutamide was somewhat more striking on ventral skin glands. In contrast, the addition of EM-800 to DHEA had no influence on the effects of DHEA on skin histomorphology (Figs 2–4), the values not being significantly different from those obtained for animals that received only DHEA.

## Discussion

The present data show that treatment with DHEA results in a significant stimulation of the sebaceous glands, characterized by an increase in both their number and their size. Since the skin is a tissue rich in steroidogenic enzymes

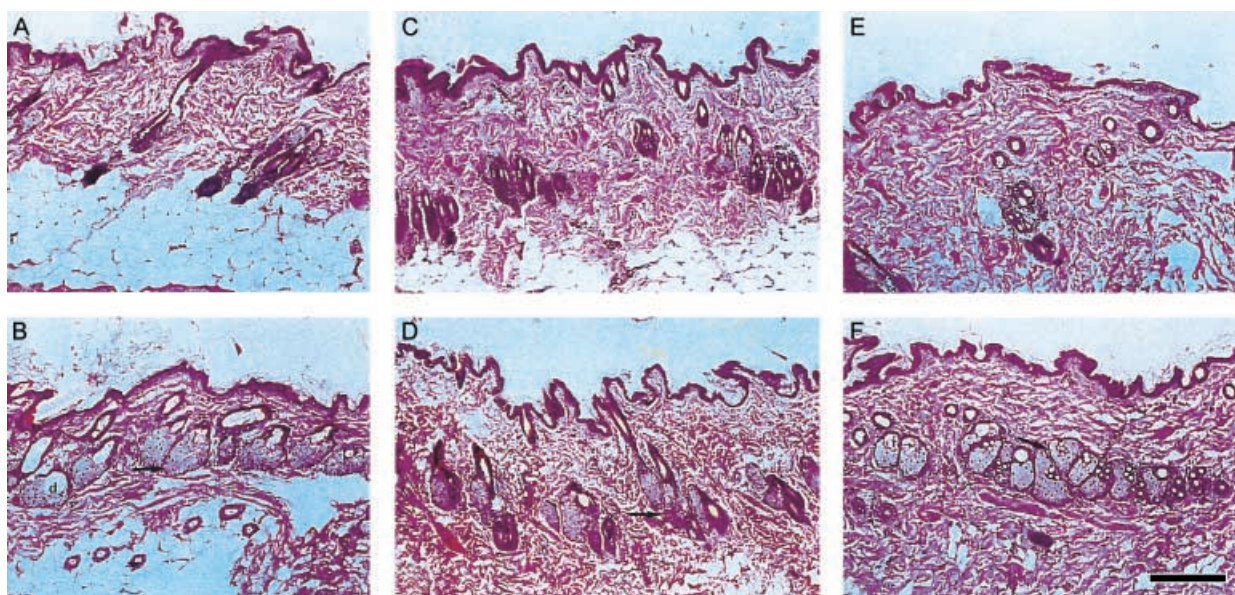
and as it is the largest organ in the body, such data strongly suggest that the skin should be considered as an important site of sex-steroid formation. As mentioned above, the skin possesses all the enzymes required for the transformation of steroid precursors of adrenal origin, namely DHEA and its sulfate, DHEA-S, into active androgens and estrogens (Baillie *et al.* 1966, Pochi & Strauss 1969, Luu-The *et al.* 1989, Labrie 1991, Labrie *et al.* 1992, Luu-The *et al.* 1994).

The sebaceous gland itself has been shown to actively convert testosterone into DHT and other 5 $\alpha$ -reduced steroids, such as 5 $\alpha$ -androstane-3 $\beta$ , 17 $\beta$ -diol and 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol *in vitro*, through the action of 5 $\alpha$ -reductase, in both rats and humans (Sansone & Reisner 1971, Bingham & Shaw 1973, Hodgins & Hay 1973, Lutsky *et al.* 1974, Bowden *et al.* 1976, Cooper *et al.* 1976). In addition, human skin and rat preputial glands have been shown to actively metabolize DHEA to various compounds including androstenedione, 5 $\alpha$ -androstenedione, androsterone, androst-5-ene-3 $\beta$ ,17 $\beta$ -diol, 7 $\alpha$ -hydroxy-DHEA and 7-keto-DHEA, whereas the conversion of DHEA to androstenedione, testosterone and DHT has been described as occurring in the sebaceous glands of human facial skin (Hay & Hodgins 1973, Thomas & Oake 1974, Hodgins & Hay 1976, Takayasu 1979, Hsia *et al.* 1983).

Androgens are well known for causing enlargement of the sebaceous glands in rats (De Graaf 1942, Ebling 1948, Haskin *et al.* 1953, Lasher *et al.* 1954), rabbits (Montagna & Kenyon 1949), hamsters (Hamilton & Montagna 1950) and mice (Lapierre 1953). Moreover, testosterone is known to prevent the reduction in volume of the sebaceous glands following castration in male rats, the effect of the androgen resulting from increased cell proliferation and reduced cell turnover (Ebling 1963). In contrast, estrogens have been reported to inhibit the growth, and reduce the size, of the sebaceous glands (Hooker & Pfeiffer 1943, Ebling 1948).

The superimposable stimulatory effect of DHEA on the sebaceous glands at distant and local sites of DHEA application indicate that DHEA is well absorbed in the general circulation. This situation is analogous to that in humans, in which DHEA secreted by the adrenal gland reaches the peripheral target sites via the circulation (Labrie *et al.* 1988, Labrie 1991). The intracrine formation of androgens and/or estrogens thus depends upon the expression of androgen- and/or estrogen-forming steroidogenic enzymes.

Interestingly, as indicated by the effect of Flutamide in the present study, the influence of DHEA and DHEA-S on sebaceous gland activity is mediated by testosterone and DHT in the skin. It should be mentioned that a significant correlation has been reported between the activity of 3 $\beta$ -HSD in human sebaceous glands and their secretory activity (Cameron *et al.* 1966, Oertel & Treiber 1969, Hay & Hodgins 1973, Simpson *et al.* 1983). The presence of androgen receptors (Kimura *et al.* 1993) in several



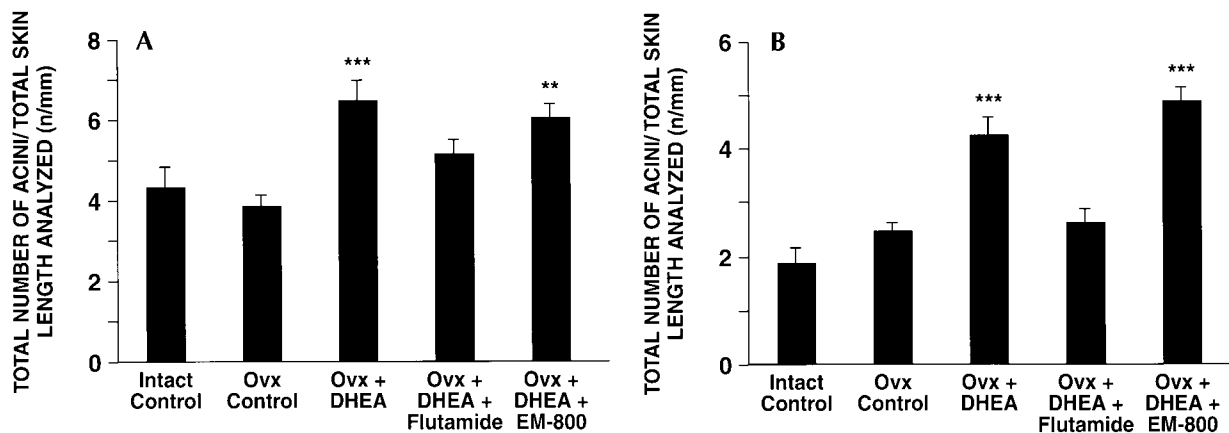
**Figure 1** Stimulation of sebaceous glands of ventral skin of OVX female rats by DHEA administered at a distant site (dorsal skin). The histology of ventral skin after 3 (D), 6 (E) or 12 (F) months of treatment with DHEA is shown. A similar mild to moderate increase in both the number and the size of the sebaceous glands ( $\rightarrow$ ) was seen, at all time intervals studied. Compare with OVX controls 3(A), 6(B) or 12(C) months after ovariectomy. Bar = 50  $\mu$ m.

structures of the skin, including the sebaceous glands, suggests that androgens regulate the activity of the sebaceous glands. This intracrine steroidogenic activity provides an explanation for the observation that the sebaceous glands develop fully in both boys and girls *in utero* and at puberty (Serri & Huber 1963, Sharp *et al.* 1976).

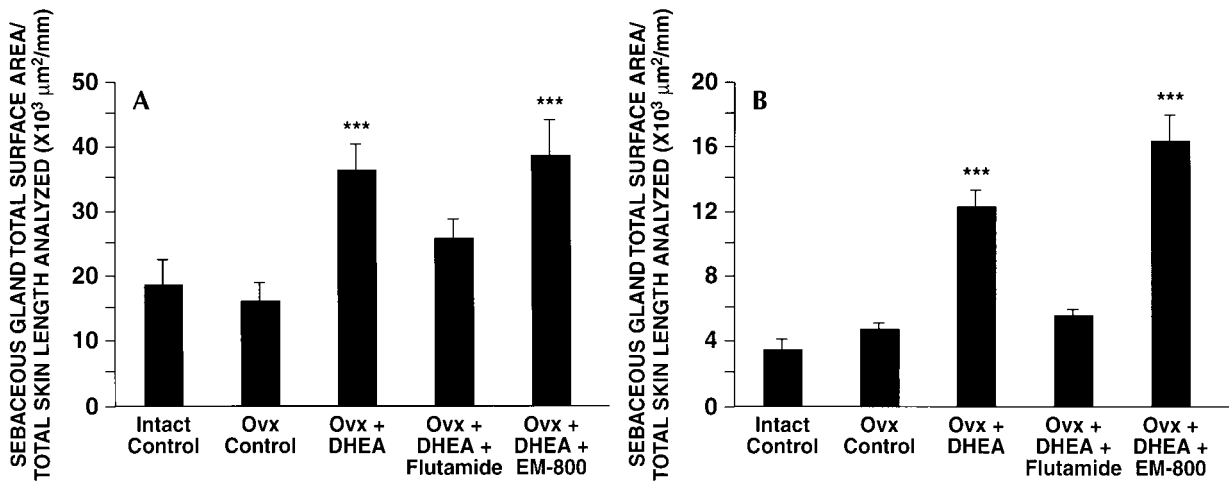
In agreement with the present data, the histomorphological changes induced by DHEA in the skin of OVX female rats, especially in the sebaceous glands, could result from the intracrine local transformation of DHEA into

steroids having androgenic activity. Pochi & Strauss (1969) and Drucker *et al.* (1972) have reported that the administration of 4-dione and DHEA stimulates sebaceous gland secretion in humans, while Chen and coworkers (Chen *et al.* 1996a) have demonstrated that the administration of DHEA and 4-dione, constantly released from silastic implants, are potent stimulators of the sebaceous glands of the flank organs and ears in the hamster.

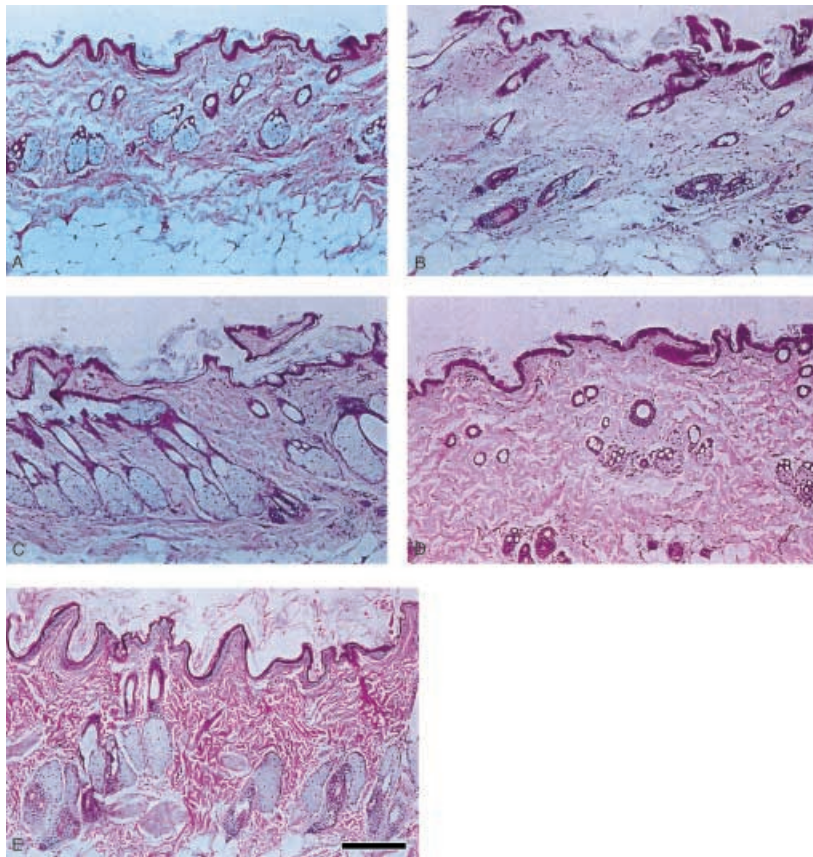
At puberty, the increase in the secretion of DHEA, and especially of DHEA-S (de Peretti & Forest 1978), is



**Figure 2** Effects of DHEA administered alone or in combination with Flutamide or EM-800 on the number of sebaceous gland acini in dorsal skin (A) and ventral (B) skin of OVX rats. The results are expressed as means  $\pm$  S.E.M. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  OVX controls versus all the other experimental groups (eight animals per group). The group receiving DHEA is significantly ( $P < 0.05$  for dorsal skin and  $P < 0.001$  for ventral skin) different from the group that received both DHEA and Flutamide.



**Figure 3** Effects of DHEA administered alone or in combination with Flutamide or EM-800 on the surface area of sebaceous glands in dorsal skin (A) and ventral (B) skin of OVX rats. The results are expressed as the means  $\pm$  s.e.m. \*\*\* $P < 0.001$ , OVX controls versus all the other experimental groups (eight animals per group).



**Figure 4** Antagonism of the effect of DHEA on ventral skin by the antiandrogen Flutamide. The histology of ventral skin in intact rats (A), OVX rats (B) and OVX rats treated with DHEA alone (C) or with a combination of DHEA and Flutamide (D) or DHEA and EM-800 (E) for 12 months is shown. The stimulatory effects of DHEA on the growth and size, as well as the secretory activity, of the sebaceous glands of ventral skin, seen after 12 months of treatment of OVX animals (C), were completely abolished by the concomitant administration of Flutamide (D). The addition of EM-800, however, had no effect on the DHEA-induced histological changes in the skin (E). Compare with intact (A) and OVX controls (B). Bar = 50  $\mu\text{m}$ .

associated with an increase in sebaceous gland size and sebum production (Ramasastry *et al.* 1970, Pochi *et al.* 1977) which frequently leads to problems of acne (Milne 1969). The activity of 5 $\alpha$ -reductase in genital skin has been found to be higher in hirsute than in normal women, the values being intermediate between those of normal women and men (Mowszowicz *et al.* 1983, Serafini & Lobo 1985, Serafini *et al.* 1985). In addition, it has been found that sebaceous gland size is increased in patients with hirsutism and acne (Burton *et al.* 1972, Plewig 1974) while a higher level of DHEA metabolism has been described in the skin of hirsute women relative to that of normal women (Thomas & Oake 1974).

Although the presence of estrogen receptors has been described in both human skin and mouse skin (Hasselquist *et al.* 1980) and estrogen has been reported to increase vascularization of skin in rodents (Reynolds & Foster 1940), the effects of DHEA on rat skin dermis appear to result exclusively from androgenic action, as demonstrated by the reversal of the above-described effects by the addition of the specific antiandrogen Flutamide. In contrast, the co-administration of the specific antiestrogen EM-800 did not alter the histological changes induced by DHEA, thus excluding significant estrogenic action by DHEA on skin.

The present study provides morphological evidence that DHEA secreted by the adrenal cortex has an exclusive androgenic influence on the histomorphology and function of the sebaceous glands, probably via its intracrine conversion into testosterone and DHT. The development of inhibitors acting locally in the skin to block the intracrine formation of androgens offers the potential for an effective treatment of common skin diseases, such as acne and hirsutism, without systemic effects.

## References

- Baillie AH, Thomson J & Milne JA 1966 The distribution of hydroxysteroid dehydrogenase in human sebaceous glands. *British Journal of Dermatology* **78** 451–457.
- Bingham KD & Shaw DA 1973 The metabolism of testosterone by human male scalp skin. *Journal of Endocrinology* **57** 111–121.
- Black MM, Shuster S & Bottoms E 1970 Osteoporosis, skin collagen and androgens. *British Medical Journal Endocrinology* **4** 773–774.
- Bowden PE, Meddis D, Cooper MF, Thody A & Shuster S 1976 Effects of 5 $\alpha$ -reduced androgens on preputial-gland size and lipogenic activity. *Biochemical Society Transactions* **4** 795–797.
- Burton JL, Johnson C, Libman S & Shuster S 1972 Skin virilism in women with hirsutism. *Journal of Endocrinology* **53** 349–354.
- Cameron EH, Baillie AH, Grant JK, Milne JA & Thomson J 1966 Transformation *in vitro* of [7 $\alpha$ -3H] dehydroepiandrosterone to [3H] testosterone by skin from men. *Proceedings of the 101st Meeting of the Society for Endocrinology*, London, UK. Abstract 19.
- Chen C, Bélanger A & Labrie F 1996a Adrenal steroid precursors exert potent androgenic action in the hamster sebaceous glands of flank organs and ears. *Endocrinology* **137** 1752–1757.
- Chen C, Li X, Singh SM, Bélanger A & Labrie F 1996b Additive *in vivo* growth inhibitory effects of flutamide and finasteride on androgen-sensitive shionogi 115 carcinoma. *Endocrine-Related Cancer* **3** 217–227.
- Choudry R, Hodgins MB, Van der kwast TH, Brinkmann AO & Boersma WJA 1992 Localization of androgen receptors in human skin by immunohistochemistry: implications for the hormonal regulation of hair growth, sebaceous glands and sweat glands. *Journal of Endocrinology* **133** 467–475.
- Chrousos GP, Peck GL, Gross EG, Cutler Jr GB & Loriaux DL 1982 Adrenal function in women with idiopathic acne. *Journal of Investigative Dermatology* **78** 468–471.
- Cooper MF, Hay JB, McGibbon D & Shuster S 1976 Sebaceous lipogenesis and androgen metabolism in acne. *Biochemical Society Transactions* **4** 793–795.
- De Graaf HJ 1942 Endocrine influences on sebaceous glands. *Acta Brevia Neerland* **12** 67–68.
- de Peretti E & Forest MG 1978 Pattern of plasma dehydroepiandrosterone sulfate levels in human from birth to adulthood: evidence for testicular production. *Journal of Clinical Endocrinology and Metabolism* **47** 572–577.
- Drucker WD, Blumberg JM, Gandy HM, David RR & Verde AL 1972 Biological activity of dehydroepiandrosterone sulfate in man. *Journal of Clinical Endocrinology and Metabolism* **35** 48–54.
- Dumont M, Luu-The V, Dupont E, Pelletier G & Labrie F 1992 Characterization, expression and immunohistochemical localization of 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase in human skin. *Journal of Investigative Dermatology* **99** 415–421.
- Ebling FJ 1948 Sebaceous glands. 1. The effect of sex hormones on the sebaceous glands of the female albino rat. *Journal of Endocrinology* **5** 297–302.
- Ebling FJ 1963 Hormonal control of the sebaceous gland in experimental animals. In *Advances in Biology of Skin. The Sebaceous Glands*, pp 200–219. Ed. W Montagna. Oxford: Pergamon Press.
- Ellis RA 1958 Ageing of the human male scalp. In *The Biology of Hair Growth*, pp 469–485. Eds W Montagna & RA Ellis. New York: Academic Press.
- Farein I, Fazekas AG, Toth I, Kokai K & Julesz M 1969 Transformation *in vitro* of [4-14-C]-dehydroepiandrosterone into 7-oxygenated derivatives by normal human male and female skin tissue. *Journal of Investigative Dermatology* **52** 357–361.
- Gallegos AJ & Berliner DL 1967 Transformation and conjugation of dehydroepiandrosterone by human skin. *Journal of Clinical Endocrinology and Metabolism* **27** 1214–1218.
- Gauthier S, Caron B, Cloutier J, Dory YL, Favre A, Larouche D, Mailhot J, Ouellet C, Schwerdtfeger A, Leblanc G, Martel C, Simard J, Mérand Y, Bélanger A, Labrie C & Labrie F 1997 (S)-(+)-4-[7-(2,2-dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl)-ethoxy]phenyl]-2H-1-benzopyran-3-yl]-phenyl 2,2-dimethylpropanoate (EM-800): a highly potent, specific, and orally active nonsteroidal antiestrogen. *Journal of Medicinal Chemistry* **40** 2117–2122.
- Hamilton JB 1951 Quantitative measurements of a secondary sex character, axillary hair growth. *Annals of the New York Academy of Sciences* **53** 585–599.
- Hamilton JB & Montagna W 1950 The sebaceous glands of the hamster. Morphological effects of androgens on integumentary structure. *American Journal of Anatomy* **86** 191–233.
- Hamilton JB, Terada H, Mestler GE & Tirman W 1969 Coarse sternal hairs, a male secondary sex character that can be measured quantitatively. I. The influence of sex, age and genetic factors. II. Other sex differing characters: relationship of age to one another and to values for coarse sternal hairs. In *Advances in Biology of Skin. Hair Growth*, pp 129–151. Eds W Montagna & RL Dodson. Oxford: Pergamon Press.
- Haskin D, Lasher N & Rothman S 1953 Some effects of ACTH, cortisone, progesterone and testosterone on sebaceous glands in the white rat. *Journal of Investigative Dermatology* **20** 207–211.
- Hasselquist MB, Goldberg N, Schroeter A & Spelsberg TC 1980 Isolation and characterization of the estrogen receptor in human skin. *Journal of Clinical Endocrinology and Metabolism* **50** 76–82.

- Hay JB & Hodgins MB 1973 Metabolism of androgens *in vitro* by human facial and axillary skin. *Journal of Endocrinology* **59** 475–486.
- Hay JB & Hodgins MB 1978 Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. *Journal of Endocrinology* **79** 29–39.
- Held BL, Nader S, Rodriguez-Rigau LJ, Smith KD & Steinberger E 1984 Acne and hyperandrogenism. *Journal of the American Academy of Dermatology* **10** 223–226.
- Hibberts NA, Howell AE & Randall VA 1998 Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp. *Journal of Endocrinology* **156** 59–65.
- Hodgins MB & Hay JB 1973 The metabolism of androgens in rat preputial glands. *Steroids* **21** 307–322.
- Hodgins MB & Hay JB 1976 Steroid metabolism in human skin: its relation to sebaceous-gland growth and acne vulgaris. *Biochemical Society Transactions* **4** 605–609.
- Hooker CW & Pfeiffer CA 1943 Effects of sex hormones upon body growth, skin, hair, and sebaceous glands in the rat. *Endocrinology* **32** 69–76.
- Hsia SL, Sawaya ME & Voigt W 1983 Transformation of dehydroepiandrosterone into dihydrotestosterone by isolated cells from rat preputial gland. *Journal of Steroid Biochemistry* **19** 599–605.
- Kimura N, Mizokami A, Oonuma T, Sasano H & Nagura H 1993 Immunocytochemical localization of androgen receptor with polyclonal antibody in paraffin-embedded human tissues. *Journal of Histochemistry and Cytochemistry* **41** 671–678.
- Kuttann F, Mowszowicz I, Schaison G & Mauvais-Jarvis P 1977 Androgen production and skin metabolism in hirsutism. *Journal of Endocrinology* **75** 83–91.
- Kuttann F, Mowszowicz I, Wright F, Baudot N, Jaffiol C, Robin M & Mauvais-Jarvis P 1979 Male pseudohermaphroditism: a comparative study of one patient with 5 $\alpha$ -reductase deficiency and three patients with the complete form of testicular feminization. *Journal of Clinical Endocrinology and Metabolism* **49** 861–865.
- Labrie C, Bélanger A & Labrie F 1988 Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate. *Endocrinology* **123** 1412–1417.
- Labrie C, Flamand M, Bélanger A & Labrie F 1996a High bio-availability of DHEA administered percutaneously in the rat. *Journal of Endocrinology* **150** S107–S118.
- Labrie F 1991 Intracrinology. *Molecular and Cellular Endocrinology* **78** C113–C118.
- Labrie F, Simard J, Luu-The V, Pelletier G, Bélanger A, Lachance Y, Zhao HF, Labrie C, Breton N, de Launoit Y, Dumont M, Dupont E, Rhéaume E, Martel C, Couet J & Trudel C 1992 Structure and tissue-specific expression of 3 $\beta$ -hydroxysteroid dehydrogenase/5-ene-4-ene isomerase genes in human and rat classical and peripheral steroidogenic tissues. *Journal of Steroid Biochemistry and Molecular Biology* **41** 421–435.
- Labrie F, Simard J, Luu-The V, Pelletier G, Belghmi K & Bélanger A 1994 Structure, regulation and role of 3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase and aromatase enzymes in formation of sex steroids in classical and peripheral intracrine tissues. In *Hormone, Enzymes and Receptors*, pp 451–474. Eds MC Sheppard & PM Stewart. London: Baillière's Clinical Endocrinology and Metabolism, Baillière Tindall Ltd.
- Labrie F, Bélanger A, Simard J, Luu-The V & Labrie C 1995 DHEA and peripheral androgen and estrogen formation: intracrinology. *Annals of the New York Academy of Sciences* **774** 16–28.
- Labrie F, Simard J, Luu-The V, Bélanger A, Pelletier G, Morel Y, Mebarki F, Sanchez R, Durocher F, Turgeon C, Labrie Y, Rhéaume E, Labrie C & Lachance Y 1996b The 3 $\beta$ -hydroxysteroid dehydrogenase/isomerase gene family: lessons from type II 3 $\beta$ -HSD congenital deficiency. In *Signal Transduction in Testicular Cells. Ernst Schering Research Foundation Workshop*, pp 185–218. Eds V Hansson, FO Levy & K Taskén. Berlin: Springer-Verlag.
- Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R & Bélanger A 1997 The key role of 17 $\beta$ -HSDs in sex steroid biology. *Steroids* **62** 148–158.
- Lapière C 1953 Modifications des glandes sébacées par des hormones sexuelles appliquées localement sur la peau de souris. *Critical Reviews of the Society for Biology of Paris* **147** 1302–1306.
- Lasher N, Lorincz AL & Rothman S 1954 Hormonal effects on sebaceous glands in the white rat. II. The effect of the pituitary–adrenal axis. *Journal of Investigative Dermatology* **22** 25–29.
- Lookingbill DP, Horton R, Demers LM, Egan N, Marks Jr JG & Santen RJ 1985 Tissue production of androgens in women with acne. *Journal of the American Academy of Dermatology* **12** 481–487.
- Lucky AW, McGuire J, Rosenfield RL, Lucky PA & Rich BH 1983 Plasma androgens in women with acne vulgaris. *Journal of Investigative Dermatology* **81** 70–74.
- Luo S, Martel C, Gauthier S, Mérand Y, Bélanger A, Labrie C & Labrie F 1997a Long term inhibitory effects of a novel antiestrogen on the growth of ZR-75-1 and MCF-7 human breast cancer tumors in nude mice. *International Journal of Cancer* **73** 735–739.
- Luo S, Martel C, Sourla A, Gauthier S, Mérand Y, Bélanger A, Labrie C & Labrie F 1997b Comparative effects of 28-day treatment with the new antiestrogen EM-800 and tamoxifen on estrogen-sensitive parameters in the intact mouse. *International Journal of Cancer* **73** 381–391.
- Luo S, Sourla A, Labrie C, Bélanger A & Labrie F 1997c Combined effects of dehydroepiandrosterone and EM-800 on bone mass, serum lipids, and the development of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat. *Endocrinology* **138** 4435–4444.
- Luo S, Stojanovic M, Labrie C & Labrie F 1998 Inhibitory effect of the novel antiestrogen EM-800 and medroxyprogesterone acetate (MPA) on estrone-stimulated growth of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat. *International Journal of Cancer* **73** 580–586.
- Lutsky BN, Koziol P & Gershaw C 1974 Metabolism and effects of androgens in hamster flank organs. *Endocrinology* **95** 882–890.
- Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC & Labrie F 1989 Full length cDNA structure and deduced amino acid sequence of human 3 $\beta$ -hydroxy-5-ene steroid dehydrogenase. *Molecular Endocrinology* **3** 1310–1312.
- Luu-The V, Sugimoto Y, Puy L, Labrie Y, Lopez I, Singh M & Labrie F 1994 Characterization, expression and immunohistochemical localization of 5 $\alpha$ -reductase in human skin. *Journal of Investigative Dermatology* **102** 221–226.
- Martel C, Rhéaume E, Takahashi M, Trudel C, Couet J, Luu-The V, Simard J & Labrie F 1992 Distribution of 17 $\beta$ -hydroxysteroid dehydrogenase gene expression and activity in rat and human tissues. *Journal of Steroid Biochemistry and Molecular Biology* **41** 597–603.
- Martel C, Melner MH, Gagné D, Simard J & Labrie F 1994 Wide-spread tissue distribution of steroid sulfatase, 3 $\beta$ -hydroxysteroid dehydrogenase/ $\Delta$ 5- $\Delta$ 4 isomerase (3 $\beta$ -HSD), 17 $\beta$ -HSD 5 $\alpha$ -reductase and aromatase activities in the rhesus monkey. *Molecular and Cellular Endocrinology* **104** 103–111.
- Marynick SP, Chakmakjian ZH, McCaffree DL & Herndon Jr JH 1983 Androgen excess in cystic acne. *New England Journal of Medicine* **309** 981–986.
- Milne JA 1969 The metabolism of androgens by sebaceous glands. *British Journal of Dermatology* **81** (Suppl 2) 22–28.
- Montagna W & Kenyon P 1949 Growth potentials and mitotic division in the sebaceous glands of the rabbit. *Anatomical Record* **103** 365–380.
- Mowszowicz I, Riahi M & Wright F 1981 Androgen receptors in human skin cytosol. *Journal of Clinical Endocrinology and Metabolism* **52** 338–344.

- Mowszowicz I, Melanitou E, Doukani A, Wright F, Kuttan F & Mauvais-Jarvis P 1983 Androgen binding capacity and 5 $\alpha$ -reductase activity in pubic skin fibroblasts from hirsute patients. *Journal of Clinical Endocrinology and Metabolism* **56** 1209–1213.
- Neri R, Monahan MD, Meyer JG, Afonso BA & Tabachnick IA 1967 Biological studies of an antiandrogen (SH-714). *European Journal of Pharmacology* **1** 438–444.
- Oertel GW & Treiber L 1969 Metabolism and excretion of C19 and C18 steroids by human skin. *European Journal of Biochemistry* **7** 234–238.
- Papa CM & Kligman AM 1965 The effects of topical steroids on the aged human axilla. In *Advances in Biology of Skin. Ageing*, pp 177–198. Ed. W Montagna. Oxford: Pergamon Press.
- Plewig G 1974 Acne vulgaris, proliferative cells in sebaceous glands. *British Journal of Dermatology* **90** 623–630.
- Pochi PE & Strauss JS 1969 Sebaceous gland response in man to the administration of testosterone,  $\Delta$ 4-androstenedione and dehydroisoandrosterone. *Journal of Investigative Dermatology* **52** 32–36.
- Pochi PE, Strauss JS & Downing DT 1977 Skin surface lipid composition, acne, pubertal development and urinary excretion of testosterone and 17-ketosteroids in children. *Journal of Investigative Dermatology* **69** 485–489.
- Puy L, Turgeon C, Gagné D, Labrie Y, Chen C, Pelletier G, Simard J & Labrie F 1996 Localization and regulation of expression of the FAR-17A gene in the hamster flank organs. *Journal of Investigative Dermatology* **106** 44–50.
- Ramaswamy P, Downing DT, Pochi PE & Strauss JS 1970 Chemical composition of human skin surface lipids from birth to puberty. *Journal of Investigative Dermatology* **54** 139–144.
- Randall VA, Thornton MJ & Messenger AG 1992 Cultured dermal papilla cells from androgen-dependent human hair follicles (e.g. beard) contain more androgen receptors than those from non-balding areas of scalp. *Journal of Endocrinology* **133** 141–147.
- Reynolds SRM & Foster FI 1940 Peripheral vascular action of estrogen observed in the ear of the rabbit. *Journal of Pharmacology and Experimental Therapeutics* **68** 173–184.
- Sansone G & Reisner RM 1971 Differential rates of conversion of testosterone to dihydrotestosterone in acne and in normal skin: a possible pathogenic factor in acne. *Journal of Investigative Dermatology* **56** 366–372.
- Schiavone FE, Rietschel RL, Sgoutas D & Harris R 1983 Elevated free testosterone levels in women with acne. *Archives of Dermatology* **119** 799–802.
- Serafini P & Lobo RA 1985 Increased 5 $\alpha$ -reductase activity in idiopathic hirsutism. *Fertility and Sterility* **43** 74–78.
- Serafini P, Ablan F & Lobo RA 1985 5 $\alpha$ -reductase activity in the genital skin of hirsute women. *Journal of Clinical Endocrinology and Metabolism* **60** 349–355.
- Serri F & Huber WM 1963 The development of sebaceous glands in man. In *Advances in Biology of Skin. The Sebaceous Glands*, pp 1–18. Eds W Montagna, RA Ellis & AF Silver. Oxford: Pergamon Press.
- Sharp F, Hay JB & Hudgins MB 1976 Metabolism of androgens *in vitro* by human foetal skin. *Journal of Endocrinology* **70** 491–499.
- Shelley WB & Hurley HJ 1953 The physiology of the human axillary apocrine sweat gland. *Journal of Investigative Dermatology* **20** 285–295.
- Simard J, Luthy I, Guay J, Bélanger A & Labrie F 1986 Characteristics of interaction of the antiandrogen Flutamide with the androgen receptor in various target tissues. *Molecular and Cellular Endocrinology* **44** 261–270.
- Simard J, Labrie C, Bélanger A, Gauthier S, Singh SM, Mérand Y & Labrie F 1997 Characterization of the effects of the novel non-steroidal antiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells *in vitro*. *International Journal of Cancer* **73** 104–112.
- Simpson NB, Cunliffe WJ & Hodgins MB 1983 The relationship between *in vitro* activity of 3 $\beta$ -hydroxysteroid dehydrogenase  $\Delta$ 4–5 isomerase in human sebaceous glands and their secretory activity *in vivo*. *Journal of Investigative Dermatology* **81** 139–144.
- Strauss JS & Pochi PE 1963 The hormonal control of human sebaceous glands. In *Advances in Biology of Skin*, pp 250–254. Eds W Montagna, RA Ellis & AF Silver. Oxford: Pergamon Press.
- Takayasu S 1979 Metabolism and action of androgen in the skin. *International Journal of Dermatology* **18** 681–692.
- Thomas JP & Oake RJ 1974 Androgen metabolism in the skin of hirsute women. *Journal of Clinical Endocrinology and Metabolism* **38** 19–22.
- Voigt W, Sawaya M & Hsia SL 1984 Mechanism of action of dehydroepiandrosterone in the androgenic stimulation of sebaceous glands. *Proceedings of the International Symposium on Regulation of Androgen Action*, Montréal, Québec, Canada. Abstract.

Received 19 May 1999

Revised manuscript received 27 October 1999

Accepted 28 March 2000