

journal of applied cosmetology

OFFICIAL JOURNAL OF

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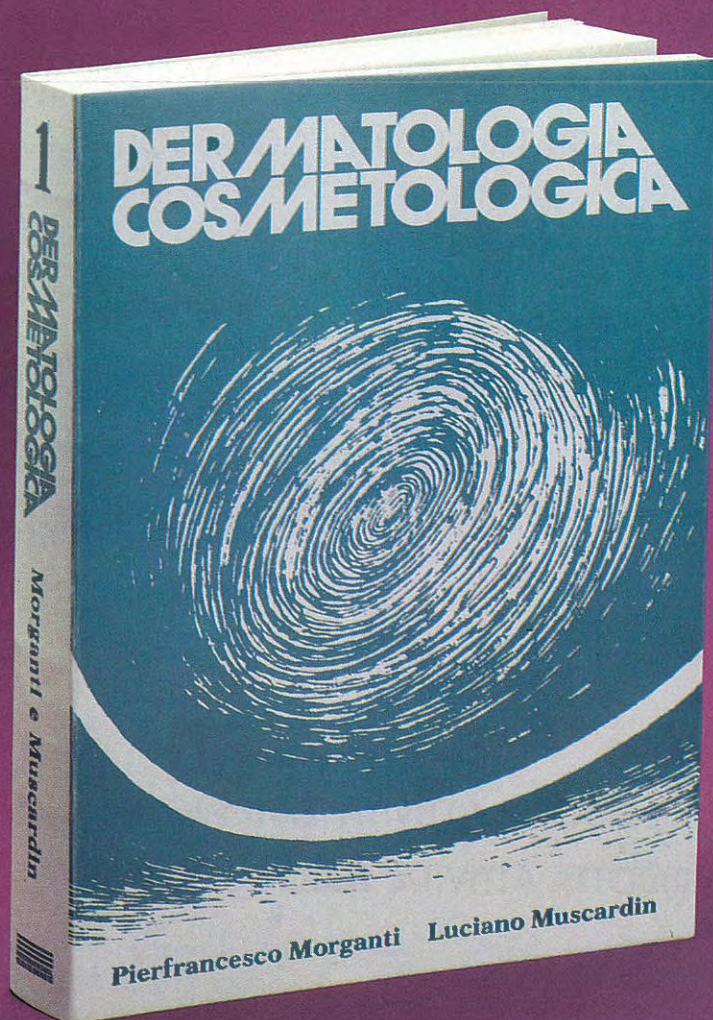


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A cura di P. Morganti e L. Muscardin
Ed. International Ediemme

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Trimestrale di Dermatologia Cosmetologica Quarterly Review of Cosmetic Dermatology

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SMOKING OUT THE SKIN

R. WOLF M.D.*, A. LO SCHIAVO M.D.** and V. RUOCCO M.D.**

*Tel-Aviv Sourasky Medical Center, Ichilov Hospital
Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv ISRAEL

**Department of Dermatology University of Naples II, School of Medicine and Surgery,
Naples, ITALY.

The opinions and assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Dermatology, Ichilov Hospital.

Received: October 16, 1994

Key-Words: Smoking, Environmental agents

Synopsis

"The Elizabethan age might be better named the beginning of the smoking area" (Sir James M. Barrie, *My Lady Nicotine*). From that time cigarette smoking increased at an epidemic pace, peaking in 1964 when more than 40% of all adult Americans smoked. Since that time smoking has decreased, however, today 28% of all adults are still smokers.

The skin is directly and intensely exposed to the cigarette smoke, and its inhalation, and is also exposed to toxic substances reaching it via the bloodstream. It is not surprising, therefore, that smoking has multiple and different effects on the skin. This review has highlighted the many and varied effects of smoking on the skin from a dermatological point of view.

It starts with the relation between cigarette smoking and facial wrinkling, beginning with the clinical and epidemiological findings and continuing with the pathogenetic aspects. The review continues with the relationship of smoking on various skin diseases, in particular, psoriasis. It further summarizes our knowledge about smoking, immune response, and infectious diseases. The relationship between smoking and defective wound repair is then discussed, and a brief review on smoking and skin cancer concludes this article. Although we have accumulated much information on this subject in recent years, we are only beginning to scratch the surface. Unfortunately, most of the articles on this subject are aimed to serve the anti-smoking campaigns. It seems that not enough efforts have been expended to investigate the pure dermatologic effects of smoking. It is our hope that this review will provide dermatologists with much of the information necessary for understanding the problem, and that the material offered here will inspire them to increased interest in the subject, since "great love is born of great knowledge of the object one loves" (Leonardo da Vinci, *Treatise on Painting*. Ch 80).

Riassunto

Attualmente il fumo di sigaretta é ancora la causa principale di malattie gravi e, spesso, mortali. L'abitudine di fumare é aumentata durante tutto il nostro secolo, raggiungendo un picco nel 1964, anno in cui il 40% degli americani apparteneva alla schiera dei fumatori. Da allora si é avuta una riduzione di questo "vizio": oggi, fuma solo il 28% degli adulti.

La pelle é di continuo esposta al fumo di sigarette ed alle sostanze tossiche, le quali la raggiungono

direttamente e per via ematica. Non sorprende, pertanto, che il fumo eserciti effetti molteplici e svariati sull'organo cutaneo. In questo articolo si vogliono evidenziare, da un punto di vista squisitamente dermatologico, gli effetti dannosi, focalizzando gli aspetti clinici, epidemiologici, patogenetici. Il primo impatto del fumo sulla cute é cosmetologico e riguarda il suo effetto favorente la formazione di rughe. Inoltre esiste una correlazione documentata tra il fumo ed alcune dermopatie, in particolare la psoriasi. Di grande interesse é la dimostrata interferenza del fumo sull'omeostasi immunitaria e, conseguentemente, su alcune patologie infettive. Anche la cicatrizzazione subisce l'influenza negativa del fumo. Infine, come molti altri apparati, anche l'organo cutaneo non si sottrae al ben noto effetto carcinogenetico di questo "antico vizio".

INTRODUCTION

Whilst the hazards of cigarette smoking on the pulmonary, cardiovascular, oral and laryngeal systems have been recognized and widely studied for the past 20 years, the effects of cigarette smoking on the skin has received little attention. Furthermore, most of the articles on this subject are aimed to serve the anti-smoking campaigns, i.e., to sell the idea of smoking cessation to our patients. Most of them are, therefore, published in non-dermatologic journals (1).

The aim of this article is to review the effects of cigarette smoking on the skin, from a dermatological point of view.

Cigarette smoking and facial wrinkling.

Although a relation between smoking and the complexion was first suggested as early as 1856 (2), to date there have been few controlled studies to test that association.

The association between complexion and smoking was first studied by Ippen and Ippen in 1965 (3). They found that 79% of female smokers had "cigarette skin", compared with only 19% of the nonsmokers. "Cigarette skin", according to the authors, exhibited a loss of turgor, a pale color with grayish hue, and prevalent wrinkles with thick skin between the wrinkles.

Their description of "cigarette skin" or "smoker's face" is similar to that of Solly (2) some 100 years earlier. Since the authors did not believe that those characteristics were present in men, they conducted their study on women only. Although this study can be criticized for many reasons — in particular, the lack of control or adjustment for age and sun exposure — its importance lies in the fact of its being a pioneer study in this field.

Four years later, Harry W. Daniell, who has become the protagonist of the idea of using the smoking-wrinkle association for the anti-smoking campaign and education, published a

"letter to the Editor" stating that "There must be a close relation between the presence of wrinkling of the facial skin and habitual cigarette smoking" (4). Indeed, he succeeded in a large, well controlled study to prove his clinical observation (5). He established a method of grading the severity of facial skin wrinkling that "can be rapidly learned and easily used by untrained students". In a study of 1104 subjects, he found that the association between cigarette smoking and wrinkling was striking in both sexes soon after age 30, was related to the duration and intensity of the smoking, and was more pronounced than was the association between wrinkling and outdoor exposure. He found that smokers in the 40-49 years age group were as likely to be prominently wrinkled as nonsmokers who were 20 years older. His study, which was a "blind" study, was well controlled for age and sun exposure.

Allen et al. (6) used Daniell's wrinkle categories to examine the association of smoking with facial wrinkling among 650 men and women. He concluded that wrinkles in the crow's foot area are caused by actinic exposure and not by smoking, because black patients had no such wrinkles whether they smoked or not. Several authors believe that this conclusion is not supported by the data presented in the article, and that although Allen et al's results appeared to refute Daniell's findings, their data actually supported an association between smoking and skin wrinkling in whites (7-9).

In spite of the criticism, Daniell's study was for many years — and indeed, still remains — a key study, and one of the most important and convincing pieces of research in the field. His findings, and the statistical analysis, are still valid today, and indeed very little new has been added or changed since then. Subsequent studies have only served to confirm and reinforce his findings.

While Daniell's study (5) was mainly concerned with grading the degree of facial wrinkling and relating it to the number of cigarettes smoked a

day, Model's study (10) emphasized and defined the characteristics of "Smoker's face" and introduced it as a clinical sign. "Smoker's face", which has features similar to those described by Ippen and Ippen (3) 20 years earlier, was found among 46% of smokers, 8% of ex smokers and none of the nonsmokers. The association of smoker's face with current smoking was significant, and remained so after adjustment for age, social class and sun exposure. In contradistinction to Daniell's study, Model made no attempt to grade or quantify the skin changes, but was more concerned with a clinical assessment of the face, and a description of a clinical sign. He was interested in a variety of skin changes, and not just wrinkles.

The effect of smoking on the induction of premature facial wrinkles was further demonstrated in a recent study by Kadune et al. (11), showing that cigarette smoking is an independent risk factor for the development of premature wrinkles, and that sun exposure had a cumulative effect.

The association of smoking with premature wrinkling has recently been confirmed in the largest study to date, involving nearly 7000 adults (12).

How does smoking bring about the observed clinical changes?

The pathogenesis of smoker's skin changes is poorly understood. It is probably multifactorial. The effects of cigarette smoking on the skin could be localized or systemic. Smoking probably exerts its deleterious effects on several components of the skin.

A recent study (13) has shown abnormalities in the elastic tissue of the non- sun-exposed skin of heavy cigarette smokers, similar to those seen in photo damaged skin.

The biochemical defects of the dermal elastic tissue in smokers still remains to be determined. Cigarette smoke can increase the plasma neutro-

phil elastase activity (14) and the release of elastase from neutrophils (15) and can also inactivate α -1-proteinase inhibitor (16). The plasma levels of active α -1- antitrypsin activity are decreased in smokers (17). Also the enzyme which catalyses the cross-linking of elastin - lysyl oxidase - is inhibited by some components of tobacco smoke (18). All these effects of smoking can explain changes in elastic tissue.

Changes in the vasculature, chronic ischemia of the dermis, and decrease in capillary and arteriolar blood flow in the skin, (discussed below) certainly play additional key roles in the pathogenesis of smoker's skin changes.

Hormonal changes, due to cigarette smoking, may also play an important role in the development of smoker's skin changes. There are today sufficient data indicating that smoking decreases estrogen secretion, and increases its metabolism and excretion (reviewed in 19).

Although most of the studies showing that smoking can cause relative estrogen deficiency were performed by gynecologists and were concerned with the reproductive system, dermatologists can draw very important conclusions from those studies. There is general agreement that decreased estrogen levels contribute to the development of age-related skin changes (20- 21). Hence, smoking-induced estrogen deficiency will accelerate aging of the skin. Furthermore, estrogen replacement therapy is less effective among smokers (19,22).

With regard to effects on the epidermis, there is no doubt that prolonged exposure of the facial skin to the multitude of toxic substances in smoke must affect the epidermis, since that layer is in much more intensive contact with the smoke than is any other skin layer. Moreover, some of the toxic substances derived from smoking almost certainly reach the epidermis via the bloodstream, in the same way as they get to other tissues in the body. From the above, it is fairly obvious that cigarette smoking must have an effect on the epidermis. It is to be noted with regret that very few dermatologists show

much interest in the effects of smoking in the skin. Most of the studies on the effects of smoking have been carried out by researchers in other disciplines — oncologists, internists, bronchologists, epidemiologists, and gynecologists.

Consequently, there are very few research studies concentrating on the epidermis — a tissue that is generally acknowledged to be the exclusive province of the dermatologist. On the other hand, effects on other tissues such as connective tissue, the immune system, or the vascular system, have attracted widespread interest from researchers in many fields.

Smoking and psoriasis.

Whilst the relationship between smoking and pulmonary or cardiovascular diseases has been extensively studied, very little research has focused on the effect of smoking on various skin diseases. Nonetheless, there are some interesting and informative studies on the association between smoking and psoriasis.

O'Doherty and MacIntyre (23) were the first to draw attention to the strong link between smoking habits and palmoplantar pustular psoriasis. Eighty percent of patients with palmoplantar pustulosis were smokers at the time their disease developed, compared with only 36% of the controls; four out of five patients were female. Whether palmoplantar pustulosis is accepted as a variant of psoriasis or not, the strength of the relationship between smoking and this disease, according to this study, is of the order of magnitude, of the well known, and generally accepted relationship of acute guttate psoriasis to streptococcal disease. Several years later Sonnex et al (24) were able to show that polymorphonuclear leukocytes (PMNs) from psoriatic smokers responded to a significantly greater degree to a standard chemotaxin than did PMNs from psoriatic non-smokers, control smokers and control non-smokers. These findings suggest that smoking has a selective effect on psoriatic PMNs, and thus might explain the mechanism underlying O'Doherty and MacIntyre's finding (23). In an earlier study no significant difference in neutrophil morphology between smokers and non-smokers with palmoplantar pustulosis could be found (25).

Further studies have suggested that smoking might be a risk factor, not only for palmoplantar pustular psoriasis, but also for plaque type psoriasis (26-30).

The results of these studies showed a striking association between smoking and psoriasis. Only two of the reports included a control for drinking behavior (26, 28). In these studies, the increased risk of psoriasis in smokers did persist after adjustment for alcohol intake. In this regard, a contradictory study of Poikolainen (30) is worthwhile mentioning. He found that increased alcohol consumption has been associated with psoriasis, including a high consumption prior to the development of the disease, whereas no association could be found between psoriasis and smoking. Although the problem of confounding by alcohol consumption is not completely resolved, there seems to be little doubt that smoking and psoriasis are associated. The question to what extent is the association causal? remains, however, unanswered.

Smoking, immune response, and infectious diseases.

Although there have been extensive studies on the effects of smoking on inflammatory and infectious diseases of the lung, and of the oral, gastrointestinal, and cervical epithelium, relatively few studies have focused on the immunologic effects of smoking on the skin.

Most of the studies in this field have been done on human papillomavirus (HPV) infection, most of them, by non-dermatologists. Several studies suggest an increased risk for cervical HPV infection and for intraepithelial neoplasia in female smokers.

A positive association between smoking and

HPV in the cervix of women has been found in these studies, an association that was present even after adjustment for measures of sexual activity (31-33). The statistically proven association between smoking and oncogenic HPV, which suggested a causal relationship between these two factors, has been criticized by some investigators. Philips and Smith (34), for example, criticized this statistical evidence in a somewhat unusual and provocative way. They presented a study demonstrating an association between HIV infection among the heterosexual population of Haiti and smoking, or having had three or more lifetime sexual partners. However, a stronger statistical association has been found between HIV and "having a dirt floor in the home". The conclusion is obvious.

In an interesting study on 20,333 Swedish adults Axell et al. (35) found that the prevalence of recurrent herpes labialis was influenced by smoking habits.

The prevalence of recurrent herpes was significantly lower among smokers, and especially among pipe smokers, compared to non-smokers. Snuff dipping did not influence the prevalence values.

Numerous clinical studies have documented smoking-induced alterations in immune and inflammatory function (36-37). Smokers have higher leukocyte counts, without an associated altered chemotactic, microbicidal or secretory function (38). The effect of smoking on lymphocyte and monocyte number and function have been extensively studied, with diverse and conflicting results (37,39-43).

Studies on the effect of smoking on the mucosal immune system showed a reduction in salivary IgA and increased IgM (44). By contrast the IgA concentrations in bronchoalveolar lavage fluid were normal in smokers compared with a non-smoker control group, whereas IgG levels were increased (45).

Obviously, the area of most interest to dermatologists is the skin immune system and its inflammatory and allergic reactivity. Clinical and

epidemiological studies have shown several interesting associations between smoking and skin diseases. Smokers showed a higher incidence of cutaneous adverse reactions to drugs compared with non-smokers (46). A statistically significant relationship could be found between cigarette smoking and vesicular palmar eczema (pompholyx) (47), as well as between smoking and the severity of reactions to latex gloves (48), and between smoking and infectious, eczematoid dermatitis (49).

Regarding the effect of smoking on atopic, IgE-mediated skin disorders — several studies have addressed this aspect of smoking. Results remain, however, controversial and the problem still unresolved. A recent study by Mills et al. (50) showed that there was no significant difference between the prevalence of smoking among patients with atopic dermatitis compared with matched controls. In an earlier study she could demonstrate that smoking clearly depresses cutaneous reactivity to UV irradiation, to histamine, and to direct irritants (51).

Jonderko et al (52) performed dermic allergometric tests with the extract from cigarette tobacco on 714 persons, 336 of them smokers. He did not find any evidence of an immediate type allergic reaction to tobacco smoke.

Burrows and coworkers have reported lower rates of allergy skin test reactivity in adult smokers (53,54), however, in apparent contradiction to that finding, several other workers (36,55) have found higher levels of serum IgE in smokers compared with non-smokers. The nature of this increase in serum IgE in smokers, which does not correlate with rises in specific IgE to common aeroallergens (53), remains, however, unclear.

In summing up, the relation between smoking and the integrated immune function remains obscure, particularly our understanding of how, or even whether these alterations play an important part in smoking-related skin diseases. One of the main difficulties in studying the immunology of smoking is how to move from the stage

of quantification of cells or other products and relate these changes to functional relevant alterations in immunological compartments (as opposed to irrelevant epiphenomena), and then link these alterations directly to specific diseases.

Finally, of particular interest is one of the most fascinating observations in this area (56), demonstrating that there are less smokers among acne sufferers than in control group. These differences are statistically significant ($p < 0.001$), and the conclusion to be drawn is that smoking exerts an anti-inflammatory effect on acne lesions.

This association between smoking and inflammatory diseases of the skin contributes significantly to our understanding of the inflammatory and immunologic response of the body to smoking. It provides one of the more impressive examples of the contribution of dermatology to the elucidation of immunologic processes and the effects of smoking.

Smoking and wound healing.

Surgeons have long noted a relationship between smoking and defective wound repair. Thus many surgeons, based on their clinical experience, routinely asked their patients to abstain from smoking during the immediate preoperative period (57). Smokers are at increased risk of wound dehiscence (58), of epidermolysis, and of other complications following surgery (59,60).

They have worse cosmetic results after surgery compared with non-smokers (61), have a higher incidence of skin-flap necrosis (62-64), and show poor healing of peripheral ulcers (65,66). These deleterious effects of smoking on wound healing have been demonstrated in animal models, as well (67-72).

There are many possible mechanisms by which smoking may affect wound healing. It seems, that a common end feature of several mechani-

sms is a decrease in tissue oxygen content or PO_2 . Wound healing, resistance to infection, collagen deposition, epithelialization and angiogenesis have all been shown to depend in part on the PO_2 content in tissue (73-79).

Smoking-induced decreased tissue oxygen may be mediated through several mechanisms: 1. Stimulation of sympathetic ganglia by nicotine that results in temporal peripheral vasoconstriction (80,81). Peripheral vasoconstriction, and decreased blood flow induced by the adrenergic effects of nicotine, contributes to the decreased tissue PO_2 .

Smoking for 10 minutes decreases blood flow and tissue oxygen tension for approximately 1 hour, therefore, a typical "pack-per-day" smoker experiences tissue hypoxia during a significant portion of each day (36, 82-84). 2. Reduced oxygen transport capacity of the blood, caused by carbon monoxide. Carbon monoxide constitutes up to 6% of cigarette smoke (85).

Carbon monoxide, which has an affinity for hemoglobin about 250 times greater than oxygen, not only reduces the oxygen transport capacity of blood, but carboxy-hemoglobin causes the remaining hemoglobin to bind oxygen with increased affinity, resulting in greater tissue hypoxia than reflected by the decrease in arterial PO_2 (85,86). 3. Hematological effects. Smoking increases plasma viscosity and fibrinogen levels. It reduces prostacycline activation, a potent inhibitor of platelet aggregation, reduces red cell deformability, bleeding time and plasminogen levels, all of which may contribute to a decrease in perfusion and oxygen supply of the subcutaneous tissue (87,88).

Finally, we would like to mention another interesting observation concerning a vascular reaction. McArthur and al. (89) found that smokers have increased incidence of palmar erythema. This is in agreement with our observation (unpublished).

Smoking and skin cancer.

Smoking has now been identified as a definite cause of cancer at many sites. Organs in direct contact with smoke — the oral cavity, esophagus, lung, and bronchus — are at the greatest risk of malignancy among smokers. As many as 90% of these cancers are attributable to smoking (90). Organs distant from smoke — the cervix, pancreas, bladder, kidney, stomach and hematopoietic tissue — are also at some increased risk (90).

Since the skin is in a very intensive contact with the smoke, and its carcinogens, one would expect an increased risk of skin cancer among smokers. Several well controlled studies have shown, however, that smoking does not appear to be a risk factor for developing of melanoma (91,92), mycosis fungoides (93), basal cell carcinoma (94-96), or squamous cell carcinoma of the skin (95,97) (not including the lips or oral cavity). Although not well-studied, Kargas (96) found an increased risk of squamous cell carcinoma, but not with BCC, associated with smoking. However, there are enough evidence indicating that smoking is not associated with an increased incidence of skin cancer.

An interesting observation by Rigel et al. (98) indicates that smoking does have an influence on the prognosis of melanoma. In a prospective study of 178 patients with malignant melanoma he found that smokers had two-years disease-free survival rates of 74.2% compared with 92.3% for the remaining patients. He suggested a smoking-related diminished host defense as a possible explanation for his finding. His results have, however, not been adjusted for other prognostic factors, in particular, tumor thickness, mitotic index and others.

Although Koh et al. (99) confirmed the results of Rigel et al., that smoking affects the prognosis and biologic behavior of malignant melanoma, they suggested a different explanation for this relationship. They found that patients with clinical Stage I melanoma who were heavy smokers presented with thicker lesions than

their nonsmoking counterparts ($P=0.037$). Whatever the mechanisms for this relationship might be, these studies indicate that, indeed, smoking plays a role in the biologic behavior of melanoma.

Concluding remarks.

Cigarette smoking, an uncommon behavior in 1900, increased at an epidemic pace throughout this century, peaking in 1964 when more than 40% of all adult Americans smoked. Since that time, smoking has decreased considerably. However, today 28% of all adults are still following Mark Twain's rules "not to smoke more than one cigar at a time... never to smoke when asleep, and never to refrain when awake (Mark Twain's Speeches, "Seventieth Birthday"). Cigarette smoking is now the chief avoidable cause of illness and death in the western world, responsible for one of every five deaths each year. "Tobacco surely was designed To poison, and destroy mankind" (Philip Freneau *Poems*, "Tobacco").

Although the deleterious effects of smoking on the skin pale into insignificance compared to the effects on other body systems, the range of damage caused to the skin by smoking cannot be ignored. The skin is directly and intensely exposed to the cigarette smoke, and its inhalation, and is also exposed to toxic substances reaching it via the bloodstream.

This review deals specifically with the effects of smoking on skin function and skin diseases, and has highlighted the many and varied effects of smoking on the skin. Although we have accumulated much information on this subject in recent years, we are only beginning to scratch the surface.

One of our failures, as dermatologists, is that most of the research has been directed at waging an anti-smoking campaign. There is no doubt that as doctors, that - should indeed be a prime goal, and our challenge is to work toward a smoke-free society, thereby reducing illness,

suffering, deaths and the health toll exacted by cigarette smoking. There is also no doubt that people are influenced by the deleterious effects of smoking on the skin — the premature aging, the wrinkling, and the esthetic damage — which provides us with very potent ammunition in the war against smoking.

Nevertheless, in spite of the above, it seems that too much effort has been expended in this aspect of the problem, and not enough into the dermatologic effects of smoking. For example, the association between premature aging of the skin and smoking should have been used to

learn about aging processes of the skin. The effects of smoking on the immunologic system in the skin can, and should be investigated, as should the effects of smoking on Langerhans cells and other system components. Other areas for investigation, for example, include the effects of smoking on the pathogenesis of various diseases such as psoriasis and acne.

Since we still live in a “smoking” world, and the dream of a smoke-free world is, unfortunately, a long way off, we can yet achieve some of those aims.

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A NEW TRANSDERMAL DELIVERY COSMETIC SYSTEM TO CONTROL THE SKIN LOCALIZED LIPODYSTROPHY.

P. MORGANTI ⁽¹⁾, L. CELLENO ⁽²⁾ AND A. VASSELLI ⁽³⁾

⁽¹⁾ Department of Dermatology, II University of Naples, School of Medicine and Surgery, Naples, Italy.

⁽²⁾ Department of Dermatology S. Cuore Catholic University of Rome, Italy.

⁽³⁾ Cosmetic Technical School S. Cuore Catholic University of Rome, Italy.

Received: November 10, 1994. Presented at the International Meeting of EADV "Skin therapy update '94" - Aghia Pelaghia, Crete, Grece, October 16, 1994.

Key Words: Cellulitis; Skin Lipodystrophy; Transdermal Delivery Cosmetic System; Centella Asiatica; Fucus Vesiculosus; 3C System Computerized Methodology.

Synopsis

Transdermal Delivery Cosmetic System (TDCS) is a combination of an occlusive adhesive and special active cosmetic compounds (ACC) on suitable cosmetic vehicle. Following the application, the ACC starts to migrate from the system through the skin, furnishing a constant active compounds concentration in the interested skin area for a prolonged period of time. The influence of the TDCS on skin pH and hydratation was recorded by the 3C System Computerized Methodology. The effects of natural extracts on localized skin lipodystrophy was controlled by an echographic methodology. The obtained results show a good transmigration efficacy of the ACC used from the TDCS to the underlying cutaneous layer (85%) after 6 hours of treatment, together with an aspected increase of skin pH and hydratation. The effect obtained on the localized skin lipodystrophy seems to suggest a high efficacy of this TDCS for the treatment of the "cellulitis" and its validity as new concept to treat such skin problems.

Riassunto

Questo nuovo mezzo per l'applicazione di principi attivi cosmetici denominato TDCS, è rappresentato dalla combinazione di uno speciale cerotto occlusivo sul quale viene spalmato un adeguato adesivo-veicolo cosmetico in grado di incorporare adatti principi attivi.

I principi attivi cosmetici applicati sulla cute mediante il TDCS trasmano nei diversi strati cutanei, ivi permanendo, per un prolungato periodo di tempo. Con questo studio si sono controllate sia le variazioni del pH che dell'idratazione cutanea, utilizzando la nuova metodica computerizzata 3C System. Gli effetti esplicati da estratti naturali sulla cellulite, sono stati verificati mediante l'utilizzazione di una metodica ecografia. I risultati ottenuti dimostrano una trasmissione molto elevata dei principi attivi dal veicolo ai sottostanti strati cutanei (85%), dopo le prime 6 ore di trattamento, assieme ad un incremento previsto sia del pH che dell'idratazione cutanea. Gli effetti ottenuti sulla lipodistrofia cutanea localizzata sembrano dimostrare una alta efficacia di questo TDSC se usato per il trattamento della cellulite.

INTRODUCTION

Transdermal Delivery Cosmetic System (TDCS) is a combination of an occlusive adhesive and special active cosmetic compounds (ACC) on suitable cosmetic vehicle. This new device may be topically and directly applied to the skin or to the mucous membranes providing a continuous infusion of the ACC used. Upon this topical application the ACC seems to migrate from the system through the skin, giving a constant active compounds concentration in the interested area for a prolonged period of time (1,2,3). The cosmetic activity can be stopped easily by just ending the skin contact. The aim of this study was to control the variations in thickness of hypodermis strata induced by local application of this new TDCS based on the activity of natural cosmetic compounds, having good skin uptake and minimal penetration into the capillaries.

MATERIALS AND METHODS

MATERIALS

The special acrylic base used for our devices

was enriched with *centella asiatica* and *fucus vesiculosus* extracts so that each plaster contained a total of 10 mg active compounds, 0,2 mg/cm² of which were *centella triterpenes* (4).

HPLC Varian Modello VLC 9010 Star System Work Station

METHOD

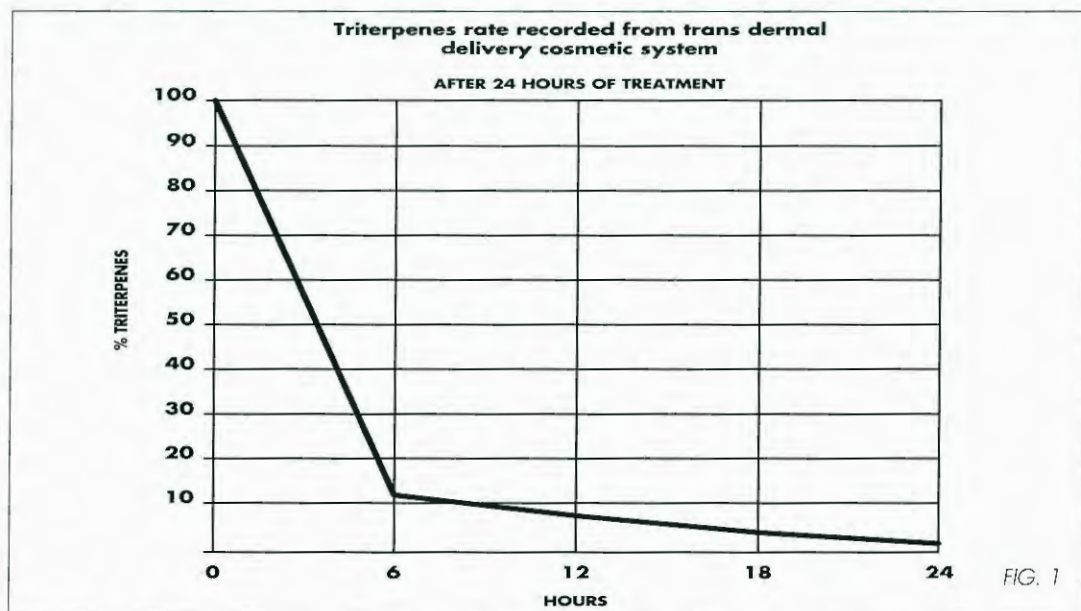
20 volunteers women ranged 27/42 years with visible sign of localized cellulitis applied the 44x57 or 50x72 cm² TDCS on the affected area for 30 days

ACTIVE COSMETIC COMPOUNDS CONTROL

Plasters devices were taken at 6,12,18 and 24 hours and analyzed by HPLC according to Morganti et al. (4) before and after the topical applications, to precisely verify any *centella triterpenes* content after their use. (Fig 1)

pH AND SKIN HYDRATION

Skin hydration and pH were monitored trough the



new 3C System Computerized equipment at day 0/1 and every 5 days. It records the values in direct redout, while environmental conditions are

automatically standardized (RH= 50% t=22°C) by the use of proper correction factors (5). Obtained results are reported in Figure 2-3.

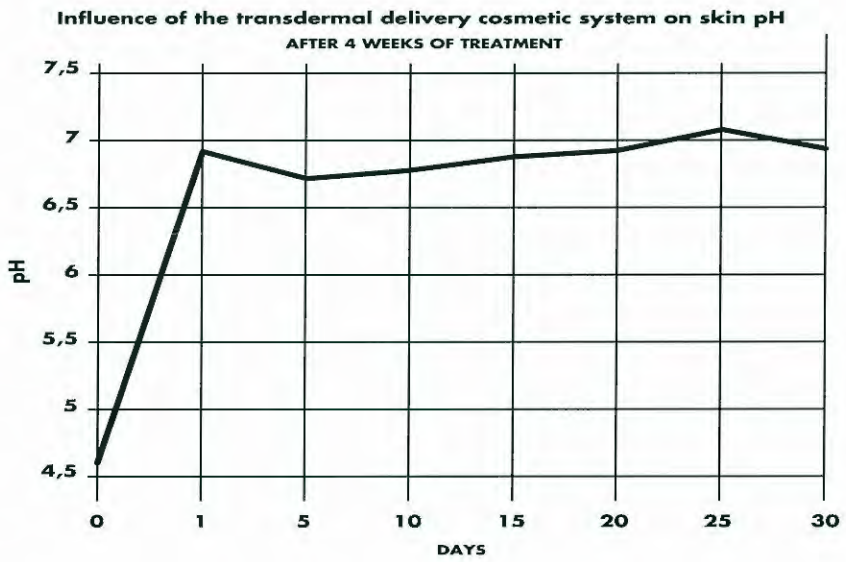


FIG. 2

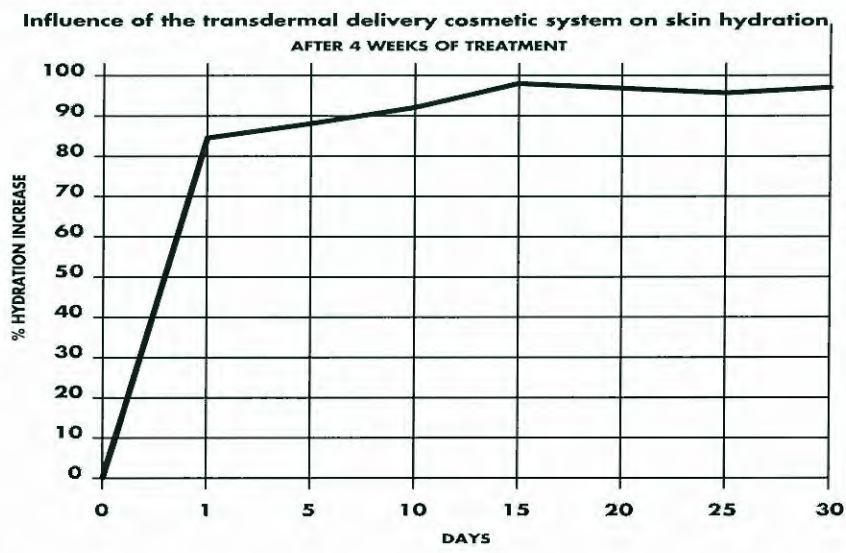


FIG. 3

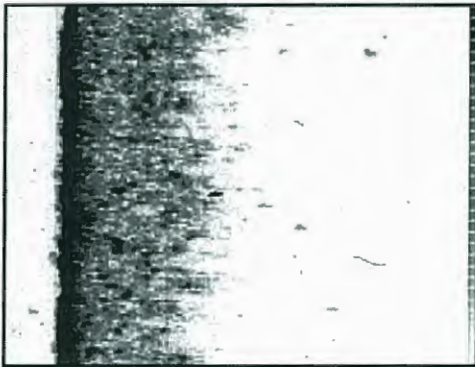
HIGH-FREQUENCY SONOGRAPHY MEASUREMENTS

Because of the limited results of some technique such as xenoradiography (6) and A-mode sonography (7) in measuring skin thickness, and according to Fornage et al. (8-9), the effects of the daily 30 day topical application of the TDCS used were evaluated by a high- frequency (20-

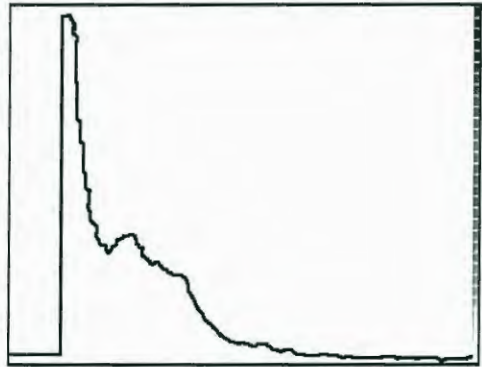
MHz) sonography equipment, providing us cross-sectional images of the skin with high resolution. With this device we were able to show the Dermal-hypodermal junctions and the modifications induced by treatment.

The obtained results are reported in Figure 4 and 5.

ECHOGRAPHY TAKEN AT THE BEGINNING OF THE TREATMENT ON CELLULITIS SKIN AREA (n=20)



B- MODE

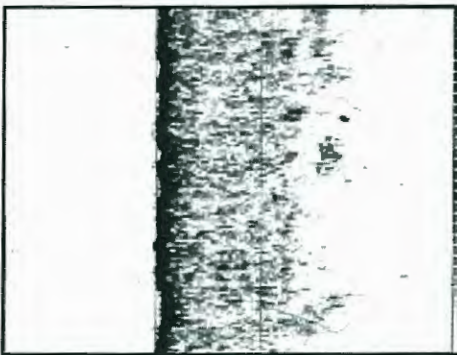


A- MODE

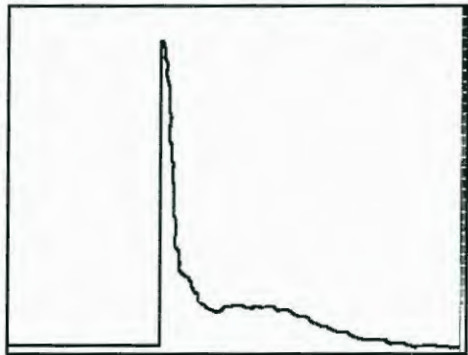
FIG. 4

ECHOGRAPHY TAKEN AT THE END OF THE TREATMENT ON THE CELLULITIS SKIN AREA

(n=20) (12 hours Daily Application x 30 Days)



B- MODE



A- MODE

FIG. 5

RESULTS AND COMMENTS

As shown in Figure 1 the triterpenes rate recorded at 6,12,18 and 24 hours of application was reduced from 88 to 98% on the average ($p < 0.001$).

On the basis of this result we may assume that there was an high trasmigration of our active compounds from the vehicle to the underlying cutaneous layers.

Obviously we don't know how much of our active compounds actually reached the hypodermis.

For this reason we are also carrying out further analitical survays on the various cutaneous layers in order to assess the indirect absorption indices of our active compounds. Local modifications in the configuration of the dermis and of dermal hypodermal junction can be seen by echography (Fig 4). Figure 4 shows the echography taken at the beginnig of the study. In the echography by B-mode it can be seen the treated area by normal ultrasound echography performed through a probe working at 20 MHz. In the A-mode it is shown the impedance obtained by the equipment in the same treated area.

The Figure 5 shows the same skin area at the end of the treatment.

The B-mode scanning demonstrates a reduction of the dermal oedema which results more omogeneous.

The A-mode shows very important modifications of the profile of the skin impedance, due in our opinion, to a reduction of the accumules of the subcutaneous fat and oedema phenomena. As shown from Figure 3 and in perfect agreement with the literature data, the skin hydration is increased by 85% to 97% due to the occlusiveness of the plaster used. pH reached mean value of 6.9 increasing by 1.4/1.5 units in 1 month treatment (Fig.2).

Furthermore to better investigate the effects of TDCS on cellulitis we undertaken a new study by normal echography which is able to reach the hypodermal tissue.

CONCLUSION

This new TDCS seems to represent a new cosmetic device which enables to achieve a sub-stained and constant action through the skin. Even if further studies have to be conducted to better understand and confirme the echography results, within short time application TDCS seems to represent an efficacious cosmeceutical therapy for the treatment of localized skin lipodistrophy, improperly called "cellulitis"

Corrispondence address:

Pierfrancesco Morganti Ph.D.
Via Innocenzo XI, 41
00165 - Rome - Italy

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SKIN AND AGEING: A MORPHO-SPECTROMETRIC STUDY

M.G. Tucci, G. Ricotti, * G. Biagini, ** P. Mengucci, *** L. Gobbi, G. Bossi

Clinica Dermatologica

* Istituto di Morfologia Umana Normale

** Istituto di Scienze Fisiche - Facoltà di Medicina e Chirurgia

*** Istituto di Scienze dei Materiali e della Terra - Facoltà di Ingegneria

Università degli Studi di Ancona

Received: October 11, 1994

Key-Words: Skin, Ageing, Chemical composition, X-ray spectroscopy

Synopsis

Ageing is a complex process influenced and determined by genetic, constitutional and environmental factors. In order to evaluate whether skin aging alterations are accompanied by a change in the chemical composition of the cutaneous tissue, we studied 8 patients. A sample of actinically damaged skin was taken from each patient. The specimens were analyzed under a Philips XL20 scanning electron microscope connected to an dispersive energy spectrometer. A semiquantitative analysis was carried out by x-ray spectroscopy considering only the value of $K\alpha$ and $L\alpha$ of each element and elaborating the data by ZAF correction method. The analysed elements were: Na, K, S, Ca, P, Zn, Mg. Our results showed that in old people there is a tendency to have elevated values for all the elements considered except for 3 parameters: P, Ca and Mg which result decreased. The work represent a new attempt to face the study of skin, from this analysis suggestive information emerged on the structural change in the aged skin which further underlines the importance of elements' microenvironment in senescence processes.

Riassunto

L'invecchiamento è un processo complesso che viene influenzato e determinato da fattori genetici, costituzionali ed ambientali. Per valutare se le modificazioni morfo-strutturali della cute senile si accompagnano ad una variazione della composizione chimica del tessuto cutaneo, è stato condotto uno studio mediante un microscopio elettronico a scansione collegato ad uno spettroscopio a raggi X. Gli elementi studiati con questa analisi semiquantitativa sono stati: Na, K, Ca, S, P, Zn, Mg, al fine di meglio comprendere l'importanza del microambiente nei processi di invecchiamento.

INTRODUCTION

Ageing is a complex process influenced and determined by genetic, constitutional and environmental factors. The majority of Authors agree on the importance of the prolonged action of external factors (such as solar exposition) in the ageing of the skin: the histological alterations in the areas exposed to the sun are much more evident even if they differ, at least partly, from those in areas not exposed.

In order to evaluate whether these aging alterations are accompanied by a change in the chemical composition of the cutaneous tissue, we studied 8 patients (4 aged under 30 years, 4 aged over 60 years)

MATERIALS AND METHODS

A sample of actinically damaged skin was taken from each patient. The specimens were fixed in 2% glutaraldehyde in 0.1% cacodilate buffer, dehydrated in ethanol concentration and subjected to a critical point drying using CO₂, set on suitable carbon-covered supports with a conductive glue and then covered in a 50 nm thick layer of carbon. The samples were studied using a Philips XL20 scanning electron microscope connected to an energy dispersive spectrometer. The semiquantitative analysis was carried out keeping certain parameters constant: voltage 25 kV, magnification 400x, spot size 1 nm, counting rate not under 2000 CPS (Counts Per Seconds), take off angle 15°, life time 600". Only the value of K α and L α of each element were considered and elaborated using the ZAF correction method which considers the atomic -Z number, the absorption-A and the fluorescence-F. For each patient two analyses were effected at the epidermal level, two at the superficial dermal level and two at the deep dermal level. The studied elements were: sodium, potassium, sulphur, phosphorus and calcium among the plastic primary elements and zinc and magnesium among the oligoelements.

RESULTS

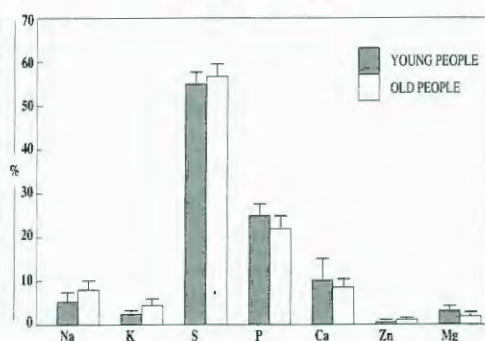
Table n°I

TOTAL VALUES OF THE SKIN

	YOUNG PEOPLE	OLD PEOPLE
Na	5.09 $\pm$ 2.10	8.25 $\pm$ 1.9
K	2.04 $\pm$ 0.64	4.08 $\pm$ 1.13
S	55.85 $\pm$ 3.32	57.58 $\pm$ 3.48
P	25.14 $\pm$ 2.58	21.41 $\pm$ 3.95
Ca	10.76 $\pm$ 5.12	9.32 $\pm$ 2.04
Zn	0.38 $\pm$ 0.08	0.60 $\pm$ 0.17
Mg	2.45 $\pm$ 1.00	1.46 $\pm$ 0.74

Table n°II

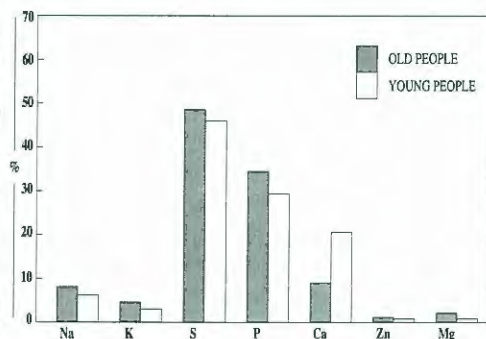
PER CENT COMPOSITION RELATED TO THE SKIN (MEAN VALUE AND STANDARD ERROR)



The data reported in table n. I and in table n. II show that in old people there is a tendency to have elevated values for all the elements evaluated, except for 3 parameters: phosphorus, calcium, and magnesium which result decreased. Table n. III, IV, V show the values obtained for single parameters in young and old people respectively at the epidermal level, superficial dermal level and deep dermal level.

Table n°III

PER CENT COMPOSITION RELATED TO THE EPITHELIAL LEVEL
(MEAN VALUE)



Studing table n. III we notice that in aged people there is an increase of Na, K, S, P, Zn, Mg and a decrease of Ca at the epidermal level.

The concentration of elements found at the superficial dermal level shows the same trend noticed before: the values obtained in old people are higher except for S and P which decreased table n.IV), while P and Mg are decreased in the deep dermal level of aged patients (table n.V).

Table n°IV

PER CENT COMPOSITION RELATED TO SUPERFICIAL DERMAL LEVEL
(MEAN VALUE)

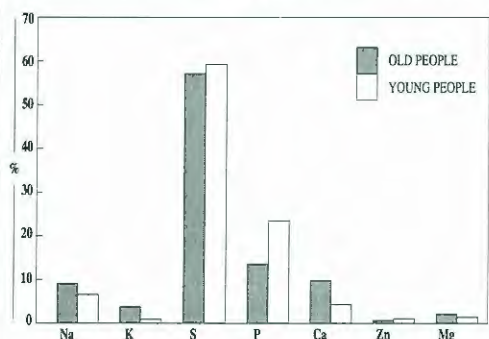
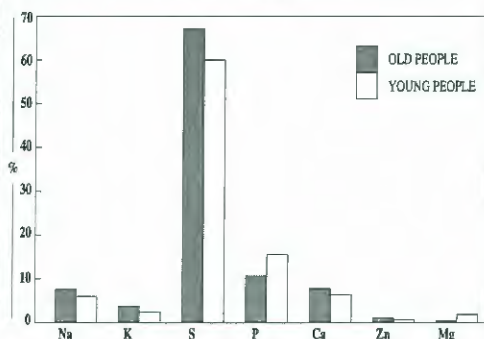


Table n°V

PER CENT COMPOSITION RELATED TO DEEP DERMAL LEVEL
(MEAN VALUE)



DISCUSSION

As far as morphology and the deepness of skin in young and old people is concerned, some Authors found as the epidermis of old people exhibits a marked variation in thickness and a disparity in the size, shape and staining quality of the basal cell nuclei.

There is a loss of the orderly alignment of cells along the basement membrane and a disruption of the gradual upward uniform differentiation present in the epidermis from younger individuals (1). The dermis of aged people seem thinner and present a lower number of cells. Despite substantial anatomic, biochemical and biomechanical studies that many Authors have done, we are still far from a consensus regarding age-associated alterations in the dermis (1).

As far as the study of the skin from a chemical point of view is concerned the decrease in total water content has always been considered one of the most important features of aged skin.

Certain analysis carried out by various Authors using RMN *in vivo*, have however shown an increase in free water in the aged skin, especially at the superior dermal level (2, 3). Moreover these Authors noticed an increase in total water contents in aged skin and a decrease of collagen amount and of proteoglycans. This last datum

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Chiuso in tipografia: 24 March 1995

Journal of Applied Cosmetology published quarterly by INTERNATIONAL EDIEMME, Via Innocenzo XI, 41 00165 Roma Italy. Direttore responsabile P. Morganti. Direzione, Redazione ed Amministrazione Via Innocenzo XI, 41 - 00165 Roma Italy. Stampa Grafica Flaminia Roma. Progetto grafico ed impaginazione STYLOgrafica Roma. Spedizione in abbonamento postale gruppo IV/70 Aut. del Trib. di Roma n. 3173/83 del 8-7-83

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