Failure of Topical Estrogen Receptor Agonists and Antagonists to Alter Murine Hair Follicle Cycling

To the Editor:
In October 1996 there appeared a publication by Oh and Smart describing experiments that were interpreted to indicate an important estrogen receptor regulation of hair growth. This report was well publicized and stimulated a worldwide response.

In essence, the report indicated that in the CD-1 female mouse topical 17β estradiol, but not 17α estradiol, blocks anagen induction and that the estrogen receptor antagonist, ICI 182780, induces anagen. This finding was of major interest to us in the field of hair biology because the conclusions implied that the hair growth cycle is under negative control, an idea that we have previously considered (Paus et al., 1990, 1991; Stenn et al., 1993). For this reason we made a concentrated attempt in two independent and widely separated laboratories to reproduce the experiments as they were described. At the outset we recognized that the putative capacity of 17β estradiol to “arrest” hair follicles in telogen as suggested by Oh and Smart contradicted the clinical use of topical estradiol for the successful treatment of telogen effluvium in humans, presumably by prolonging the duration of anagen (Orfanos and Vogel, 1980).

In our studies we used two pigmented female mouse strains, C57BL/6 and C3H, because (i) we wanted to test the phenomenon in two different mouse strains, and (ii) we have found that careful hair cycle studies using a nonpigmented animal, such as the CD-1 mouse, are very difficult to interpret.

In Experiment I, we treated the animals following the Oh/Smart protocol precisely. For the induction study hair-clipped animals were treated twice weekly for 2 wk [ICI 182780, 10 nM, as well as Tamoxifen, 10 nM (Sigma, St Louis, MO)]. For the inhibition studies, anagen was depilatory-cream-induced (NeetTM) and the mice were treated twice weekly with 17α estradiol (10 nM, Sigma) or 17β estradiol (10 nM, Sigma) for 2 wk. In addition, we treated hair-clipped mice with all back skin follicles in telogen, and treated them with either 17β estradiol, 17α estradiol, or vehicle alone, twice weekly for 5 wk. Each experimental group contained 5–7 mice and was repeated once. On completion, in no experiment were the treated mice significantly different from the controls.

In Experiment II, the estradiol studies were repeated using the same preparations as in Experiment I but the drugs were applied daily for 2 wk. Again, experimental animals showed no difference from controls.

In Experiment III, conditions were identical to Experiment I but the applications of the receptor antagonists [ICI 182780 or Tamoxifen, 10 nM (Sigma)] or the agonists (17α estradiol and 17β estradiol) were given daily for 20 d (agonists) and 9 d (agonists). Again, the experimental mice showed no difference from the controls.

It is always difficult to interpret a negative result. One possibility in explaining these discrepancies is that the phenomena described are unique to the animal strain used, namely, CD-1. Nevertheless, whatever the complete explanation for the differences observed in the three laboratories, the experiments reported here indicate that the estrogen effects are not applicable to two of the mouse strains routinely used in hair research, and conflict with common clinical experience. The Oh and Smart (1996) conclusions on the role of estrogen receptor stimulation or blockade in hair growth control therefore will require rigorous further testing and analysis before they can be critically accepted.

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On Photo-Stability of Oxybenzone

To the Editor:
I refer to a paper by Schallreuter et al. (1996) entitled “Oxybenzone oxidation following solar irradiation of skin: photoprotection versus antioxidant inactivation” that was published in the March issue of Journal of Investigative Dermatology. In this paper it is suggested that oxybenzone (2-hydroxy-4-methoxy benzophenone) is unstable to sunlight: there is a significant increase of the CO group concentration indicative of the oxidation of oxybenzone to its semiquinone. This conclusion encouraged us to perform some investigation by Fourier Transform Infrared Spectroscopy (FTIR) on the stability to light of oxybenzone.

An acetone solution of the product was deposited on sodium chloride windows and on a KRS-5 crystal. The solvent was evaporated and a very thin layer of the product obtained. The layer on the crystal was exposed to sunlight for 2 h and the layer on the sodium chloride windows was exposed to light in a Suntest CPS+ apparatus (Xenotest GmbH, Hanau, D) for 15 h. The multiple internal reflectance FTIR spectrum of the layer on the crystal and the transmission FTIR

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Figure 1. Oxybenzone on NaCl windows, FTIR spectra before (1) and after (2) exposure to light (15 h, Suntest CPS+, Xenon test, 500 Watt per m²).

The spectrum of the layer on the sodium chloride windows were recorded, before and after exposure to light, on a P.E. System 2000 instrument (Perkin Elmer, Beaconsfield, U.K.).

In both cases no change in the intensities of the carbonyl stretching bands or other changes in the IR spectrum were observed; Fig 1 shows the transmission FTIR spectra (i) before and (ii) after exposure to light.

Both experiments show that oxybenzone is very stable under light and no significant semiquinone is generated.

Finally, if Fig 1 of Schallreuter et al. (1996) is considered it seems that oxybenzone itself is absent in the Sultan facial cream as many of the oxybenzone absorbances are not present in the FT Raman spectrum of the cream. This observation, together with the results of our investigation, lead us to suspect that the study described in the paper has not been performed on oxybenzone, but on some other product.

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Reply:

We wish to respond to Dr. Santoro’s comment on our paper “Oxybenzone oxidation following solar irradiation of skin: photoprotection versus antioxidant inactivation” (Schallreuter et al., 1996). There are two major points to address: (i) the stability of oxybenzone to photo-oxidation in sun protection formulations, and (ii) whether the Sultan facial cream as used in the above study indeed contained oxybenzone.

High performance liquid chromatography (HPLC) of Sultan facial cream SPF 25 purchased in June 1995, proves the presence of oxybenzone as claimed on the label of this product (Fig 1A). The absence of some Raman absorbances in Sultan facial cream has been elucidated in more detail and is most likely due to π–π interactions with other aromatic compounds such as Parsol 1789 and Parsol MCX. HPLC analysis of the recovery of oxybenzone after ultraviolet (UV) exposure (5 d exposure time) shows 80% photodegradation of this ultraviolet A (UVA) filter (Fig 1B). Recently, Heimsohn et al. claimed

Figure 2. FT Raman spectra of Sultan sun lotion containing 3.83% oxybenzone (α) before ultraviolet B (UVB) irradiation, (b) after 0.3 J UVB irradiation per cm², and (c) after 0.9 J UVB irradiation per cm². The peaks are assigned as aromatic ring stretches with the mono-substituted ring stretch at 1598 per cm and the 1,2,4-tri-substituted aromatic ring stretch at 1625 per cm. After 0.3 J UVB irradiation per cm², there is a 9% decrease in the intensity of the 1,2,4-tri-substituted ring stretch indicating a loss of aromaticity with no effect on the mono-substituted ring stretch. After 0.9 J irradiation per cm², there is a considerable decrease in both tri- and mono-substituted ring stretches showing a loss of aromaticity in the ring structures of oxybenzone.