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EXPOSURE CONSIDERATIONS RELATED TO HAIR DYE SAFETY

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The chemistry, formulation characteristics, and mode of use of hair dyes present a rather unique situation with respect to their potential for skin permeation, and pose a challenge in reliably estimating their systemic body burden under use conditions. For example, the oxidative (permanent) hair dye process involves the uptake of colorless, lower molecular weight precursors into the hair cuticle where they react to form colored polynuclear species that become permanently trapped in the hair. Thus, during the exposure period the concentrations of the more permeable dye precursors are reduced, while the increased molecular size of the reaction products serves to limit their penetration potential. In contrast, direct (semi-permanent) hair dyes are preformed colored materials usually of higher molecular weight and greater structural diversity. After coloring they can diffuse from the hair cuticle, resulting in more protracted skin exposure, at least at low levels, as they leach from the hair, from perspiration and showering during wear.

Under use conditions, the hair itself serves as a large competing absorptive surface for the hair dyes. Hair may also modify the rate and/or extent of oxidative color reactions. While some of the applied dye may be adsorbed onto the *stratum corneum* (as evident from skin staining), or absorbed into the viable *epidermis*, loss of surface corneocytes due to the normal desquamation process serves to reduce the potential for systemic dye absorption.

Carefully conducted studies in human volunteers using a series of ^{14}C-ring-labeled oxidative and direct dyes have shown distinctive temporal patterns of systemic absorption characteristic of the chemistry involved. These historical *in vivo* data have served as useful benchmarks to evaluate the reliability of *in vitro* percutaneous penetration models using both human and animal skin. Such *in vitro* models facilitate more precise control or systematic variation of experimental conditions that may influence skin permeation potential. Such variables include dye concentrations and combinations, vehicle formulation components, and exposure time. Moreover, reliable *in vitro* models, especially those involving the relevant species (i.e., human), are useful to evaluate new dye molecules early in the development state to facilitate dose setting in toxicological studies.

Accumulated data from a number of *in vivo* and *in vitro* systems has demonstrated that, overall, the systemic absorption of hair dyes is relatively low, with only fractional percentages of the applied amounts penetrating the skin. Because of possible substantial differences in product application rates between the in-use situation and in some *in vitro* studies, expression of results as a percentage of the applied dose can sometimes be misleading. Therefore, cumulative mass absorbed per unit area (ug/cm^2) is advocated as a preferred measure of percutaneous penetration. Expressed this way, most hair dye penetration data appear to fall within a relatively narrow "dynamic range" from about <0.1 ug/cm^2 to about 5-10 ug/cm^2 following a single application.

From a toxicological standpoint, such low penetration values generally support relatively large safety margins for hair dyes, even for those dyes with no-observed-adverse effect levels (NOAELs) from toxicology studies as low as 10 mg/kg/day. Consideration of the temporal aspects of hair dye use on a discontinuous basis (e.g., once every 4-6 weeks) serves to further reduce estimated body burdens. As the denominator in the calculation of safety margins, where the numerator (NOAEL) reflects the results of extensive and expensive toxicological evaluations, reliable estimates of the extent of percutaneous penetration are of critical importance in the risk assessment process.

PHOTO-OXIDATION OF HUMAN HAIR PHOTO DAMAGE: ITS CHARACTERIZATION/ QUANTIFICATION AND ALLEVIATION

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Introduction -

When exposed to sunlight, the human hair undergoes changes in morphological, chemical, mechanical and cosmetic properties. The hair becomes dry, brittle, dull in appearance and rough to the touch. The work in this study involves simulated sunlight, optimized at 340 nm in the UVA region. The extent of UV radiation-induced damage to unprotected human hair is characterized as well as quantified using different methods. In addition, the performance of UV absorbers (sunscreens) is evaluated in terms of their diffusion behavior and their effectiveness in being able to provide protection to the keratin fiber against photodegradation. The results clearly show the importance of diffusion behavior, distribution and concentration (uptake levels) of the UV absorbers throughout the hair fiber cross sections with regard to their protective effect. Uniform distribution and high concentration levels are the prerequisite for successful performance.

Techniques and Results -

Scanning electron microscopy is used to highlight drastic changes in the **physical nature** of the hair fiber surface and interior as a result of long-term and short-term UV exposure. Longterm exposure to UV radiation/humidification cycling leads to collapsed and thinned out surface cuticle cells, fused firmly to the underlying cuticles, Figures 1 a-b.

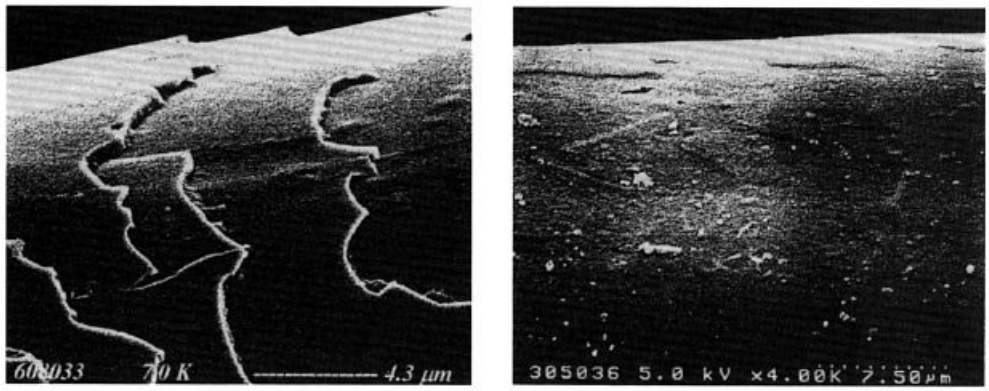

Fig. 1 a-b Typical topography of unaltered hair before and after exposure to UV radiation/humidification cycling.

Gradation of damage in the cortex as a function of progressive UV exposure is characterized and quantified using a microspectrophotometric technique. Changes in dye diffusion rates characterize/quantify UV radiation-induced **morphological changes** in the keratin fiber. The higher the dye diffusion rate, the greater the changes in fiber morphology, the greater the damage.

The degradation of the main classes of hair proteins by UV radiation is characterized by gel electrophoresis. The results strongly indicate that along with the degradation of proteins, there may be a parallel reaction, which leads to crosslinking. This decreases the solubility and eliminates the extractability of the **crosslinked protein** network. Figure 2 shows easy extraction of matrix, intermediate filament and high-molecular weight proteins from untreated different ethnic hair (lanes 2, 4, 6 and 8), and the elimination of extractability of all proteins after UV exposure (lanes, 3, 5, 7 and 9).

UV microspectrophotometry is used to characterize and quantify UV radiation-induced **degradation of the hair proteins**, especially of the aromatic and ring amino acids, which absorb at the lower wavelengths but shift to higher wavelengths after UV exposure. These higher wavelengths represent the photo-oxidized amino acids (Figure 3). UV microspectrophotometry is used to map the formation of photodegradation products within the fiber cross section, to trace the diffusion behavior of UV absorbers in the fiber, and to establish their effectiveness in providing photostability to the keratin system.

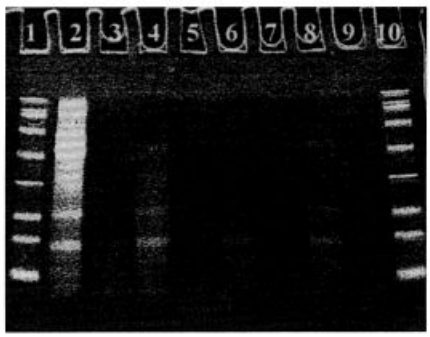

Fig. 2 UV radiation-induced elimination of protein extractability.

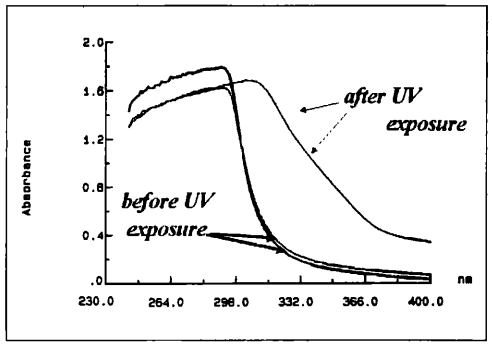

Fig. 3 UV radiation-induced photo-oxidation of the hair proteins (spectrum shifts to higher wavelengths).

The extent of long-term UV radiation-induced **damage to the cortex** and the effectiveness of UV absorbers in reducing such damage are evaluated by measuring the changes in **tensile mechanical properties**. Changes in torsion, bending, shearing and fracture behavior may be used as alternate techniques to measure the damage.

We show that already short-term UV exposure severely modifies the properties of the cuticula. Such UV radiation-induced changes in **surface chemistry** are characterized by measuring changes in **surface wettability** (surface energy), as well as in **fiber friction** and **coarseness**.

Conclusions - UV damage inflicted upon the cuticula, such as dryness, dullness and increases in fiber friction (roughness), may be repaired/remediated by the use of conditioners, humectants and moisturizers. Damage to the cortex may be prevented or at least retarded by the use of UV absorbers (sunscreens). However, their diffusion behavior, distribution and concentration in the keratin fiber are all important.

PROTECTION OF ARTIFICIAL HAIRCOLOR

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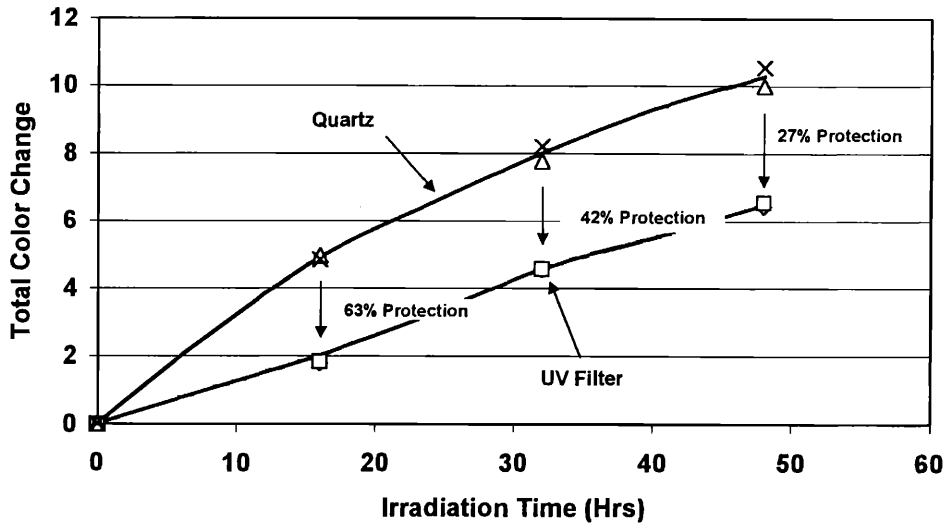
Prevention of fading of natural and artificial haircolor is subject of intensive research in many cosmetic laboratories. Several papers and patents related to this area were published in recent years [1-3]. There were also a number of commercial products, which claimed efficacy in color loss prevention, introduced into the marketplace. This area is also connected with a development of new photo-absorbers of the UVB and especially UVA type, and with introduction of antioxidants and/or free radical scavengers into haircare products. It is generally agreed that the process of color loss of dyed hair has several contributing elements such as (1) removal of a dye during shampooing, (2) decomposition of hairdye upon absorbing light, and (3) decomposition of the dye in the dark. In addition to this, the dyes exhibit different sensitivities to UVB (280 – 320 nm), UVA (320 –400 nm), Visible (370 – 780 nm), and IR (750 – 2800 nm) portions of solar radiation. It has been demonstrated that Visible and UVA light are mostly responsible for the photo-fading of artificial hair-color.

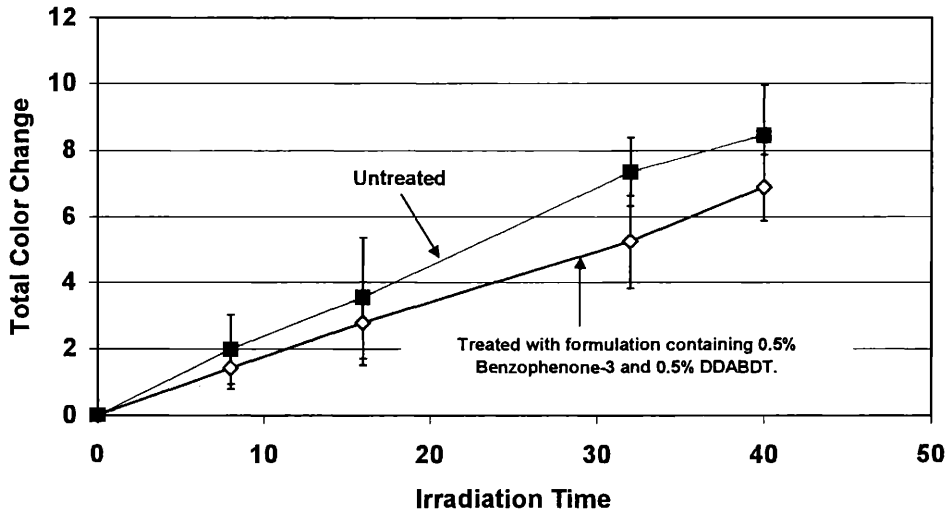
In this work, we have reassessed the contributions of various portions of radiation to the process of photo-fading by using special filters which can effectively block UVB and UVB/UVA light. Results indicate that considerable color protection can be achieved by employing combinations of UVB and UVA photo-absorbers. Figure 1 demonstrates color protection offered by glass UV filters which completely block light below 400 nm. In this work color protection ranged from 27-63% depending on the time samples were exposed for.

Figure 1: Total color changes in dyed hair samples covered with UV filters or quartz plates exposed to artificial sunlight.



We have also carried out experiments to demonstrate the color protection effects by employing selected absorbers in the concentration range 0.5% - 6.0% in leave-in and rinse-off products. In particular, we have explored the use of dodecyl-[3-(p-dimethylaminobenzamido)propyl] dimethylammonium p-tosylate (DDABDT), and PVP/DMAPMA Copolymer – Benzophenone-4 in several cosmetic formulations. Furthermore, we have performed theoretical calculations of photoprotection for individual absorbers and their mixtures. A good agreement between theoretical model and experimental data was established. Figure 2 illustrates color protection offered by a formulation containing 0.5% Benzophenone-3 and 0.5% DDABDT. This example, along with other work to be presented, illustrates the extent to which UV absorbers can be expected to protect dyed hair color.

Figure 2: Color change for dyed hair as a function of irradiation time.



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EFFECT OF MINERAL, SUNFLOWER AND COCONUT OIL ON PREVENTION OF HAIR DAMAGE

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SYNOPSIS

Previously published results showed that coconut oil application to hair prevents hair damage. Using the same methodology, the properties of mineral and sunflower oil on hair were studied.

Taguchi Design of Experimentation was used to include all the variables and factors such as hair types, treatments, oils and to reduce experimentation, cost and effort without impacting the results. Among three oils, coconut oil was the only oil found to reduce the protein loss remarkably. (both as a pre/post-wash).

This difference in results could be because of composition of each of these oils. Coconut oil, being a triglyceride of lauric acid has a high affinity for hair proteins, additionally low molecular weight and straight linear chain, enhances its penetration into the hair shaft. Mineral oil, being a hydro-carbon and sunflower oil, being a triglyceride of linoleic acid with a bulky structure (presence of double bonds), do not penetrate the hair.

INTRODUCTION

Morphologically, a fully formed hair fiber contains three and sometimes four different units or structures, i.e. cuticle, cortex, medulla and intercellular cement. Because of extensive cross-linking, cuticle cells tend to be brittle and therefore, are susceptible to damage by grooming procedures, especially wet combing (1). The loss of cuticle cells by gradual chipping impairs the cosmetic qualities of hair such as smoothness and shine. Grooming methods involving abrasive procedures are known to damage hair and its appearance.

Historically, coconut oil has been used as a hair dressing in the developing countries in the tropical regions of the globe where coconut is cultivated extensively. Prolonged use of coconut oil has been known to lead to healthy looking long hair, suggesting that it may prevent damage to the cuticle in grooming procedures involving abrasion. However, in modern times, the trend is more towards usage of non-sticky oils such as mineral oil or less greasy oils such as sunflower oil in hair oil formulations.

This investigation is aimed at comparing the effects of these two oils along with that of coconut oil in preventing hair damage. Although, several methods involving scanning electron microscopy (SEM), measurement of combing forces and tensile mechanical properties have been used earlier to characterize hair damage, we have used protein loss and water uptake methods for this purpose. Furthermore, these methods have been extended to study the beneficial effects of these oils on in preventing chemical, thermal and UV damage. Efficacy of these methods has been established in an earlier paper from this laboratory (3).

MATERIALS AND METHODS

MATERIALS

Samples of straight, curly, wavy and perm hair of Indian origin, 25cm in length were used in this work. For each of the treatment per hair type, 25 hair tresses were used. The reagents for protein estimation were obtained from Sigma Chemicals Co. of St. Louis, MO. The other reagents such as buffers, salts, etc were of analytical grade. Whereas the oil samples were the way they are available commercially.

SAMPLE PREPARATION

Hair tresses of 3±0.5 g were prepared for this investigation. They were standardized. Both Undamaged and damaged hair tresses were used. The damaging treatments were Bleaching, UV and boiling water treatment. These hair tresses were treated both before (pre) and after (post) these treatments with coconut/mineral/sunflower oil.

The entire study involved a large no. of variables such as oils, hair types, oiling sequence, etc. resulting in quite complex study. In order to simplify the complexity of the experiment without compromising on the quality of results, a statistical tool termed as Taguchi Design Of Experimentation was used.

METHODS

The tresses were wetted under running tap water (28°C), were washed with a 20% solution of Sodium laureth (3 moles of EO) sulfate (SLES) and thereafter rinsed with water. After this treatment the tresses were subjected to the following investigations.

COMBING DAMAGE - The protein loss method of Sandhu and Robbins (2) was used .

WATER RETENTION INDEX (WRI) - The method for determination of water retention index specified in the earlier publication (3) was used.

HALF - HEAD TEST - Half-head test as mentioned in the earlier publication (3) was used.

The outcome of all above experiments was analyzed statistically using parametric test termed as t-test..

RESULTS AND DISCUSSION

PROTEIN LOSS

The data in Fig 1 – 4 clearly show that the performance of coconut oil in reducing protein loss was better than those of mineral and sunflower oils. The difference between coconut and mineral oil is probably due to the difference in their ability to penetrate the hair (4). The smaller effect of sunflower oil may be due to the presence of unsaturation in the molecule. The same effect was seen in Half-head test in Salon as seen in figure (9). The entire data was analysed statistically (t-test) which clearly indicates that damaged as well as undamaged hair benefit from application of coconut oil whereas in case of sunflower oil and mineral oil, there was no effect.

WATER RETENTION INDEX

From the data of water retention index as seen in fig (5), it is evident that coconut oil reduces the WRI of undamaged hair to the tune of 44% whereas there was no effect case of mineral oil and sunflower oil. The ability of coconut oil to penetrate hair (4) supports this observation. The data for the bleached, Heat and UV damaged hair are shown in figures (6 – 8 resp.). The entire data was analysed statistically (t-test) which clearly indicates that for damaged samples, WRI is much higher than that for the undamaged hair. However, these damaged samples show a reduction in the WRI only when coconut oil was used for treatment.

CONCLUSIONS

This study has firmly established the superiority of protective effect of coconut oil on hair damage in grooming processes as well as damaging processes as compared to mineral oil and sunflower oil. The penetrative ability of coconut oil into hair cuticle and cortex seems to be responsible for this effect. Thus coconut oil really acts as a hair damage protectant whereas mineral oil and sunflower oil do not exhibit this property.

ACKNOWLEDGEMENT

The author would like to thank Mr R.B. Mohile, Head – Technology, R & D, Nature–Care Division, Marico Industries Ltd. for giving an opportunity to work on this project and Dr Yash Kamath, Director – Research, T.R.I., Princeton, U.S.A. for his valuable guidance in writing this paper.

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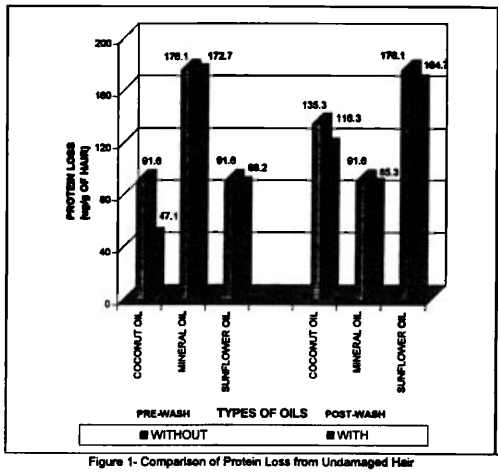

Figure 1- Comparison of Protein Loss from Undamaged Hair

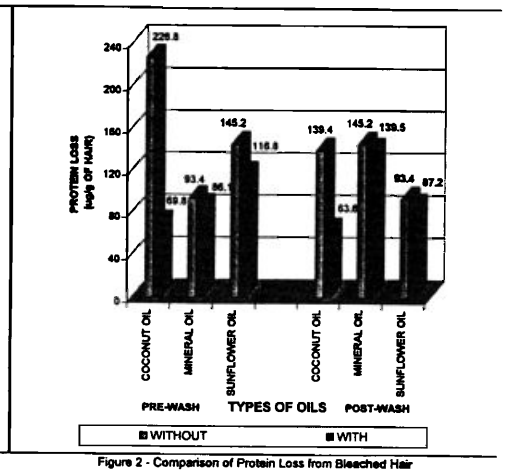

Figure 2 - Comparison of Protein Loss from Bleached Hair

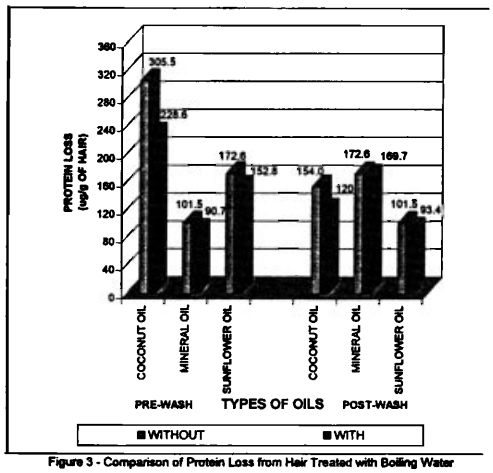

Figure 3 - Comparison of Protein Loss from Hair Treated with Boiling Water

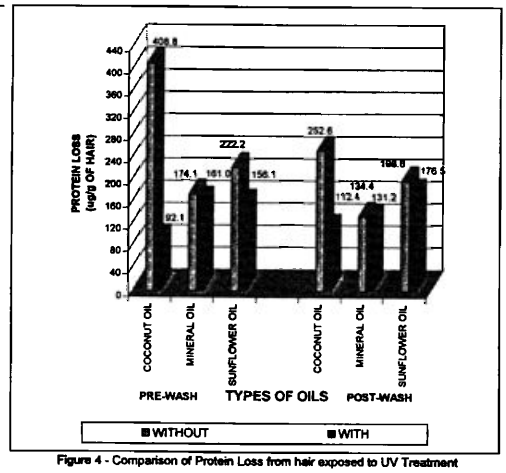

Figure 4 - Comparison of Protein Loss from hair exposed to UV Treatment

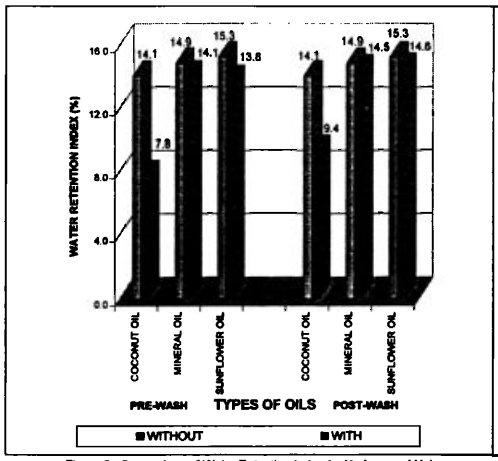

Figure 5 - Comparison of Water Retention Index for Undamaged Hair

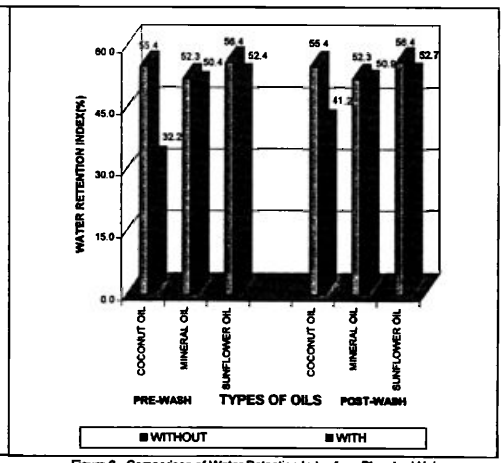

Figure 6 - Comparison of Water Retention Index from Bleached Hair

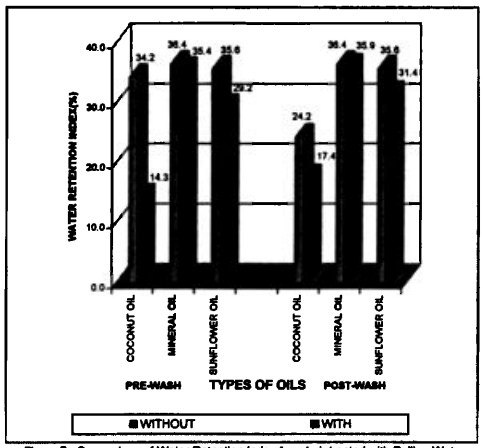

Figure 7 - Comparison of Water Retention Index from hair treated with Boiling Water

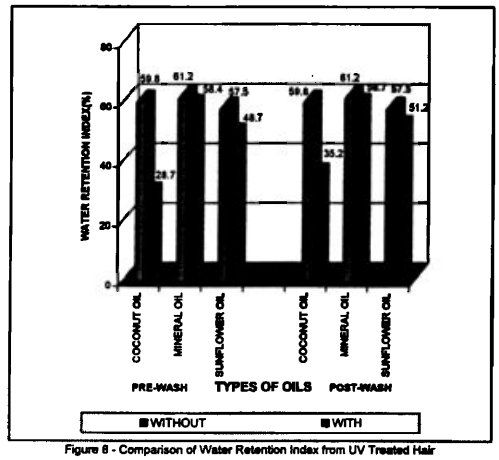

Figure 8 - Comparison of Water Retention Index from UV Treated Hair

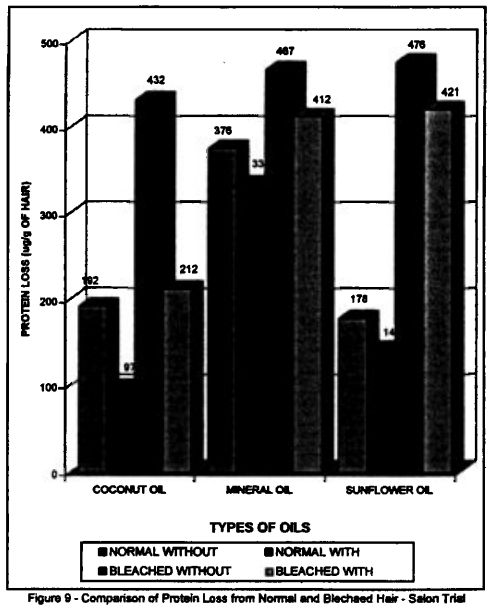

Figure 9 - Comparison of Protein Loss from Normal and Blechaed Hair - Salon Trial

ELLIPTICALLY OF BODY HAIR AND HOW TO MEASURE ITS APPARENT DIAMETER

F. Anthony Simion[1], Ph.D., Pamela S. Witt[1], Matt Klein[1] and Sidney Hornby[2]

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Humans grow hair on many parts of their bodies. The amount and density of the hair varies as a function of age and gender. Indeed within an individual the hair at different body sites is not the same. There are differences in how steroid hormones effect the growth cycle, and as will be discussed below, differences in the shape of the hair. This presentation will focus on:

1. Differences in hair parameters from different body sites.
2. How leave-on products affect the hairs' cross-sectional profile.
3. How to reproducibly measure the major axis of body hair.

For many years the focus of hair research in our industry has been on the care and treatment of scalp hair. Indeed in 2001, U.S. consumers spent $6 billion dollars on hair products (cleansers, conditioners, styling aids and color combined)[1]. In comparison, shaving and hair removal are much smaller businesses.

It has long been known that unlike Asian scalp hair, Caucasian and African-American hair is not circular in cross sectional profile. Published data showing the ratio of major/minor axes for scalp hair for the 3 major ethnic groups is shown below [2]:

	Asian	Caucasian	African-American
Major/Minor Axes Ratio	1:1	1.3:1	1.7:1

However, when Caucasian scalp hair is compared with other body sites, it appears to be less elliptical and more circular.

Major/Minor Axes Ratio: (Mean + SEM [n =])

	Scalp	Underarm	Leg
Male	1.37 ± 0.07 (10)	2.48 ± 0.14 (10)	2.58 ± 0.13 (10)
Female	1.38 ± 0.03 (50)	ND	2.23 ± 0.07 (16)

ND - Not Determined

Hair is frequently treated with 'conditioning' leave-in and 'cleansing' wash-off, products. A priori, we would expect leave-in products to have the larger effect.

The effects of a leave-in product (a lotion) on hair ellipisicity were assessed in vitro, using a laser micrometer. The hair was measured, and then treated once with lotion. After drying at 30% RH for twenty-four hours, the major and minor axes were reassessed. A second set of hair was treated for 5 cycles of lotion application before the hair was re-measured. Results show that while the lotion causes the hair to swell, the effects are proportionate for both axes and there was no effect on the ratio.

Since body hair is highly elliptical, how do you ensure that you can rapidly and consistently measure one axis in situ? Since the hair shaft usually exits the skin at an angle, with the major axis parallel to the surface, we hypothesized that using a Plexiglas plate to press the hair flat against the skin would enable consistent photography and measurement of the major axis.

This was tested experimentally and proven to be the case. Caucasian women allowed their leg hair to grow (no shaving) for 5 days. It was then photographed using a camera fitted with a non-glare Plexiglas plate to press the hair down and to fix the focal length. The diameter of the hair was then measured from the 35 mm photographs by image analysis. Meanwhile the hair was shaved off, collected and the major and minor axes were determined using a laser fiber micrometer. For fifteen of sixteen samples analyzed, there was no significant difference in the major axis measured by the micrometer and the diameter measured by image analysis.

Therefore we concluded that the photographic method described, measures the hairs' major axis. This method has the advantage of simplicity, being able to assess many panelists quickly and easy archiving.

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RETINOIDS AND ACNE...A PERSPECTIVE

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Vitamin A is so named because it was the first lipid fraction isolated in the extraction of plasma constituents. In 1926, the observation was made in Vitamin A-deprived mice that their skin was scaly and inflamed, and when the mice were fed Vitamin A, these effects were reversed. This Vitamin A deficiency resulted in an intense episode of nutritional research into the metabolism and biochemistry of the precursor to Vitamin A, beta-carotene and its metabolites containing the beta ionone motif, collectively referred to as retinoids.

The first report of treatment for acne with oral Vitamin A, a disease of the pilo-sebaceous unit of unknown etiology, appeared in 1949 by Straumfjord. In 1969, Kligman reported the successful use of topical Vitamin A for acne. Lack of significant efficacy led the same team to study the more potent metabolite, Vitamin A acid, the active ingredient in the current marketed product family, Retin A®.

In parallel to these discoveries in dermatology, retinoids became the focus of research in developmental biology. It was discovered that retinoic acid was found to play a key role in the signaling pathway in embryonic development of skeleto, cardiovascular and neuronal systems. For some years it was thought that retinoic acid was a morphogen and that limb development was controlled by a tissue gradient concentration of retinoic acid and its precursors in a space-temporal relationship. Although the retinoids have been fount to play a key role in limb development, they are no longer considered a morphogens, but these findings do relate to the teratology issues that surround this group of compounds and present a major drawback to a wider pharmaceutical usage of these versatile molecules.

In the early 90's, even more attention focused on this family as a result of the identification of nuclear receptors called Retinoic Acid Receptors (RAR's). These receptors are related by homology in their DNA binding region to a super family of ligand activated transcription factors comprising, among others, estrogen, Vitamin D, and steroid hormones. Following this discovery, came the finding that these hormones acted through a common mechanism related to a second receptor, RXR, which formed heterodimers with the receptors (RAR's) recognizing all the other hormones in this super-family.

This discovery unified much research at the time and, in turn, led to the identification of another natural retinoid, isomer 9-cis retinoic acid. As research progressed, new transcription dimer partners were discovered, notably the Peroxisome Proliferating Activating Receptors (PPARS). Ligands binding to PPARS are now the focus of research in adult onset diabetes. Further this field has benefited from cancer research, which produced some very encouraging clinical observations with another natural retinoid, 13-cis retinoic acid.

Drawing upon this fertile scientific environment, investigators in dermatology were able to study the mode of action of retinoids in skin-related models. Using these

models calibrated with the parent substances, researchers saw the creation of synthetic analogues with improved clinical performance in acne. In particular, the models developed in the NIH cancer program, based on the action to induce differentiation in a mouse teratocarcinoma cell line F9 and the reversal of keratinocytes differentiation in a Vitamin-A deprived hamster tracheal organ culture, were replaced by test systems assaying the formation of terminally differentiated human skin derived keratinocytes and the rhino mouse as an in vivo model of the comedone.

The major line of research has focused on modulating cell proliferation and differentiation by retinoids. The primary lesion in acne is the micro-comedone, which forms at the orifice of the pilo-sebaceous unit by the formation of a cellular plug formed from debris which obstructs this unit. This plug consisting of corneocytes, skin lipids, and proteins rapidly build up through accelerated proliferation of the keratinocytes located at the orifice of the sebaceous unit. Bacterial colonization follows, creating, in turn, a chronic inflammatory state. Although the cause of acne is unknown, and may be related to an enhanced sebum production through hormonal imbalance, the retinoid target has been primarily viewed as an action to reverse the comedogenesis.

Interestingly enough, topical retinoids have no effect on sebum production. In contrast, when 13-cis retinoic acid is given by mouth, sebum production is rapidly reduced. However, through research at the molecular level, retinoids were found to inhibit the formation of gene products regulated by AP1, a transcription factor controlling the expression of metallo-proteases and strongly linked to inflammatory mechanisms and dermal matrix architecture. The anti-inflammatory pathway was also invoked to explain the action of topical retinoids in moderate acne and psoriasis. This mechanism was also related to another observation from the Kligman group in 1986 that topical application of tretinoin could diminish fine lines and wrinkles and reduce mottled hyper-pigmentation, which are signs of photo-induced skin damage.

Pharmaceutical research has produced many different dosage forms of retinoids including micro-sphere, vesicular and host –guest complexes. The retinoid armamentarium, as a group of drug products, have a combined a market value of around $700 million annually, and if the oral forms of these drugs were included, this figure would approach $1 billion annually. The combination of serendipity, rational science and astute marketing has given rise to a family of drug products that treat not only acne but may prevent skin cancer, enhance therapeutic control of type II diabetes, and benefit life-style skin disorders such as photo-induced skin damage.

SYNERGIES IN COMBATING ACNE PRONE SKIN: OLEANOLIC ACID AND NORDIHYDROGUAIARETIC ACID INHIBIT 5-ALPHA REDUCTASE, KERATINOCYTE PROLIFERATION AND INFLAMMATORY RESPONSE: IN VITRO AND IN VIVO STUDIES*

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Key words : oily skin, P. acnes, 5α-reductase, prostaglandin

Introduction

Mild (i.e. non-pathological) acne and oily skin constitute a multifaceted aesthetic problem with mainly four interrelated causes and symptoms: hyperseborrhea, hyperkeratinisation, cutaneous bacterial proliferation and local inflammation. A successful approach to treat acne prone skin should take into account all four factors in a concerted manner by combining active ingredients that tackle all biochemical and physiological aspects. **Hyperseborrhea** is most often observed as a consequence of increased concentration of dihydrotestosterone, a steroid hormone generated by the reduction of testosterone by 5-α reductase and responsible for the stimulation of sebum production and secretion. The same hormone testosterone also leads to increased proliferation of keratinocytes (**hyperkeratosis**) which then causes the skin pores of the pilo-sebacious canal to clog and to the formation of microcomedos. These sites become ideal grounds for **bacterial colonization.** Chemicals produced and released by the microorganisms finally cause an **inflammatory response** that is typical of the papules, pustules and macrolesions of acneic skin.

Materials and Methods

Oleanolic acid is obtained in pure form by extraction (>95%) from the leaves of olive trees. NDGA is obtained either by synthesis or by extraction (>95%) from *Larrea divaricata*. 5-α-reductase type I assay follows the protocol of Zu-Yue (detection of NADPH [1]) and is confirmed by HPLC quantification of testosterone. Keratinocyte proliferation (neocultured human skin cells) is measured by BrdU incorporation and ELISA assay. Microbial inactivation is visualized on agar gel inoculated with 10^4 or 10^5 cfu/ml and incubated in presence or absence of the products. *P. ovale* is cultured in Sabouraud gel with olive oil.

In vivo tests are carried out on various panels of young volunteers with informed consent. Sebum secretion is assayed with the Sebutape® method.

Results and Discussion:

Hyperseborrhea:

Large-scale screening of plant derived pure molecules has yielded oleanolic acid as a powerful candidate for the inhibition of 5-α-reductase type I activity. Figure 1 shows the concentration dependent inhibition of this enzyme by as low as 3 and 9 ppm (I and II) of oleanolic acid (OA). The synergy with NDGA (4 ppm and 12 ppm) is also evident from this graph, as NDGA alone has no effect on 5-a-reductase.

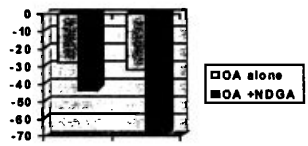

Fig. 1 : inhibition of testosterone conversion

Hyperkeratosis:

To check the rapid proliferation of keratinocytes, a growth-moderating molecule is needed that is, however, devoid of cytotoxicity. NDGA, by acting on the protein processing in the Golgi apparatus, and on the intracellular pool of ATP, has a reversible (and thus non-toxic) effect on keratinocyte multiplication as is shown in figure 2. Again this effect is powerful and concentration dependent.

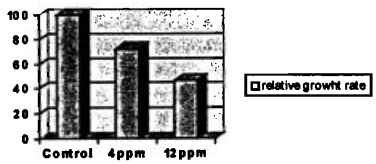

Fig. 2 : keratinocyte growth rate reduction in presence oof NDGA

Inflammation:

NDGA is well known for a second, intrinsically unrelated biological activity, that is as a non-steroidal cyclooxygenase inhibitor. Effects on prostaglandin synthesis (PGE_2) inhibition can be seen at concentrations as low as 3 ppm, once more in concentration dependent manner (fig. 3). This *in vitro* observed anti-inflammatory activity is of the same order of magnitude as acetyl salicylic acid, the active ingredient of aspirin.

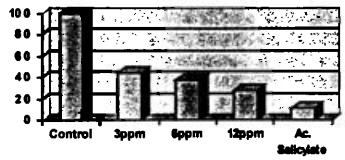

Fig. 3 :Prostaglandin PGE2 synthesis rate in presence of NDGA or Ac. Salicylic acid (3ppm)

Bacterial colonization: 5 strains of bacteria commonly found in acne lesions and acne prone skin (*Propionebacterium acnes, Coynebacrterium minutissimum, Staphylococcus epidermidis, Staphylococcus aureus and Acetinobacter calcoaceticus)* and a yeast (*Pityrosporum ovale,* also known as *Malassezia furfur*) were investigated. The strains were inoculated in agar gelose in presence or absence of oleanolic acid (15ppm) and NDGA (20ppm) dissolved in butyleneglycol, and a combination of the two at these concentrations levels formulated in a carbomer gel containing <1% of butyleneglycol, glycerin and caprylylglycol. Whereas selective bacterial inhibition by OA or NDGA or the excipient gel could be observed, a total bacterial and yeast growth inhibition was only obtained in the final synergistic mixture.

***In vivo* studies:**

Clinical studies were undertaken to confirm and substantiate the observations from the *in vitro* data. At first, the connection between 5-α-reductase inhibition with sebum production, was established in a clinical test on 9 volunteers with oily skin. The Sebutape® method was used in this double blind vehicle controlled study to measure sebum secretion rates before and after a 6 week treatment with a mixture of Oleanolic acid (9 ppm) and NDGA (12) ppm formulated in a light emulsion. Table I shows the decrease in the number of active sebum glands and the concomitant decrease in quantity of sebum produced (-44%). These values are significantly different from the placebo effects.

Table I	T0		T42	
	Active glands	Collected Sebum (mm²)	Active glands	Collected Sebum (mm²)
Mean + s.d.	181±23	6.98±1.49	125 ± 17	3.±1.25
% variation	-	-	-31	-44

The lesional aspects of acne prone skin (comedos, papules, pustules, microcysts, so called retentional and inflammatory lesions) were evaluated in a second clinical study on 20 volunteers of 18-35 years of age, presenting with symptoms of acne type skin. A two month treatment with a Carbomer /butylen glycol/polyol based gel containing 9 ppm of OA and 12 ppm of NDGA gave the following results (dermatological assessment: counting of lesions before and after the trial period (table II).

Table II	D0	D58	Decrease %
Microcysts	14.9±2.4	10.9±1.9	-27%
Comedos	7.9±1.8	5.2±1.1	-35%
Papules & pustules	5.8±1.0	3.9±0.7	-32%
Total lesions	28.6±3.3	19.9±2.5	-30%
Significance		P<0.05	P<0.05

This result compares favorably with retinoic acid treatment [2]; the panelists observed the improvement, too, and noted particularly the comfortable skin feel all throughout the day.

Conclusion:

Four causes and interrelated symptoms of acne prone skin led us to develop a blend of active molecules with which it is possible to act upon all four as we have shown in the specific *in vitro* and the related *in vivo* tests. The reduction in sebum production and in cell proliferation, coupled with a light antibacterial effect and anti-inflammatory activity leads to a visible improvement of the skin, without leaving scars or provoking irritation and side effects (fig. 4).

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COSMECEUTICAL PROPERTIES OF FERMENTED SAGE COMPOUND

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R. J. Reynolds

Background: Adipose cells in the hypodermis play two important roles in the maintenance of healthy skin (1, 2). The first role of adipose tissue is to protect the skin. Subcutaneous adipose is an energy storage site and insulating layer necessary for healthy skin. The second role of adipose tissue is to nurture the skin. Lipolysis is the process of breaking down stored triacylglycerol in the fat cell to release free fatty acids and glycerol. This occurs by triggering Hormone Sensitive Lipase (HSL) which is under acute hormonal and neural control. Catecholamines like adrenaline and nor-adrenaline activate HSL, the rate-limiting enzyme of lipolysis, through cAMP-dependent phosphorylation (3). Cellular communication involves the interaction of neurotransmitter, hormones, and hormonal factors with receptors expressed on the cell surface. Following interaction with receptors particularly those belonging to the metabotrophic super family, hormones are secreted from the pituitary gland and cellular transduction occurs through activation of second messenger pathways including the adenylyl cyclase system and the calcium-phospholipid pathways (3). The modulations of these second messengers result in changes in the phenotype and /or genotype of the target cell resulting in the biological response.

Significance: Fermented sage compound (FSC), a natural product (Molecular weight - 250) is a bicyclic terpenoid, white crystalline compound. FSC is an adenylyl cyclase activator like Forskolin, obtained from the roots of *Coleus forskoli* (2). cAMP assays indicate (3) that FSC has a similar activity profile and potency as that of forskolin, a well established adenylyl cyclase activator, in interaction with ligand gated ion channels (Figure 1). It is believed that it may also act as a therapeutic agent by selectively activating secondary messenger pathways. Forskolin is known to promote lean body mass (4).

Results: FSC elicits physiological effects consonant with its ability to stimulate adenylate cyclase and increase intracellular cyclic AMP. Other responses which are elicited by FSC and have been associated with increased intracellular cyclic AMP include lipolysis in adipocytes, inhibition of platelet aggregation, and relaxation of smooth muscle and stimulation of steroidogenesis. FSC increases sodium transport in cultured epithelia (Figure 2). The ability of FSC to induce lipolysis/lipogenesis is an integral component of anti-wrinkle, anti-aging, and cellulite creams and lotions. It is believed that FSC and analogs can selectively activate adenylyl cyclase system making analogs suitable as selective probes.

The activities associated with increased cellular c-AMP include lipolysis in adipocytes and inhibition of platelet aggregation. FSC increases sodium transport (89%). The exact site of action of FSC still remains unclear, and it is possible that FSC acts directly at the catalytic subunit of adenylate cyclase or at another subunit which has not yet been identified.

Other cosmeceutical properties of FSC: FSC has very significant antimicrobial properties in *in vitro* assays against *Propionibacterium acnes* indicating that this compound could be very effective to treat acne (5, 6). Another study using FSC (0.1%) showed lipid barrier-enhancing properties by strengthening the natural lipid barrier of the skin as well as other methods of skin treatment that are made possible by the strengthening of the barrier (7). In addition, the report also mentions that FSC reduces the skin's response to irritants and sensitizers and could be used for tanning the skin without exposure to the sun. FSC is known to cure disorders characterized by excessive cell proliferation in patients with benign hyperplasia and psoriasis (8).

Safety studies: The safety test show FSC with low irritancy potential (safe as saline); RIPT-no indication of irritation or sensitization at 1.0%. Orally it was non-toxic-acute oral LD50 >5g/kg (in rat; as per MSDS); no observed adverse effect level-50 mg/kg bw/day for 28 wks (FEMA GRAS no. 3794). Further, RTPCR studies showed no sensitization or irritation.

Conclusion: Subcutaneous adipose forms the deepest layer of skin, or hypodermis, and is responsible for protecting and nourishing the outer layers. Subcutaneous adipose tissue helps to keep skin soft and supple by insulating it from the elements and providing vital moisture and nutrient supply. FSC has potential use in personal care products, primarily anti-wrinkle, "orange peel" and other signs of aging. These compounds work in harmony with the metabolism of adipose cells in that they stimulate lipolysis/lipogenesis, thereby altering the outward appearance of the skin. FSC has been shown to breakdown lipid (triglycerides) barrier which is unique and could have application in the

cosmetics industry. In conclusion, FSC could become an alternate for Forskolin and could be used in creams and body lotions as well as in weight loss management.

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Figure 1. Effects of FSC on human nAChR functions

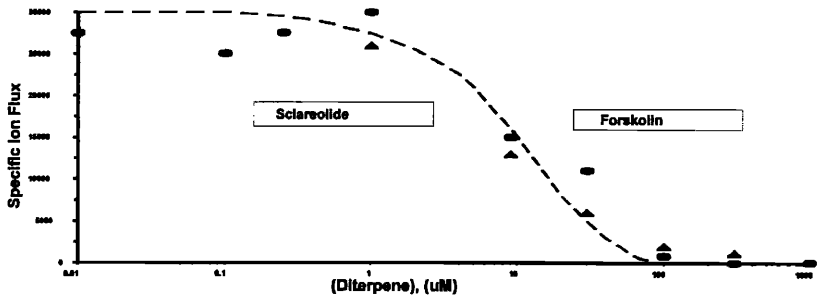
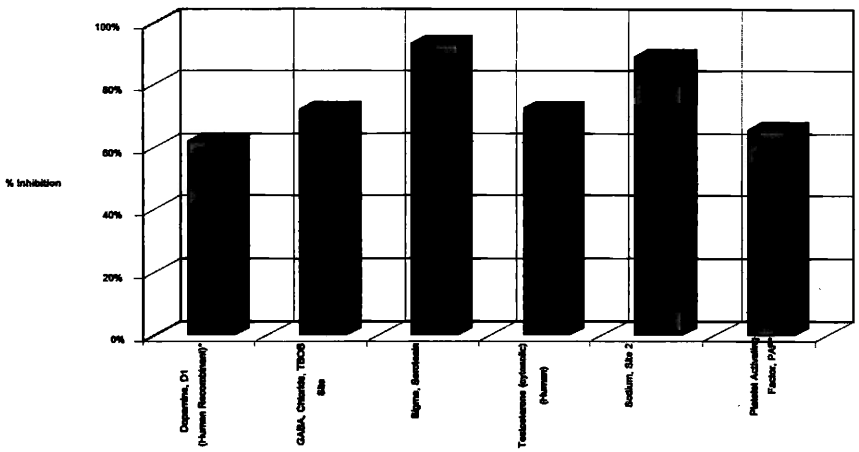


Figure 2. Other cosmeceutical properties of FSC



POST ADOLESCENT ANCE: COSMETIC RELEVANCE

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Acne has traditionally been viewed as predominantly affecting adolescents but in fact the age range goes up to and sometimes beyond 45 years, particularly in women. This presentation summarises recent literature challenging the belief that acne is primarily an adolescent problem, and discusses the importance of cosmetics in either exacerbating or improving acne.

There is much information describing the clinical picture and incidence of teenage acne, but relatively few reports on post adolescent acne. This might be because concern over adult acne has only grown in recent years. Nevertheless a 1945 study of 500 adult women aged 17-40 showed that 69% had acne ranging from slight to clinical[1]. In 121 men with clinical acne 25% were over 25 years and 9% were over 30 years. Epstein in 1968[2] examined the faces of 778 new patients over 1 year and reported that both males and females over 25 presented with clinical acne with the females showing the higher incidence. O'Loughlin[3] proposed that 2 subgroups existed based on his examination of clinical acne in 53 females aged 24 years and over. There now seems to be general agreement that there are individuals with acne continuing from their teenage years ('persistent acne'), whilst for other the phenomenon is new ('late onset acne') first occurring after age 25 years. Premenstrual acne flares seem to occur in either group.

More recent studies provide insights into the occurrence of adult acne: two were large scale community-based studies[4,5] and the third study describes findings from an acne clinic[6]. Both community-based studies emphasize the fact that acne continues well into adulthood for both males and females. In one study, a total of 749 subjects over 25 years were examined for facial acne. The prevalence of acne remained constant between 24 and 44 years in both males and females, and did not decrease significantly until after the age of 44 years. Facial acne was reported in 231 (54%) women and 130 (40%) men. The acne observed was mainly very mild which the authors refer to as physiological acne. Higher grade clinical facial acne was recorded in 3% of subjects and was worse in females. Persistent acne was the most common (82% of patients) compared with late onset acne. In the second community based study[5] acne was present in all age groups investigated up to 87 years and was detectable in 13% of people above 59 years. In the 20-29 year group, 64% have acne and 43% still had acne between the ages of 30-39 years. In an acne clinic the mean age of patients attending increased from 20.5 years in 1984 to 26.5 years in 1994.[6] The trunk area was most affected in males whilst the face was mainly affected in women. This may explain why more females are seen in clinic compared to males, despite the fact that women had a lower mean grade. Most of these patients had acne that had persisted from the teenage years. Acne appearing for the first time after age 25 years was reported by 18.4% of women and 8.4% of men.

The causes of adult acne are not clear. Persistent acne could be explained as a continuation of teenage acne and could therefore share similar pathogenic features: increased sebum production, ductal hypercornification, inflammation and increased bacterial activity. There is a significantly higher sebum excretion rate among adult women with persistent acne, compared to non-acne female adults, suggesting that at least in persistent acne there may be an underlying increase in sebogenesis.[7] It is more difficult to explain late onset acne which starts well after the hormonal changes accompanying puberty.

Factors put forward to explain adult acne include the use of cosmetics, stress, resistant bacteria, smoking, oral contraceptive usage and underlying hormone levels. One study investigating the causes of adult acne found that 37% of the women had additional clinical features of hyperandrogenicity; 82% had failed to respond to multiple courses of antibiotics, and 32% had relapsed after treatment with one or more courses of isotretinoin.[6] In this study, cosmetic use and occupation did not seem to be significant contributing factors. Clinical signs of acne occur at puberty concomitant with an increase in circulating adrenal and gonadal androgens. Androgens play a role in stimulating the sebaceous glands to enlarge and produce sebum but the role of hormones in adult acne is debated. In addition, the increased use of oral contraceptives, particularly those containing androgenic progesterone may play a role in the persistence of acne in women.

Whether cosmetics are wholely or partially causative of adult acne is unclear. At one time it was believed that cosmetic use could explain 95% of the cases of adult women presenting with a mild acneiform condition for which Kligman coined the term "acne cosmetica".[8] One study reported an increase in acneiform eruptions after a beauty treatment consisting of a facial massage of cream, steaming, application of a face pack. In this case, the most common acne lesions were nodules with infrequent occurrence of closed comedones.[9] Certain cosmetic ingredients are comedogenic in both human and animal and such substances include lanolins and certain vegetable oils. However, Cunliffe reported finding no correlation between the amount of time over which cosmetics were used and the severity of the acne.[10] Stopping the use of the suspected cosmetic did not produce an improvement. Nowadays, many cosmetics are thoroughly assessed for comedogenicity and acnegenicity, perhaps some cosmetics may not be the cause of adult acne, but may exacerbate or aggravate acne prone follicles or low grade acne.[11]

One line of investigation which has not been thoroughly explored, is whether the use of cosmetics can actually help to reduce acne. One could speculate that daily cleansing and moisturization maybe beneficial in acne and may underlie the placebo response observed in many acne studies. One study[12] examined the effects of a regime consisting of 6 formulations of low predicted acnegenic potential in 10 young women. No increase in acne was noted, on the contrary there was an improvement (i.e. decrease) in the total number of comedones and papules.

In our group, we have assessed different moisturizers and cleansers to evaluate whether they were beneficial for adult acne. A mild bar cleanser was tested on 32 female subjects between the ages of 18-45 with mild – moderate acne. A twice daily wash of 45 seconds was followed for 12 weeks. A small, but significant decrease in non-inflamed lesions (27.7%) and inflamed lesions (15.5%) was observed by week 12 compared to baseline and in addition this effect was perceived by subjects. In a comedolytic study on the back, a cleanser formulation was shown to reduce the number of follicular impactions following 8 weeks of twice daily washing. A moisturizer was tested and compared with 2% salicylic acid for antiacne efficacy. After 4 weeks, both treatments produced a significant decrease from baseline in total non-inflamed lesions. By week 12, moisturizer produced a 28% decrease in non-inflamed lesions and 2% salicylic acid produced a 33% decrease: these changes were not significantly different from each other. For inflamed lesions, both treatments produced a significant decrease from baseline by week 2 reaching a maximum decrease of about 36% by week 4-8. At week 12, though, total inflamed lesions returned to baseline for moisturizer treatment. At all time points, neither product was significantly different from each other.

Treatments for female adult acne are similar to those used for adolescent acne. For example, topical treatments for mild cases (benzoyl peroxide, salicylic acid) with antibiotics for inflammatory acne and retinoids for comedonal or more severe forms of the disease. Newly developed cosmetics such as foundations and blushes have included actives designed to ameliorate acne and improve overall skin condition. In conclusion. there is now good evidence that acne can continue well into adulthood and there is some indication that unlike teenage acne were males tend to show the most severe forms of the disease, adult acne mainly affects females. The role of cosmetics is debated in the literature, but new data supports the role of some cosmetics in reducing the appearance of acne.

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