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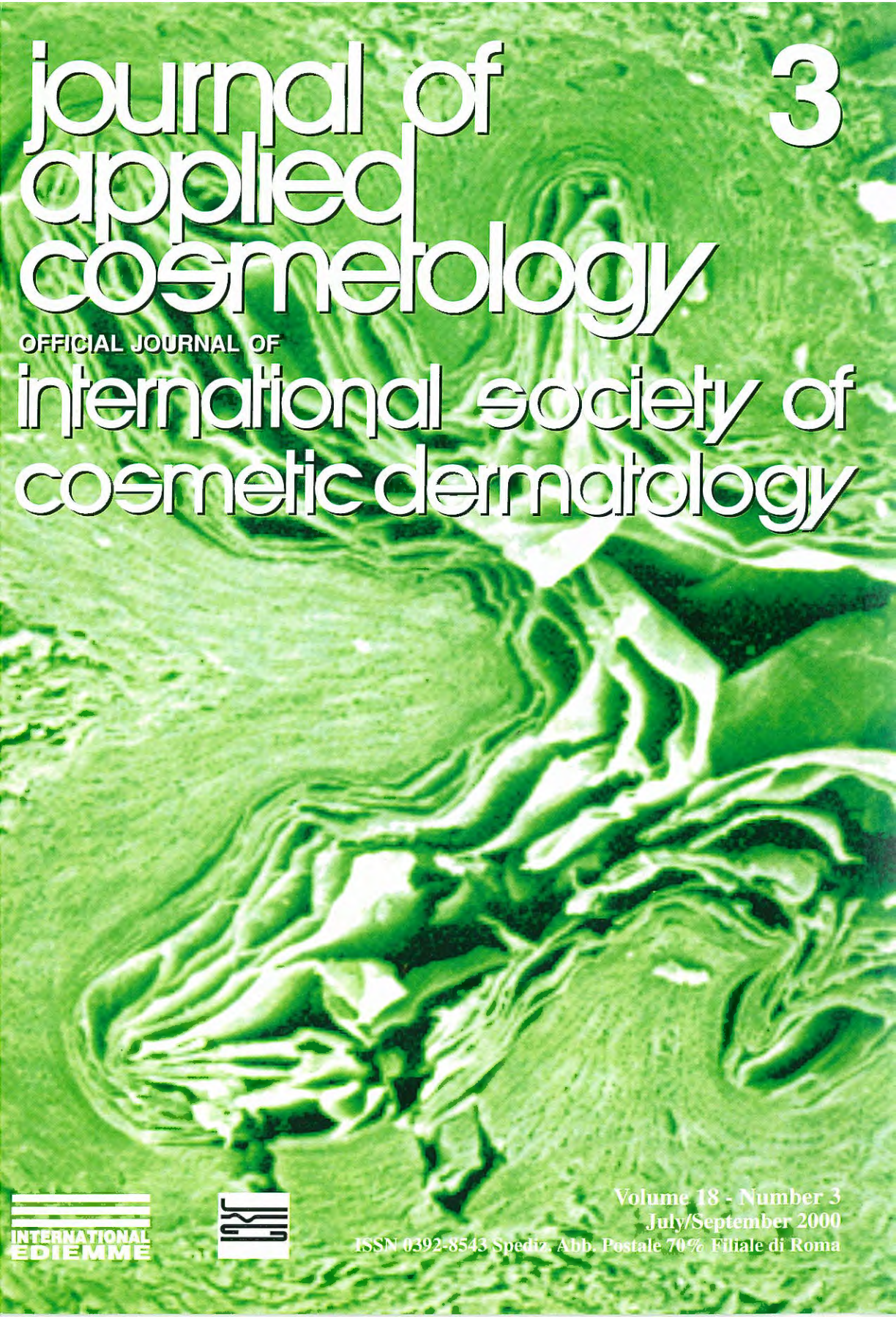

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July/September 2000
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The **scientific approach** to [AGE-SPOTS] (1-4)

UNA SOLUZIONE [chiara] per le iperpigmentazioni (1-4

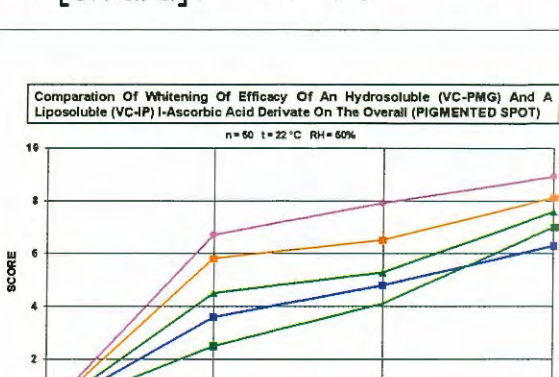

Fig. 1

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1) MATOBA O., HASC, MOTOS, KOHATA Y., et al (1999), A new lipofhilic l-ascorbic acid derivative, the synthesis, physical property and dermatological efficacy. Proceedings of 4th scientifica Conference of the Asian Societes of Cosmetic Scientists, Bali, Indonesia, 7-8 April, 1999.
2) KEMEYAMA k., SAKAC, KONDOH S., et al (1996), Inhibitory effect of magnesium l-ascorbiyl-2 phosfate (VC-PMG) on melanogenesis in vitro and in vivo, J. Am, Acad.Dermatol.,34,29-33.
3) P. MORGANTI, FABRIZI G., MORGANTI G. (1999), an innovative cosmeceutical with a skin lwhitening activity, Presented at the 4th International symposium on Cosmetic Efficacy, New York, May 10-12. In print on J. Appl. Cosmetol.
4) MORGANTI P., FABRIZI G., MORGANTI G., (1999) A new cosmeceutical with a skin lightening activity: Second note, The combining whitening activity of hidrosoluble and liposoluble vitamin C derivatives.
Presented at the E.A.D.V.-Congress, September 29-October 3, 1999 (Fig.1)

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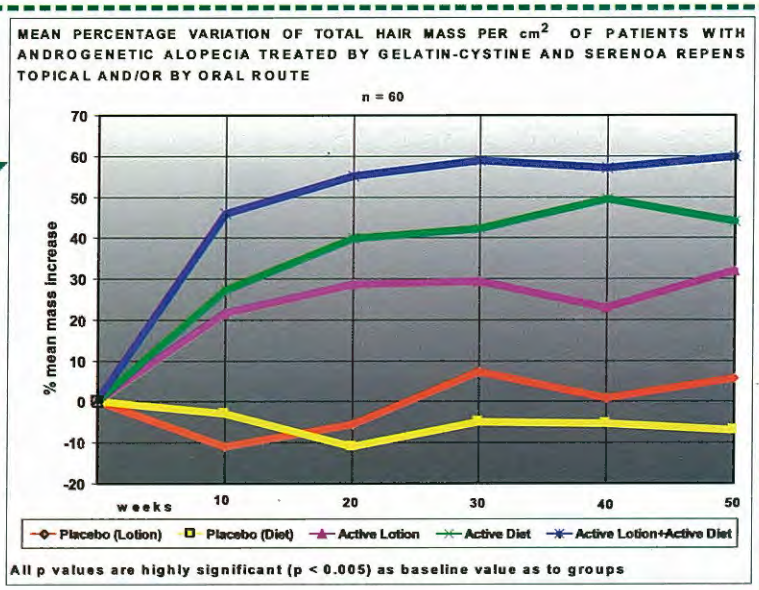
Clinical studies established efficacy with mild to moderate hair loss in the frontal - parietal scalp[1]

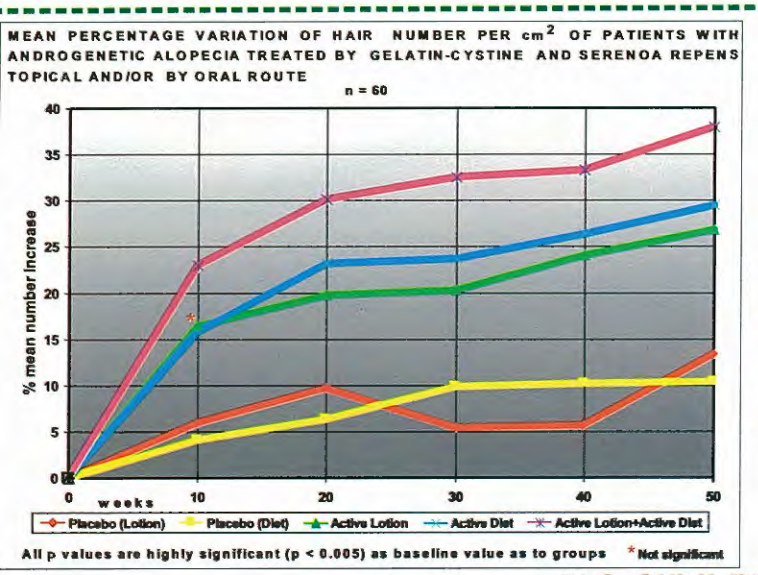
Increases mass and numbers of hair in a short period[1]

BIOESSE



60%

increase in hair mass



38%

increase in hair number

NO SIDE EFFECT WAS RECORDED

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1) Morganti P., Fabrizi G., James B., Bruno C. *J. Appl. Cosmetol.* **16**,57,1998

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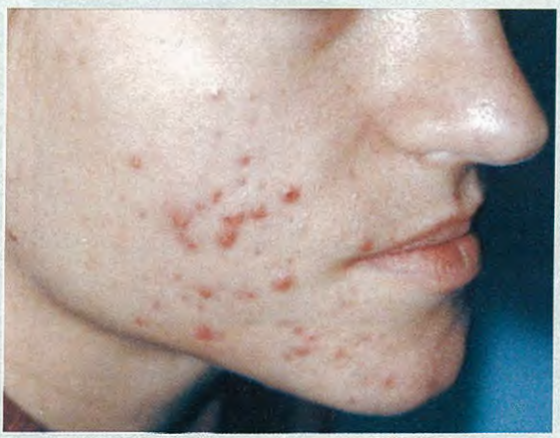

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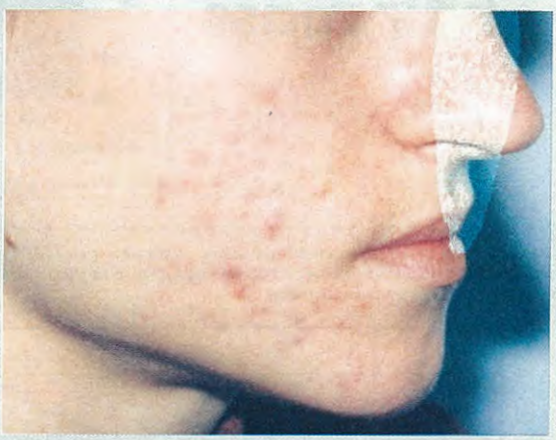

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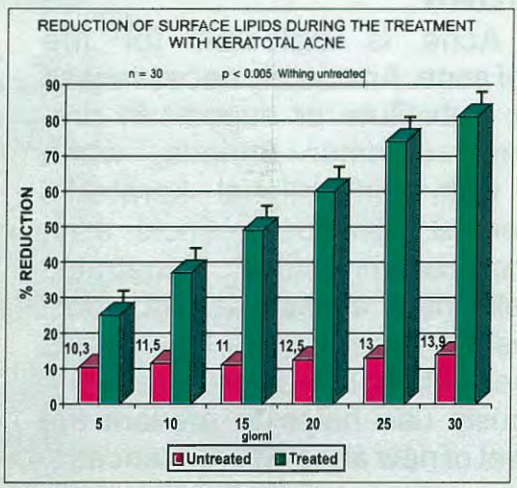

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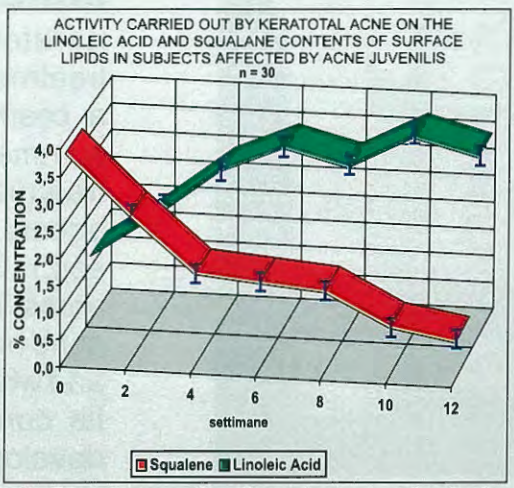

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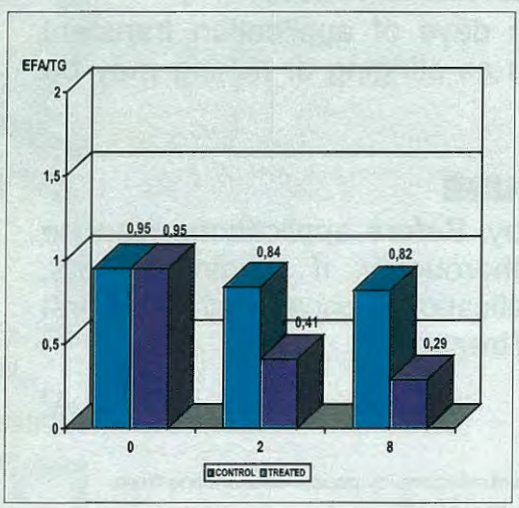

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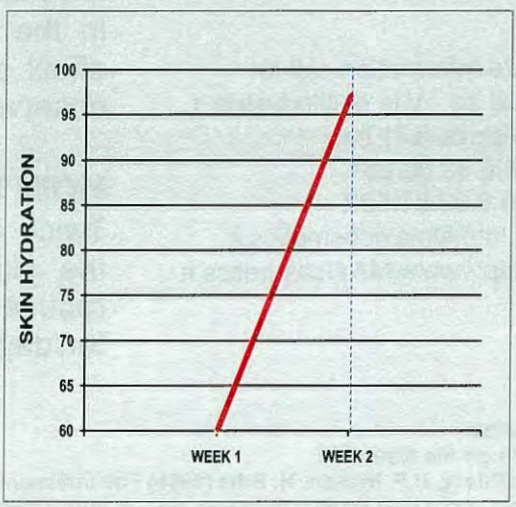

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HOW TO USE

Twice a day. Before applications cleanse the skin thoroughly; if stinging occurs, reduce application to once a day for the first ten days of treatment

REFERENCES:
*1,2 - **Data on file Mavi Sud***
3 - **M. Ghiczy, H.P. Nissen, H. Biltz (1996)** The treatment of Acne Vulgaris by phosphatidilcholine from Soybeans, with a high content of linoleic acid. *J. Appl. Cosmetol.* **14,** 137-145

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2) Strehler BL (1977) Time, cells and aging 2nd edn. Academic Press, New York
3) Ebling FJ, Rook (1972) Ciclic activity of the follicle. In: Textbook of dermatology 11, Blackwell, Oxford, p. 1567-1573.

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Trimestrale di Dermatologia Cosmetologica
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Contents

J. Appl. Cosmetol. 18, 105-111 (July/September 2000)

COSMETIC EFFICIENCY ASSESSMENT THROUGH CONFOCAL LASER SCANNING MICROSCOPY

S. MAKKI[1,2], A. ETTALEB[1], J. MILLET[2], P. HUMBERT[1]
[1] Laboratory of Engineering and Cutaneous Biology
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Faculty of Medicine and Pharmacy
25030 Besançon - France

Received: *July 2000. Presented at the 13th International Congress of the Society of Bioengineering and skin, Jerusalem, March 26-30, 2000*

Key words: *skin microtopography, confocal microscopy, visualization, assessment, cosmetic efficiency.*

Summary

Nowadays, the determination of human skin microtopography is currently curried out by profilometric and surfometric methods which are based on mechanical or optical conceptions. The skin microstructure assessment is performed through a skin replicas made of silicone rubber (Silflo®) or with cast in epoxy resin (Araldite®). Profilometric and surfometric methods allow to quantify skin relief by using parameters such as Rtm, Sm ... Scanning electron microscopy shows images and gives qualitative information.

In the other hand the confocal scanning microscope (CLSM), gives simultaneously images of the skin surface associated with quantitative measurements of the microtopography. With this apparatus, it is easy to perform a skin images with an assessment of the microstructure before and after applications of topical medicine or cosmetic for evaluating their efficiency. It is possible to work in vitro by using replicas, or in vivo, directly on the skin surface. The major inconvenient of this technique is a limited area to explore (3-5 plateaus). We are progressing in solving this problem by using a special device for increasing the studied surface. Moreover, it is indispensable to study the same area (same plateaus and same furrows). For this reason it is necessary to localize exactly the same area (1.2 x 2mm) before and after a product final application, whatever the period extend of treatment.

Riassunto

La determinazione della microtopografia della cute umana viene normalmente effettuata mediante l'utilizzazione di metodologie meccaniche (profilometria) o ottiche (surfometria).

La microstruttura della pelle viene replicata con l'utilizzazione di resine siliconiche (Siflo®) o epossidiche (Araldite®). Per ottenere valori quantitativi è necessario utilizzare assieme a queste tecniche, la microscopia elettronica a scansione.

Con la microscopia a scansione confocale (CLSM) è possibile invece ottenere contemporaneamente sia le immagini che i valori quantitativi sia "in vitro" che "in vivo". Il vero inconveniente di questa

metodica è che si possono controllare soltanto piccole aree. Il nostro gruppo di lavoro sta cercando di risolvere questo problema.
E' però indispensabile che le aree controllate prima e dopo il trattamento siano sempre rigidamente le stesse, qualunque sia la durata del trattamento utilizzato.

INTRODUCTION

Cosmetics are daily used by a large number of people of different ages. For this reason, it seems necessary to control the efficiency of these products and to verify their attributed good characters as skin smoothness, skin hydration, etc... In addition, it is important to protect the consumers from false and misleading or exaggerated claimed qualities related to some cosmetic products. A cosmetic efficacy is credible by the determination of the skin restoration after a cosmetic topical application during the indicated period of time.

The aim of this work was to establish a simple technique for a precise location of the skin surface to study, followed by visualization and assessment of the skin microtopographical relief after a cosmetic application. Using a replica technique associated with a confocal laser scanning microscopy, a simultaneous imaging and a quantitative determination of the human skin microtopography were performed.

MATERIALS AND METHODS

1. Replication of the skin surface

An impression of the skin microstructure was taken by using a silicone rubber material (Silflo®, Flexico Development Ltd, London, GB). The area to be replicated was shaved and exactly localised. Two ml of the fluid impression material, mixed with 3-5 drops of a specific catalyst, was applied on the skin surface of the chosen area. A glass microscope slide was placed onto the unset material in order to provide uniform thickness, and to facilitate the removal of the skin surface replica (Makki et al, 1979).

2. The confocal laser scanning microscope (CLSM) (Figure 1)

The CLSM employed in this work was the TCS 4D of LEICA (Paris-Lyon). It has a resolution power of 0,2 µm in the plane X/Y and a 0,4 µm in the Z axis for a wavelength of 488 nm. This apparatus offers a good bi-dimensional image in the two axis X and Y and in the three axis X,Y and Z, giving a three dimensional representation.

The specimens are illuminated through imaging lenses. A diffraction limited light spot is produced within the sample with a beam source (a). A pinhole (b) focuses the spot. Re-emitted light is deflected by a beam splitter (c) and is detected by a photomultiplier (d). In order to produce an image, the light point is moved in X and Y directions by a mirror driven by two galvanometers over a defined area of the sample. A high precision focusing stage and a computer allow the user to produce whole series of sectional images and store informations on optical disks. Topographic measurements, as the distance between two points or the width of plateaus surfaces and the depth of furrows, can be easily calculated. (Corcuff and Lévêque, 1993 ; Rajadhyaksha et al, 1995 ; Sheppard and Shotton, 1995 ; Paddock, 1999).

EXPERIMENTAL

1. Location of the skin surface (Figures 2a, 2b)

A simple accurate marking method, using a special marker, was performed for indicating easily the skin surface area to replicate and to reproduce exactly, after 60 days and even more.

2. Procedure

In this work, the skin surface studied was an area near the right eye of a volunteer female, 45 year-old. The skin surface was replicated with Silflo® before applying a moisturizer (c). Replicas were taken from the same skin surface. After one month of the cosmetic product (c) daily

application on the replicated surfaces, according to the instructions written on the cosmetic bottle, the replicas were analyzed qualitatively and quantitatively through the CLSM. The apparatus moves the sample from the top reference to the bottom reference which have been previously manually fixed and memorized each point of the surface. The depths of furrows were calculated by a computer program, sectioning optically the image from the top to the bottom, in ten layers. The distance between two points or two lines (furrows) could be measured by the same program. The acquisition was automatically performed. A clear image could be given by the CLSM of the skin surface representing 4-5 plateaus, separated by crossing furrows.

RESULTS

The surface of the area (plateaus and furrows) treated by the cosmetic was relocated easily after 30 days. Photographs of the skin surface replicas were taken, with the CLSM, before and after the moisturizer application. The restoration of the skin surface was clearly noticeable from the images. There was a diminution of furrows (A,B,C,D) depths and the surface profile became smoother (Figure 3a, 3b). This was confirmed by the measurements of furrows depths before and after the product application (Table I).

DISCUSSION

The results of this work demonstrate a technique which assures a good location of the surface to study (Figures 2a, 2b). There was a replication of the same surface area after 30 days of a cosmetic application which proves the accuracy of the localization method. Our study was carried out on skin surface negative replicas which assured an economy of time and money. When profilometry, surfometry and scanning electron microscopy are employed for determining skin microtopography, skin positive replicas are necessary. The surface analysis with the CLSM gives the possibility to have an image of the skin microtopography associated simultaneously with measurements of furrows depths and plateaus widths (Figure 3). This apparatus has a remarkable advantage compared to the scanning electron microscope which gives skin surface images of very good quality, but unfortunately, without measurements.

From the present results, it is clear that the control of a cosmetic efficiency can be performed perfectly by the CLSM. The moisturizer employed in this work showed its efficacy by decreasing the depths of furrows (Table I). A noticeable difference could be observed between the profiles of surfaces before and after the product application (Figure 3a, 3b, Table 1). The skin surface became smoother.

It is important to mention that there are some essential aspects to consider :

- The surface area studied was not very large (3-4 plateaus). Thus, it is advisable to increase the surface analyzed by the CLSM.
- The exact location of the area (same plateaus, same furrows) is absolutely necessary, otherwise the visualization and the assessment of another area have no interest and give false results.
- It is difficult to compare the results of this work with our previous studies in this field (Makki et al., 1979 ; 1987), unless the same quantitative parameters (Rtm : average of furrows depths ; Sm : average of plateaus widths) were employed. For this reason, it is necessary to exert in this technique, the same parameters of profilometry and surfometry. Then, we can be able to compare the different methods and to use the most sensitive one.

Table I *Furrows depths measurements before and after 30 days of a daily cosmetic (C) application*

Furrows (10 measurements)	Furrows depth (µm)	
	D0 (x ± S.D.)	D30 (S.D.) (x ± S.D.)
A	80 ± 5	30 ± 1
B	60 ± 3	10 ± 1
C	40 ± 2	10 ± 1
D	80 ± 3	50 ± 1

Each furrow was measured 10 times

D0 The day before cosmetic (c) application

D30 After 30 days of the cosmetic (c) daily application

x The mean

S.D. Standard deviation

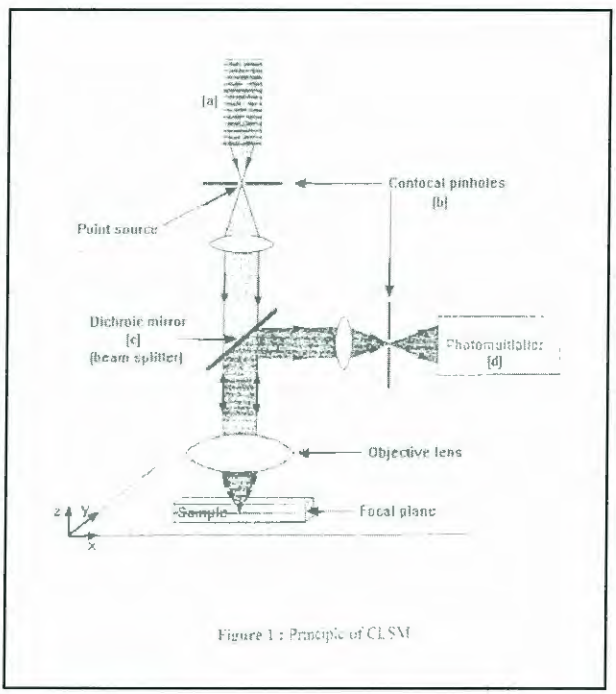
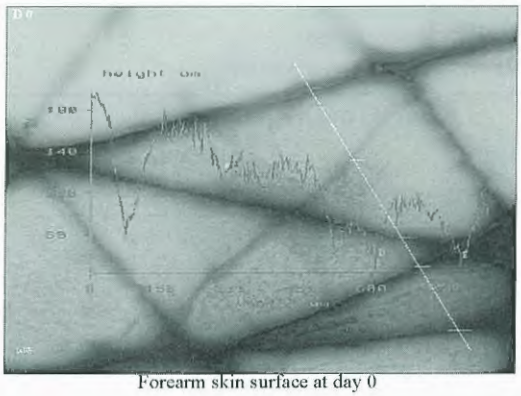
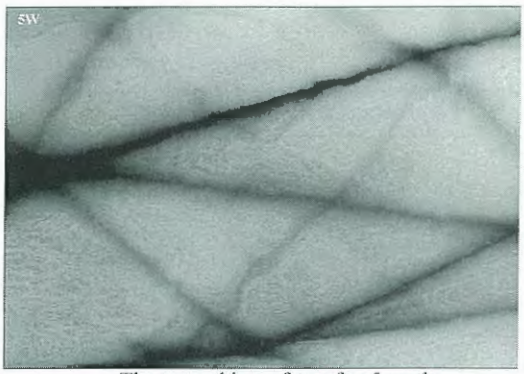


Figure 1

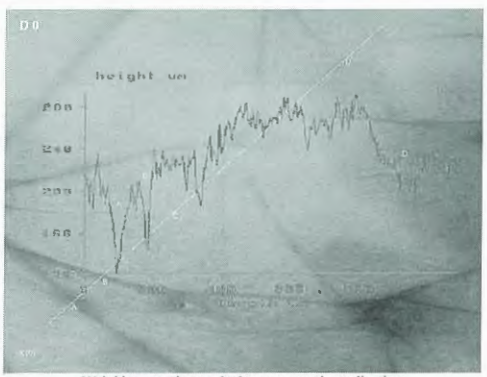


Forearm skin surface at day 0

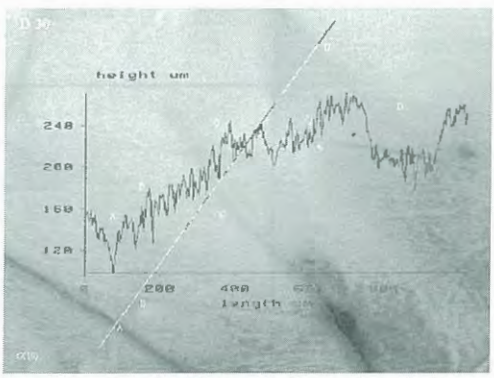


The same skin surface after 5 weeks

Figure 2 Skin surface localised the first day (D0) and 5 weeks after (5W)



Wrinkles near the eye **before** a cosmetic application



The same skin surface **30 days after** the cosmetic application

Figure 3 Wrinkles near to the eye before and after application of a cosmetic (c)

3a Before treatment (D0) : the surface profile is showing different incidents and furrows are noticeable.

3b The same eye wrinkles after 30 days of the daily cosmetic (c) application : the skin surface profile becomes smoother and furrows are narrower and tight.

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SKIN LIPID ABNORMALITIES IN GAUCHER'S DISEASE

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Summary

In the epidermis of terrestrial vertebrates stratum corneum lipids are known to play an important role as regulators of skin permeability; particularly ceramides, some of which with very long chain, hydroxy fatty acids, are important components of this "barrier" function. Ceramides are derived from the hydrolysis of previous existing glucosylceramide during the terminal stages of epidermal differentiation, due to the presence of a lisosomal-like glucosylceramidase localised in the lamellar bodies in the space between stratum granulosum and the inferior layer of stratum corneum: the deficiency of glucosylceramidase, localized in lysosome, is responsible of Gaucher's disease.
In this study we investigated the glycolipids and ceramide distribution in the epidermis of 4 Gaucher's patients (two of type 1 and two of type 3), compaired with the same parameters in 4 healthy donors.
The comparison between the pathological samples and the healthy ones, indicates an impairment of the degradation of glucosylceramide to ceramide in the lamellar bodies of Gaucher's patients stratum corneum, as shown by the high percentage of the former (range: 48-69% of the total glycolipids), and the complete absence of the latter.
We are now evaluating the usefulness of topical therapy (in addition to the replacement therapy with Ceradase) with the use of a lotion containing commercially available 3 hydroxy ceramide, which fatty acids composition is closely related to that of the main ceramide present in human skin.

Riassunto

Nell'epidermide dei vertebrati terrestri i lipidi dello strato corneo giocano un ruolo importante nella regolazione della permeabilità della pelle; in particolare le ceramidi, alcune delle quali contengono acidi grassi idrossilati a lunga catena, sono elementi importanti della funzione "barriera". Esse derivano dall'idrolisi di preesistenti glucosilceramidi durante le fasi terminali della differenziazione dell'epidermide, dovuta alla presenza di una glucosilceramidasi localizzata nei corpi lamellari presenti nello spazio tra lo strato granuloso e il margine inferiore dello strato corneo: il deficit di glucosilceramidasi, localizzata nei lisosomi, è responsabile della malattia di Gaucher.
In questo studio abbiamo analizzato la distribuzione dei glicolipidi e delle ceramidi nell'epidermide di 4 pazienti affetti dalla malattia di Gaucher (2 del tipo 1 e 2 del tipo 3), confrontati con gli stessi parametri di 4 donatori sani.

Il confronto tra i campioni prelevati da soggetti malati e soggetti sani, rivela uno squilibrio nella degradazione del glucosilceramide a ceramide nei corpi lamellari dello strato corneo dei pazienti affetti da Gaucher, come dimostrato dall'alta percentuale dei primi (dal 48 al 69% dei glicolipidi totali) e dalla completa assenza delle ceramidi.
Stiamo adesso valutando gli eventuali vantaggi ottenibili con una terapia topica (in aggiunta alla terapia sostitutiva con Ceradase) basata sull'uso di una lozione contenente 3-idrossiceramide la cui composizione in acidi grassi è assai simile a quella della principale specie ceramidica presente nella pelle umana.

INTRODUCTION

Lipids are considered to play an important role in the structure, differentiation and function of the epidermis.

During the process of keratinisation and epidermal differentiation the lipid composition of the skin changes dramatically (1,2). These changes are consistent with the requirement for cutaneous waterproofing (2-5).

In this view, many reports have shown the importance of sphingolipids in maintaining the optimal mammalian epidermal permeability barrier function (6-11), and solvent extraction studies have shown that the progressive removal of sphingolipids, rather than non-polar lipids, is associated with proportional abnormalities in barrier function (11).

Stratum corneum lipids major constituent, ceramide, has been shown to be associated to both water retaining capacity of the skin and permeability barrier (10-13); ceramide is thought to derive primarily from glucosylceramide which is practically absent in the exterior layer of the stratum corneum. This suggests the presence in the stratum corneum of hydrolytic enzymes; in fact, data from the literature show that a number of catabolic enzymes, such as sphingomielinase, triacylglycerol hydrolase (14), phospholipase A (15), steroid sulphatase (16), and b-glycosidase (17-19) are localised in the lower part of the horny layer.

Together all these data lead to the hypothesis that the hydrolysis of glucosylceramide to ceramide plays an important role in the formation and the maintenance of epidermal permeability barrier (12). This hydrolizing steps however, is only one aspect of the complex process by which the permeability barrier is build-up. Other possible biochemical events associated with stratum corneum barrier formation might be the hydrolysis of sphingomyelin by sphingomyelinase and/or the breakdown of phospholipids by a variety of phospholipase.

For these reasons we analysed the distribution of glycosphingolipids in the stratum corneum of 4 skin biopsies from patients affected by Gaucher's disease, the commonest lysosomal storage disorder, characterised by an accumulation of glucosylceramide in the reticuloendothelial system and, in the neuronophatic forms, in the central nervous system.

Three phenotypes of Gaucher's disease are recognised: type 1, without neurological involvement; type 2, with acute neurological involvement and lethal within the first 2 years of life; type 3, with chronic neurological involvement.

The association of cutaneous abnormalities with Gaucher's disease is still a controversial problem. Skin manifestations are reported to be infrequent and non-specific, such as diffuse brown or yellow-brown pigmentation and easy tanning (20). Hyperpigmentation is probably due to a greater deposit of melanin; infact iron metabolic alterations characteristic of the disease may interact with tyrosinase activity (19,20,21,22,23).

On the other hand cutaneous alterations are particularly evident in Gaucher's disease type 2; this phenotype causes death after few days or months from the birth and shows the characteristic symptoms of "collodion baby" such as ectropon, eclabion, petechias, purpura, and bright and tight skin.

Purpura, ecchimosys, telangiectasia, pallor and sometimes a yellow skin colour can be caused by hepatic invasion of Gaucher's cells (20,21), but it is not at all clear that a cause - and - effect relationship exists between the glycolipids storage in different organ and the cutaneous disorder.

The histological examination of the cutaneous biopsy in these patients has shown the presence of exfoliative lamellar ichtyosis with normal derma and epidermis, but with a great thickening of stratum corneum and follicular hyperkeratosis.

A female type 1 Gaucher's disease patient, dia-

gnosed when 50 years old, had thickened, hardened and xerotic skin; palms of hands and soles had hyperkeratosis and rhagades; on the contrary unguis modifications were not noticed; hair appears dry without dandruff. A biopsy on the right forearm with punch 4.0, showed little thickened skin, without the ichtyosic desquamation such as seen in type II (Celleno L. and Melchiorre M. personal communication).

MATERIALS AND METHODS

PATIENTS AND CONTROLS

The skin biopsies were taken from patients with type 1 and type 3 Gaucher's disease under enzyme replacement therapy with Ceradase and were kindly provided by Dr. B. Bembi (C.H. "Burlo Garofalo" and Institute of Pediatrics, University of Trieste); the skin from healthy controls were surgically removed from a 52, a 54 and a 67 years old women abdomen and a 52 years old woman arm.
Gaucher patients' features in terms of age, type, severity of the disease and period of therapy are reported in Table I.

REAGENTS

Commercial chemicals were of the highest available grade; Silicic acid (200-400 mesh) for column chromatography, standard neutral glycolipids and ceramides for thin-layer-chromatography were from Sigma Chemical Co. (St. Louis, USA); Silica gel plates (Kieselgel 60, HPTLC 10x10) were from Merck GmbH (Darmstadt).

LIPID EXTRACTION AND PURIFICATION

The lipids were extracted according to the method of Brunngraber E.G. et al. (24).
Briefly, each sample was handly accurately homogenised in a mixture of phosphate buffer (10mM, pH 6.8) / tetrahydrofuran (THF) 1:8. After centrifugation, at 10000 rpm for 10 min., the pellet was extracted three more times with the same solvent mixture in a different ratio (phosphate buffer / THF 1:4).
The 4 supernatants collected after each centrifugation were pooled and partitioned by addition of diethilether (1/3 of the total volume). The mixture was gently shaken and centrifuged at 2000 rpm for 10 min.; the lower phase was removed; to the remaining upper phase, 1/10 of the total volume of water was added and the mixture was centrifuged again in the same conditions. The two lower phases were collected to-

Table I Patients' features

NAME	AGE (y[a])	GAUCHER TYPE	TIME OF THERAPY (y[a])
R.L.	20	3	1
F.A.	28	1	_
P.M.	10	3	3
S.M.	33	1	_

[a]: years
_ / : not yet in therapy with Ceradase.

gether and were referred to as "aqueous phase". The remaining organic phase was dried under vacuum (Rotavapor Büchi 461 Water Bath, Switzerland) at room temperature, resuspended with 2-3 ml of chloroform and applied to silicic acid (previously activated for 12 hr at 80°C) chromatographic column (1x10 cm). The column was conditioned with 50 ml of chloroform before loading the sample (25).

A previous passage with chloroform, in order to elute cholesterol and neutral lipids, was performed. The column was than eluted with two different kinds of solvent mixtures: chloroform / methanol 95:5 (v/v, 100 ml), which eluted ceramides, and acetone / methanol 9:1 (v/v, 100 ml) which eluted neutral glycolipids and sulphatides. Each fraction was collected and taken to dryness under vacuum: the ceramide fraction was then resuspended in 2-3 ml of chloroform / methanol 2:1; the second fraction was dissolved in 2-3 ml of chloroform.

To obtain a complete purification, an appropriate aliquot (1 ml) of the neutral glycolipid fraction was hydrolysed in methanol / NaOH 0.6N, at 37°C for 1 h. After hydrolysis, the sample was partitioned with 1.2 ml methanol / HCl 0.5N, 1.7 ml bidistilled water and 3.4 ml chloroform, vortexed and centrifuged at 2000 rpm for 10 min. The upper phase was eliminated; the lower phase was rinsed twice with 4.5 ml methanol / bidistilled water 1:1, discarding the upper phase each time. The lower phase was taken to dryness and resuspended in 2-3 ml of chloroform / methanol 2:1.

The qualitative and quantitative percentage distribution of each class of neutral glycolipids was achieved by high performance thin layer chromatography (HPTLC) on 10x10 cm silica gel plate previously activated at 120°C for 1 hr. The developing solvent system was: chloroform / methanol / water (110:40:6, by vol., running time: 40 min) and the visualising reagent was the diphenylamine spray (0.25g of diphenylamine in aniline / acetone / ortophosphoric acid, 0.25:25:2.5, by vol.). After spraying, the plate was heated at 120°C for 20 min.

The densitometric analysis was performed with a Camag II TLC Scanner (l = 660nm), connected to a Shimadzu C-R 3A Chromatopak system; the concentration of each compound was determined using a standard calibration curve, obtained spotting pure reference standards in different amounts.

The quali-quantitative evaluation of ceramides was also achieved by HPTLC. Plate was activated as above described. Developing solvent system: chloroform / methanol / water (95:5:0.5, by vol.). The compounds were then visualised by spraying the plate with a mixture of anisaldehyde / acetic acid / sulphuric acid (0.25:25:0.5, by vol.). After spraying, the plate was heated at 120°C for 10 min.

The densitometric analysis was performed at 610nm; the concentration of each compound was determined as above described.

RESULTS

Tables II and III, respectively, show our results concerning the content of each neutral glycolipid, as µg/mg of fresh tissue and their percentage of the total. Total glycolipids as well as glucosilceramide amount are higher in patients as compared to control simples (Table II).

Glucosylceramide was the most remarkable neutral glycolipid in all of our samples, both as indicated by its absolute amount or by its relative percentage (Table III); its range was 35-46% of the total glycolipids in three out of controls, and 48-69% in the pathologic samples. In one control its percentage reached the value of 70%; this subject showed also the highets amount on the µg/mg tissue basis.The other glycolipid content and distribution do not seem to have any apparent correlation with the disease. Lactosylceramide was missing in two out of the four samples while pentahexosylceramide was ab-

Table II Glycolipids content in the skin biopsies analyzed.

NAME	TISSUE WEIGHT (mg)	TOTAL GLYCOLIPIDS	GLUCOSYL CERAMIDE	LACTOSYL CERAMIDE	SULPHATIDE CERAMIDE	TRIHEXOSYL CERAMIDE	TETRAHEXOSYL CERAMIDE	PENTAHEXOSYL
R. L.	40,390	0,180	0,087	0,030	0,022	0,032	0,009	n.d.a
F. A.	15,490	0,170	0,114	n.d.a	0,007	0,009	0,026	0,014
P. M.	9,400	0,744	0,415	n.d.a	0,019	0,022	0,288	n.d.a
S. M.	8,600	0,648	0,449	0,023	0,011	0,027	0,139	n.d.a
F.M.	334,200	0,124	0,088	0,011	0,015	0,006	0,001	0,002
P.A.	393,060	0,086	0,030	0,031	0,008	0,003	0,006	0,007
C.J.	65,450	0,096	0,042	0,015	0,004	0,006	0,004	0,026
B.J.	826,000	0,026	0,012	0,003	0,003	0,004	0,002	0,003

The results are given as µg/mg of fresh tissue; the samples referred to as R.L., F.A., P.M. and S.M. are the pathologic samples;
F.M., P.A., C.J. and B.J. are the healthy controls.
[a]: not detectable.

sent in three out of the four Gaucher's samples.
One of the patient in therapy with Ceradase (R.L.) showed a decrease in glucosylceramide absolute content if compared with the other patient (Table II); percent distribution of the stored compound in the two patients under enzyme replacement therapy (R.L. and P.M.) are more similar to the normal ones (Table III).
According to the method we used, total ceramides were only detectable in control samples (Table IV, panel a), where we analysed also the distribution of different molecular species; the presence of higher amounts of OH-ceramide and 3OH-ceramide was noticed in all the four samples. In C.J. subject the hydroxy species are the only one present.

DISCUSSION

In this study we have investigated the amount of ceramide and glucosylceramide in the skin of Gaucher's disease patients, compared with healthy control samples.

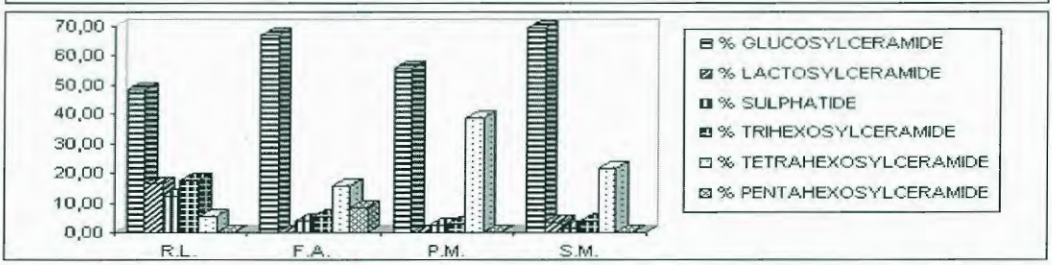
Gaucher's disease is due to the deficiency of glucosylceramide ß-glycosidase, (E.C.: 3.2.1. 45, ß-D-glucosyl-N-acylsphingosine glycohydrolase) a lysosomal enzyme: on the contrary the hydrolysis of skin glucosylceramide takes place in the lamellar bodies at the interface between the upper part of stratum granulosum and the lower part of stratum corneum. However it was suggested that the lysosomal and the lamellar bodies enzymes should have similar characteristics, even though their topology is different; this suggestion is further supported by the ascertained presence in the lamellar bodies of a proton pump (26) to secure the acidic conditions for the optimum of activity of the enzyme. The similarities between the two enzymes is further supported by the demonstration that in an animal model for Gaucher's disease, one of the most impressive clinical sign was the collapse of water barrier. Holleran W. M. and coworkers (23) showed the correlation between the deficit of glucocerebrosidase and the cutaneous barrier permeability, the ultrastructural

Table III

Glycolipid percentage and distribution in the pathologic samples (a) and in the healthy ones (b).

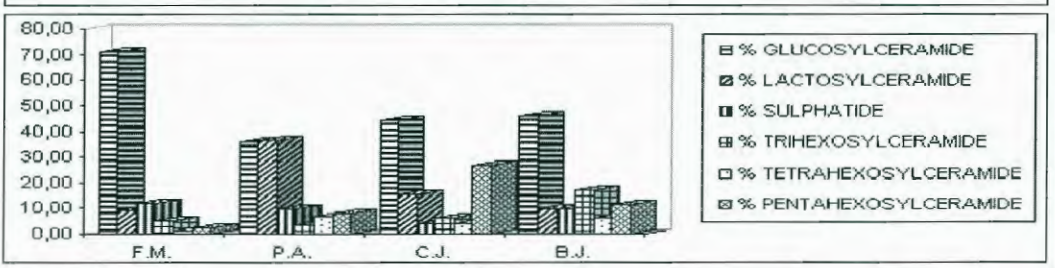
a)

	R.L.	F.A.	P.M.	S.M.
% GLUCOSYLCERAMIDE	48,25	66,84	55,77	69,28
% LACTOSYLCERAMIDE	16,41	n.d.a	n.d.a	3,53
% SULPHATIDE	12,43	3,88	2,51	1,58
% TRIHEXOSYLCERAMIDE	17,71	5,28	2,99	4,10
% TETRAHEXOSYLCERAMIDE	5,19	15,53	38,72	21,51
% PENTAHEXOSYLCERAMIDE	n.d.a	8,47	n.d.a	n.d.a



b)

	F.M.	P.A.	C.J.	B.J.
% GLUCOSYLCERAMIDE	70,83	35,70	44,04	46,10
% LACTOSYLCERAMIDE	8,83	36,07	15,23	9,70
% SULPHATIDE	12,12	9,84	3,78	9,90
% TRIHEXOSYLCERAMIDE	5,14	3,53	6,23	16,80
% TETRAHEXOSYLCERAMIDE	1,00	6,71	4,03	6,00
% PENTAHEXOSYLCERAMIDE	2,06	8,15	26,70	11,50



a: not detectable

aspect of stratum corneum and the epidermis lipid content. This study was performed using healthy mice and homozygous mice for the Gaucher's disease gene. In these ones Odland bodies are modified and incomplete, there is an increase of trans epidermal water loss, a decrease of epidermal ceramide and of glucerebrosidase activity, which was reported to be no more

Table IV

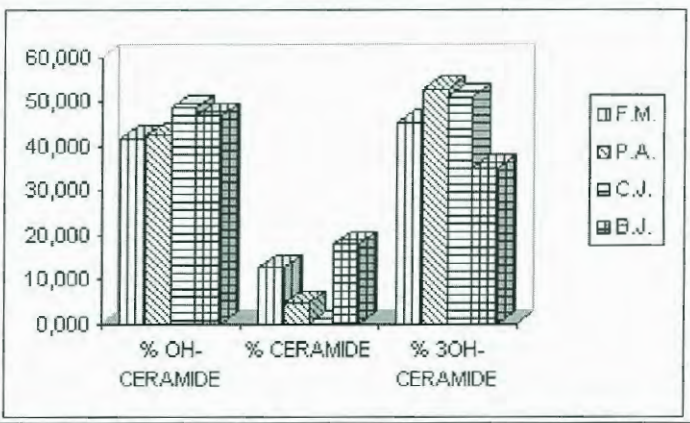
a) Ceramide content (a; mg/mg fresh tissue) and distribution (b; %) in healthy controls.

NAME	OH-CERAMIDE	CERAMIDE	3OH-CERAMIDE	TOTAL CERAMIDES
F.M.	0,109	0,033	0,118	0,260
P.A.	0,064	0,007	0,079	0,150
C.J.	0,171	n.d.a	0,179	0,351
B.J.	0,055	0,021	0,041	0,118

b)

NAME	% OH-CERAMIDE	% CERAMIDE	% 3OH-CERAMIDE
F.M.	41,842	12,668	45,530
P.A.	42,508	4,606	52,886
C.J.	48,834	n.d.a	51,166
B.J.	46,963	17,990	35,040

[a]: not detectable



than 1% as compared to normal animals

This experimental model could justify the alteration found in newborns skin with Gaucher's disease type II. Infact these modifications should derive from incomplete intercellular lamellae formation caused by a reduced transformation of glucosylceramide into ceramide.

However experimental data indicate also that the "storage" of glucosylceramide in the skin was more responsible of the barrier disruption rather than the absence of ceramide (12).

Also in other lisosomal pathology the epidermal barrier function is compromised by tissue accumulation of ceramide: clinical expression of the classic form of Fabry disease, due to the deficiency of α-galactosidase A, are cutaneous vascular lesions (angiectases) that progressive increase in the number and size with patient's age (27). On the other hand Farber disease, another lisosomal pathology, associated with deficiency of acid ceramidase leading to tissue accumulation of ceramide, shows granulomatous lesions in the skin (28).

Possibly more than one processe are involved in

the skin barrier formation. Small differences in these catabolic processes seem to exist among the different regions of the keratinizing epithelium, resulting in subtle variations in lipid composition of the stratum corneum, so explaining the different physico-chemical organization and fluidity of intracellular lipids (29). However the conversion of glucosylceramides to ceramides remains one of the more important step in the formation of the barrier, also due to the topology of these complex lipids (30). Holleran et al. found that the induced barrier abnormalities were not reversed by coapplication of ceramide (8). No indications on the chemical structure of the used ceramide were presented. It is noteworthy that the most abundant species of ceramide present in the skin has a peculiar fatty acid composition as shown by Uchida et al. (31). Due to the small amount of extracted lipids we were unable to perform reliable analysis on the fatty acid composition of our samples

In conclusion our results seem to indicate that the glycosidase, normally present in the lamellar bodies, failed in our patients to hydrolyse the glucosylceramide to ceramide, as shown by the complete absence of the latter in the pathological samples but its presence in a relative high amount in the healthy subjects. Due to the relevant role of ceramide in the cutaneous permeability barrier function, these findings might justify the presence of a mild to a severe dry skin in our patients. To our knowledge this is the first report on glucosylceramide and ceramide content in the skin of Gaucher disease patients. Infact despite many studies of glycolipid content in various tissues of these patients it appears that skin has been never analyzed, probably because, mainly in type I, the alterations in epithelia are not of sufficient clinical significance compared to the other more severe problems associated to the disease.

In order to solve the question whether or not the coapplication of ceramide could improve the skin abnormalities in Gaucher's disease patients, we prepared a special emulsion containing 0.5 - 1% 3OH-ceramide, which fatty acid composition is more closely related to what reported by Uchida et al.(32)

The treatment of the patients is now started and the preliminary results seem very encouraging.

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NEW PROSPECTS FOR CUTANEOUS WOUND HEALING AND KELOID TREATMENT

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Summary

The early steps of skin tissue repair are sustained by fibroblast proliferation, collagen deposition, and re-epithelization. On the contrary, injury or surgery in predisposed individual can result in hypertrophic scar.

Keloid is characterized by an increased number of fibroblasts, mast cells and a greater collagen synthesis with irregular bundles conformation which contributes to the formation of a disordered repair tissue.

However some medical-grade chitosans seem to have a reparative action on wounded tissues, inducing the formation of ordered repair tissue where collagen bundles had a regular direction. In this way, wounds did met slow evident scar formation or the wound contraction proper of the keloid.

The aim of this study was to control the activity of a gel based on a new patented hydrosoluble chitosan derivative of medical grade to repair and improve the cutaneous microenvironment on wounded tissues.

Dermo-epidermal grafts were performed with the aid of a dermotome on the frontal part of the thighs of 10 patients aged 30 to 50 years. In every patient a donor site was dressed with N-carboxybutylchitosan pads, the other was treated with phytostimuline gauze.

On day 10 and 30, when the surgical wounds were clinically healed, bioptie fragments were obtained from both areas.

Ultrastructural analysis of the areas treated by our chitosan derivative, showed a regular distribution of the collagen network and the general aspect of the derma gave an overall impression of histoarchitecture order which was more evident than in controls.

Overall, the epithelium was organized and citologically normal.

The studied chitosan derivative seems to favour the cell proliferation, the rapid re-epitheliazation of the tissue and optimizing the cutaneous repair, limitating the process of wound contraction.

It seems this gel could be considered as an innovative cosmeceutical or medical device able to speed up the tissue repair and to avoid the keloid formation

Riassunto

Il processo di riparazione della cute □ caratterizzato da alcune fasi principali: proliferazione di fibroblasti, deposizione di collagene e riepitelizzazione. In soggetti predisposti, tuttavia, insulti e/o interventi chirurgici possono dare origine a cicatrici ipertrofiche (cheloidi). Il cheloide □ caratterizzato sia da un aumento del numero di fibroblasti e mastociti, sia da una maggiore sintesi di fibre collagene i cui fasci tendono ad assumere una conformazione irregolare determinando la comparsa di un tessuto di riparazione disordinato.
Alcuni "medical-devices" a base di chitosano sembrano avere, tuttavia, un'azione riparativo/ricostitutiva inducendo una riparazione tissutale ordinata, con fasci di collagene disposti in maniera regolare, riducendo le possibilit□ di formazione di cicatrici ipertrofiche e/o di cheloidi.
Scopo del presente studio □ stato la valutazione della capacit□ di un nuovo chitosano idrosolubile, applicato quale gel, migliorare il microambiente cutaneo di un tessuto lesionato.
Lo studio □ stato condotto su 10 pazienti, aventi un'et□ compresa tra 30 e 50 anni, sottoposti ad innesti dermo epidermici a medio spessore. Le zone donatrici di innesti sono state realizzate sulla parte anteriore di entrambe la cosce in tutti i pazienti; un□area □ stata medicata con il prodotto in studio, l□altra con fitostimolina come area di controllo. Ø stata eseguita una biopsia delle aree trattate e di quelle di controllo al 10¡ e 30¡ giorno.
Le analisi ultrastrutturali condotte sulle aree trattate con il gel di chitosano, hanno mostrato una distribuzione dei fasci di collagene regolare e un derma con un ordine istoarchitetturale pi□ evidente rispetto alle zone di controllo. Inoltre l'epitelio si mostrava organizzato e citologicamente normale.
Questo derivato del chitosano sembra pertanto favorire la proliferazione cellulare e la rapida riepitelizzazione del tessuto, ottimizzando cos□ la riparazione cutanea e limitando il processo di contrazione della ferita. Tale gel potrebbe quindi essere utilizzato in medicina o in cosmetologia come mezzo per accelerare la riparazione tissutale e per evitare la formazione di cheloidi.

In superficial lesions, the rebuilding of a normal tissue is made possible by the basal cells of the epidermis, which proliferate and cause the tissue to regenerate. This process becomes more difficult for lesions, which penetrate deeply into the dermis, because the layer of basal cells whose proliferation would create a new epidermis is lost, and its formation is entrusted only to cells bordering the injured area. This process, however, is very slow and frequently interrupted by infections that occur in this type of lesions. When the lost of tissue is severe this is replaced by healing tissue, as it is impossible to obtain a perfect "restitutio ad integrum". The scar tissue is a particular connective tissue, frequently poor in elastic fibers, which can not be compared to the dermis because its has no normal elasticity and yields easily, with pathological stretching of its matrix structure. Another evident characteristic of scar tissue is tendency to retract. Wound healing alterations, i.e. excessive cicatrization, leads to cheloids.

Aim of the present study was the evaluation of the following materials abilities: a) favour formation of physiologically and histologically valid extracellular matrix; b) enhance vascularization and ordered collagen fibre formation; c) favour basal cell migration and proliferation; d) protect against infections; e) accelerate healing in slowly healing wounds (1-4).

The use of glycosaminoglycans in wound treatment is not new, and was justified by the occurrence of hyaluronic acid, chondroitin-6-sulfate, and dermatan sulphate, among other compounds, in the extracellular matrix. Nevertheless chitosans were found to be biocompatible and degradable in the human body, and their unique basically made them more promising for tissue regeneration and microbial growth depression than other polysaccharides. In this study, 5-8 high molecular weight N-carboxybutyl chitosan (700,000 Da) was used either as a transparent membrane or as a freeze-dried material having a sponge-like appearance, the diameter of pores being in the range 1 - 500 microns.

The pads were obtained sterile after the chemical manipulation and lyophilization; thus the freeze-dried dressing can be packaged under sterile conditions with no further treatment. In any case, however, sterilisation was done by irradiating with 60-cobalt gamma rays (1.4 Mrad) the freeze-dried N-carboxybutyl chitosan in sealed plastic envelopes: this treatment did not affect the chemical and physical characteristics of N-carboxybutyl chitosan.

The chemical treatment of chitosan leading to water-soluble N-carboxybutyl chitosan included ultrafiltration and dialysis (membrane cut-off 100,000 Da) which permitted removal of foreign matter, contaminants, pyrogens and salts from the N-carboxybutyl chitosan solution. Such treatments also permitted to refine the average molecular weight by eliminating small polysaccharide fragments. Thus, the resulting freeze-dried wound dressing was found to be well suited for applications in vivo and, thanks to its sponge-like and expanded form, it interacted in an active manner with cells migrating from adjacent tissues into the implant, while the chemical structure of N-carboxybutyl chitosan acted as a template for the extracellular matrix reconstruction.

The macromolecules present in the extracellular matrix probably exert two main functions in the histoarchitectural organisation: they provide a medium where cells can migrate, and mediate adhesion between cells and substrate. Specific binding sites confer to those molecules the ability to interact with each other; for instance fibronectin binds to hyaluronic acid, collagen and heparin.

Substances released by platelets and macrophages stimulate the proliferation of fibroblasts, which characterises wound repair morphologically. Fibroblasts then secrete collagen that initially is immature but able to provide a structural support to fragile newly formed capillaries.

The morphological role of glycosaminoglycans

is known to lead to cellular proliferation, stromal collagen network and functional characteristics close to normal. The ordered deposition of collagen and the histoarchitectural reconstruction of cutaneous wound tissues can be modulated by N-acetylglucosamine polymers (chitin derivatives) supplied to the wound. Similarities between some modified chitins and hyaluronic acid have been underlined.

CUTANEOUS REPAIR IN PATIENTS

Patients undergoing plastic surgery were subjected to medium thickness dermo-epidermal explant. The donor site (ca. 50 cm2) was dressed with freeze-dried sterile pads of N-carboxybutyl chitosan; the control donor site on the same patient was treated with phytostimuline gauze (5).
During the healing period, the square shape of the wound was preosserved while its size decreased progressively, as a point of difference from controls where the square shape was lost soon. In no case infections were reported and complete healing occurred after 8 days (7 for controls).
Ultrastructural analysis identified fibroblasts of elongated shape, arranged according to precisely oriented parallel lines. Vascular structures were largely present, while the inflammatory cellular component was occasional. The rather loose collagen network showed a regular distribution. The general aspect of the derma had an overall histoarchitectural order more evident than in controls. The epidermis showed in general a linear junction with derma, deprived of marked offshoots. The epithelium was in general organised and cytologically normal, even though a Malpighian layer appeared less extended than in controls. The skin reached its final differentiation with superficial aspects of normal keratinisation.

HEALING OF ULCERS IN AGED PATIENTS

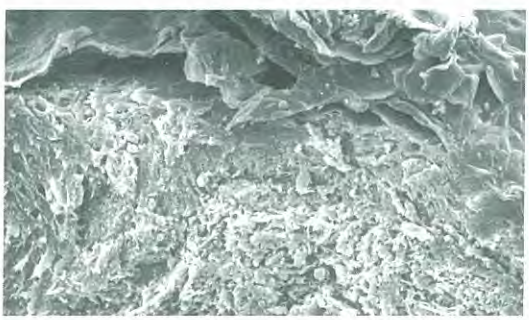
In aged skin, collagen undergoes cross-linking reactions and physico-chemical alterations; proteoglycans decrease in general and their percent ratios are altered. Vascular walls undergo thickness increase and a reduced quantity of oxygen reaches the cutaneous tissues with unfavourable consequences in case of ulcers and burns. Hypertension, oedema, atherosclerosis and diabetes further reduce the quantity of oxygen, nutrients and cells having defensive action (leukocytes and macrophages) reaching the cutaneous tissue.
10 Patients (average age 62) affected by ischemico-ulcerative lesion of the legs were studied; every patient had altmost two ulcerative lesions: the bigger one was treatedwith N-carboxybutyl-chitosan, the smaller with phytostimuline gauze. If the lesions had the same size, the one in the lower site was treated with N-carboxy butyl-chitosan, the upper one with phytostimuline gauze. Bioptic specimens were kept far to morphological study on 30° days when the lesions were all healed.
Compared to controls, a more rapid epithelialisation was remarked (7 days instead of 15-20 days). In no case infections occurred and good haemostasis was observed. From a morphological point of view, controls showed the usual disordered deposition of collagen fibers, whilst in patients treated with N-carboxybutyl chitosan a correct histoarchitecture of the regenerated skin was observed with, in particular, an ordered organisation and vascularization of the derma. In these patients, N-carboxybutyl chitosan exerted favourable effects on the partially altered metabolism presented by senescent cutaneous tissues and favoured functional recovery.

CONCLUSIONS

Sterile, pyrogen-free and pure N-carboxybutyl

chitosan is affordable via a chemical treatment of chitosan, which introduces carboxybutyl groups on some of the polymer glucosamine units. Water-solubility is achieved while preserving the cationic character that is important from several standpoints, including antimicrobial action (6).

The novel group artificially introduced into chitosan was expected to be non toxic and proved to be so. Experimental evidence indicated that a major part of the carboxybutyl groups is in the methyl pyrrolidinone form which introduces an aspect of similarity with synthetic polymers and monomers widely accepted in the pharmaceutical, medical and cosmetic field, such as poly(vinyl pirrolydone) (7,8) and pyroglutamate (9). In practice, in our study N-carboxybutyl chitosan was well tolerated by all patients.



Regenerative Aspects of Human Skin

Chitosan is an insoluble, partly crystalline substance in the form of irregular and abrasive grains. Even though it can be put into an amorpbous form, its hydrogen-bonded structure lives little chance for effective interactions with wound tissues. On tbe other hand, chitin/chitosan dressings recently developed as non-woven tissues can be used on wounds but there is no evidence of any biological action (10-14). N-Carboxybutyl chitosan, on the contrary, combines in itself desirable chemical characteristics and biological significance. By acting as a template for the extracellular matrix regeneration, it heals wounds and ulcers with little or no scar formation, leading to better functionality and histoarchitectural organisation than with other dressings.

N-carboxybutyl chitosan favoured an orderly reconstruction of dermal architecture, a rapid reconstitntion of the stromal network. This study demonstrates that it is possible to modulate the wound healing processes with the aid of biomaterials to produce replacement-like processes, rather than scar tissue formation. The biomaterial here examined took an active part in the repair processes. The biomaterial exhibited particular biological significance, suggesting different therapeutical application in relation with specific morpho-structural properties: it conld be used, as structural modulators in wound dressing.

Finally, as the application of chitin and chitosan to wounded human tissues has been experimented only in recent years, different kind of molecular modification of this polysaccharide must he performed.

Regenerative Aspects of Human Skin

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GENETIC SKIN DISEASES IN BEAUTY CARE PRACTICE

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Summary

Cutaneous benign tumors and disorders of pigmentation (hipopigmented as well as hyperpigmented spots) are most common skin symptoms in hereditary skin diseases. At the same time these are the basic cutaneous problems which cosmetologists have to deal with in their practice. They are mostly seen at birth or develop during the first years of life. In the majority of cases they indicate the presence or, more often, the increased risk of various systemic disorders development. The fact that such skin lesions are seen several years before systemic alterations occur determine the importance of correct interpretation of such skin symptoms for the complications preventive purposes.

Riassunto

I tumori cutanei benigni e le discromie (aree ipopigmentate ed iperpigmentate) sono sintomi cutanei molto comuni nelle malattie ereditarie della pelle. Allo stesso tempo esse rappresentano i primi problemi cutanei che i cosmetologi devono affrontare nella pratica.

Le discromie si riscontrano maggiormente alla nascita o si sviluppano durante i primi anni di vita. Nella maggioranza dei casi indicano la presenza o, più spesso, il rischio crescente dello sviluppo di varie malattie sistemiche.

Il fatto che tali lesioni cutanee si ritrovano alcuni anni prima che le alterazioni sistemiche si sviluppino, determina l'importanza di una corretta diagnosi di tali sintomi cutanei al fine di prevenire future complicazioni.

Hereditary skin diseases represent the most serious among cutaneous disorders. As a rule genodermatoses are associated with various systemic disturbances. In most cases cutaneous lesions develop a long time before systemic alterations become evident. The importance of correct interpretation of such skin symptoms is beyond doubt. It is a prereqiusite for the early diagnosis which in turn determines further prognosis.

The data discussed below were obtained during the long-term clinical observation of patients with various hereditary skin pathology. All of them developed serious systemic complications, partly as a result of misinterpretation of thier cutaneous lesions prior to their referral to our department.

For the purposes of this article we have subdivided the most common skin changes in two groups: disorders of pigmentation and benign cutaneous tumors.

Analysis of over 150 case histories revealed that such lesions often do not attract the attention of paediatritians, general physicians and sometimes even that of dermatologists. Moreover, the lesions are often being treated as a simple cosmetic problem. Absence of appropriate treatment results in development of systemic complications, inreversible in most cases, including malignant transformation of benignant tumors.

Hyperpigmented spots (cafe-au-lait macules), as well as freckling in auxillary and/or inguinal folds are the earliest skin symptoms of von Reklinghausen disease (neurofibromatosis type I) (5). It is known that various skeletal abnormalities, not equally severe in all cases, are commonly seen in neurofibromatosis. These abnormalities tend to progress with advancing age and sometimes lead to chronic cardiac and pulmonary insufficiency. Fractures of the bones easily develop. In the boy shown on Fig. 1 a very slight trauma resulted in the vertebral column fracture and in a severe 4th degree kyphoscoliosis with subsequent cardiovascular and respiratory impairment and pronounced physical retardation.

Multiple lentigines, predominantly localized periorificially, on mucous membranes, digits,

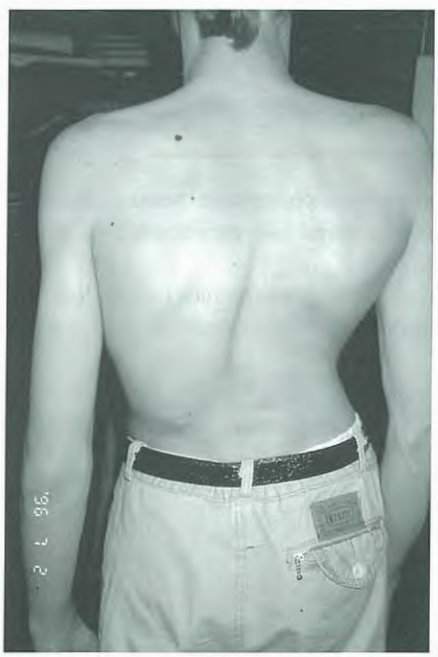

Fig. 1

Fig. 2

palms and soles are also seen at birth or appear during the first years of life. They are asymptomatic. In some patient these spots were diagnosed as moles which did not require further medical observation. But being a symptom of Peutz-Jegers-Touraine syndrome (periorificial lentiginosis) (4), they are the markers of the small and large intestine polyposis which develops several years later with the increased risk of malignant transformation of polyps into adenocarcinoma.

Maple leaf - like hypopigmented spots, seen in children, are the earliest cutaneous signs of Burneville-Pringle disease (Fig. 2). As we could see, some of the patients consulted at our department due to the previous misdiagnosis had an advanced epilepsy and pronounced mental retardation. It should also be remembered about the increased incidence of malignant brain tumors development as well as cardiac and renal complications in these patients. The vital importance of therapeutic investigation of such children before the systemic pathology develops is obvious.

Cutaneous changes in the girl, shown on Fig. 3 were diagnosed as multiple pigmented naevi. But lentigines of generalized pattern are the earliest symptom of LEOPARD-syndrome. Patients must be under clinical supervision to prevent the development of cardiovascular, neurologic complications etc.

Pigmented spots in neurofibromatosis I type are seen at birth and in some patients almost 15-20 years before the development of neurofibromas, which sometimes, especially in case of plexiform neurofibromas may transfer into neurofibrosarcoma. The misdiagnosis in patient shown of Fig. 5 and removal of just a single dermal neurofibroma at a beauty care center resulted in development of thousands of neurofibromas which covered most of body surfaces including the face.

Inherited cutaneous tumors, especially if they develop in early childhood may be helpful in preventing serious systemic complications. For instance, it is known that in overwhelming majority of patients with neurofibromatosis type 2 bilateral acoustic neuromas result in deafness on average at the age 20-30 (6). That is why it is very important for the patient to be under medical supervision, in case cutaneous schwannomas are diagnosed in association with multiple cafe-au-lait spots.

Spitz nevus (Fig.4) is not a genodematosis per se, but we have seen several cases of congenital spindle and epithelioid cell nevi, that were treated with electrocoagulation as angiomas when solitary, and as molluscum contagiosum when multiple in infants. As it is, Spitz nevus is one of the most common simulators of malignant melanoma of the skin, both clinically and histologically (1,3). However, when it recurs after such unnecessary medical manipulations (with a great deal of inflammation, as we have seen), it may acquire more atypical features that become misleading for the pathologist.

We have suggested that multiple cutaneous leiomiomas (Fig. 5) indicate the increased risk of uterine myoma. Such association was present in all family cases observed by us. It is very impor-

Fig. 3

tant to say that all out patients underwent hysterectomy at child-bearing age. As cutaneous changes are seen several years before the uterine myoma develops, the importance of timely diagnosis cannot be overestimated.

The presence of multiple sebaceous tumors (adenomas as a rule) should rise suspicion and prompt examination should be made to reveal the internal malignancies (gastrointestinal most commonly), which usually precede cutaneous lesions.

Thus certain skin lesions can be helpful in correct diagnosis of internal pathology, which, if made in time, improves further prognosis and course of the disease.

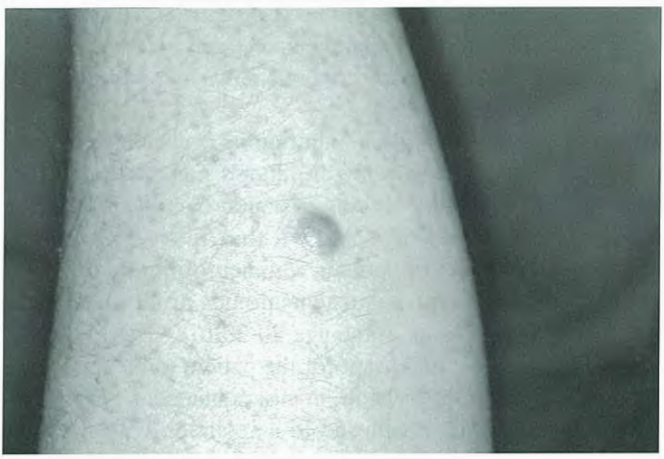

Fig. 4

Fig. 5

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NUTR(A)CEUTICAL OR NUTR(I)CEUTICAL?

Pierfrancesco Morganti
Editor - in - Chief

To abbreviate the two words/concepts of nutrition and pharmaceutical, recently in USA has been coined the new word nutraceuticals to underline the importance that should be given to a correct diet, that, as it is well known, contributes to the quality of life in a key way.
Why nutraceutical and not nutriceutical?
Since the words nutrition or nutritive come from the Latin "nutrio-is" and "nutrimentum", the substitution of the vocal "i" with the "a" has been probably done for sound easiness, because English, differently from Italian and Latin, very often is written in a way and pronounced differently.
From a semantic point of view I still remain doubtful considering more correct to write nutriceutical instead of nutraceutical: the English words "nutrition, nutrient and nutritive" are written with the "i".
Apart from that, during "The 3rd "International Exhibition and Conference on Nutriceuticals food for vitality" held in Geneva this year, was stressed the necessity of developing functional food in order to improve the general health of the world population, reducing the risk of the early onset of life-threatening diseases.
According to Prof. Marcel Roberfroid, from the Catholic University of Louvain in Belgium, "the development of functional food is a unique opportunity to contribute to the improvement of the quality of the food offered to consumer's choice for the benefit of his well-being and health". But functional food has to be well defined and need specific low regulations. At this purpose, although the US does not have a specific regulatory category for functional foods, the food and drug administration in USA has authorized different "health claims" to be allowed on food labels and which may highlight the relationship between a food substance and a disease or health-related condition. That's what has been reported by Clare M. Hasler from University of Illinois, who also affirmed how the consumer interest in functional foods, nutriceuticals and dietary supplements is at an all time high in the USA and is expected to continue as baby boner dominate the market for the next 30 years.
Regulatory initiative related to nutriceuticals and functional food are currently underway in Canada, according to what said by Kelley C Fitzpatrick, president of Sa of Saskatchewan -nutraceutical Network.. The Canadian Standing Committee on Health (SCH) provided recommendations for the regulation of Natural Health Products (NHP) as a separate category from food and drugs. NHPO has to include "substances or combinations of substances consisting of molecules and elements found in nature as well as homeopathically prepared products for the purpose of maintaining or improving health and treating or preventing disease conditions. NHPs would include, but would not be limited to vitamins, minerals, enzymes, co-enzymes, co-factors, herbs or botanicals and animal source substances, and a variety of molecules extracted from natural substances such as amino acids, polysaccharides, peptides, naturally occurring molecules and synthesized one by chemical or biological means".
According to Tsuneo Hirahara from ISSI "the concept of functional foods was proposed in Japanese academic society since 1980 and the legislation of these foods was first implemented as FOSHU, which stands for "food for specific health use". FOSCHU is a permission system for labeling the chain that a food claim, helps maintaining or is suitable for a health condition when the claim is substantiated based on medical and nutrition science.

Based on the concept of traditional Chinese medicine (TCM), foods have to maintain and improve health status, to prevent disease and help in treating disease; and to facilitate rehabilitation.
In TMC food and drug are from the same sources, are based on same theories and have the same uses.
According to the Food Hygiene Law of the people's Republic of China reported by Junhi Chen from ILSI, the food claimed to have specific health functions shall not be hazardous to human health and the functions and ingredients of the product shall be identical with the information given in the product description material, and there shall be no deceiving information.
The debate on health claims largely extends beyond the frontiers of Europe and the origin of differences observed in the European or international law situations lies in the different nutrition and health context. Though the existing processes leading to harmonization in Europe preclude rapid change in regulatory texts, the scientific substantiation of health claims the frameworks for communication are evolving. Therefore, the common objectives are to permit industrial innovation vehicle protecting public health by proposing operational rules, according to what reported by Ambroise Martin from French Food Safety Agency and Tage Offertsholt from Food Group Denmark..
Both industry and consumers stand to gain control and endorsement of health by government authorities and it ought to be possible to communicate health benefits to consumers when true and documented. As a matter of fact, consumers have to know, for example, that our body harbors a natural ability to re-transform malignant cells into normal healthy transcription without apoptosis or lysis. This is what reported by Thomas Tallberg from the Institute of Bioimmunotherapy of Helsinki, Finland. Moreover food allergy is increasing in the world due to an increase in the last years of the use of several allergising foods because of a diffusion of different food all over the world, once confined to certain countries.
Food allergy interest about 8% of the children and 2% of the general population of adults and the hypoallergenic functional foods can be an important solution, according to C. Ortolani from Niguarda Cagranda Hospital in Milan, Italy.
A correct diet is fundamental to live better and longer hut it's necessary that we all learn to know better and deeper our body and all the substances active included in the foods in order to select them according to our needs.
Development of novel or functional foods is one of the most exciting and promising way in which the future food supply may address human health needs and well being. For this to happen, there will be need to be a conjunction of food governance according to what reported by Prof. Mark L. Wahlqvist from University of Adelaide, Australia. The extent to which the new and emerging food supply will draw upon technologies, like that greater communication between scientists and the community, will allow future progress. For the successful progress will be mandatory an engagement through education and the direct intervention of the governments and international agencies to emanate and diffuse appropriate regulations.
At this purpose a new class of biologically active food additives has been worked out in the research conducted at the St. Petersburg Institute of Bioregulation and Gerontology, as reported by Prof. V. KH Khavinson.
These new bioregulators, named cytamins TM, seem to be able to correct the cellular metabolism in damaged cells of the tissues. This results in production of morphologically normal cells with an enhanced resistance to pathogenic agents.
Cytamins application regulates and restores the mechanism of organic defense, thus enabling prevention of various diseases and pathological states.

Also the "good old" vitamins are on the spotlight again, such as vitamin E recognized as the human body's most important lipophilic antioxidant. Furthermore, it should be remembered the importance of the "antioxidant networking", i.d. the co-operation between vitamin E, vitamin C and further antioxidants such as glutathion, lipoic acid and selenium-containing enzyme glutathion peroxidase, as reported by Dr. Christine Gartner from Cognis- Deutchland.

What makes beta-carotene unique?

Its bioprotective effects. For the antioxidant and immune system modulating properties.

Observational epidemiological studies have clearly shown an inverse relationship between dietary intake of beta-carotene, or blood levels, and risk of cancer, risk of coronary heart disease and stroke, and age-related eye diseases.

According to Dr. Ruud Schmeink from DSM Food Specialties - the Netherlands, unfortunately beta-carotene has not been proved to be the "magic single ballet" to prevent disease. In fact it should be add to other carotenoids as licopen, which strengthen also its melanin-stimulant activity.

Many others are the botanicals usable as food for vitality, as some polyphenolic structures from Citrus fruit, that have promising anticancer properties with chemically-inducing, tumor-inhibiting characteristics and strong activity against well-known chemical carcinogens, such as benzopyrene. A unique extraction process capable of isolating from citrus fruit fractions rich in polyphenolic components was presented by Nissin Garti from The Hebrew University of Jerusalem, Israel.

Another family of polyphenols are the grape procyanidolic oligomers, also called leucoanthocyanins. These products seem to improve the biochemical and structural properties of the whole circulatory system, endowing with an antioxidant activity stronger than vitamin-E, according to the data presented by Paolo Morazzone from Indena, Italy.

Nevertheless the pharmacological and ecotrophological significance of flavonoids and isoflavonoids is still a point of intensive discussion and other studies have to be done for better understanding their biological role, according to all the scientific data reported by Jörg Heimann of the Institute of Pharmaceutical Science in Zurich.

Another universal antioxidant a-lipoic acid may help to alleviate the effects of oxidative stress for the human body. Due to its unique chemical structure, a-lipoic acid is capable of attaching the so-called free radicals, improving also the overall cellular function of the human body. Schubauer from SKW Trostberg AG in Germany presented the data on a commercially available a-lipoic acid, which does not contain any traces of organic solvents.

Ralf Jäger from SKW, proposed a novel unique nutritional molecule combining the energy and endurance booster effect for short-term and long-term exercise of Creatine and Pyruvate, in a ideal manner. It was synthetized a new chemical compound, creative pyruvate resulting in a "stabilized and improved Pyruvate" and a "highly bioavailable Creatine" proved to be very active as neuroprotective agent.

Food manufactures are aware of the potential for developing a broader range of products which apply nutrition science and innovative food technologies and which have demonstrable health benefits"

Diet should be always well balanced in order to avoid any health problem.

If we eat in a balanced way many problem, as for example, the Premenstrual syndrome (PMS) affecting women, can be cured, as reported by Georg Boonen of the Swedish Zeller AG.

PMS causes psychological changes and this pathology cannot be efficiently treated with a single-mechanism-based-drug.

At this purpose a new extract has been developed from the fruit of vitex agrus castus, which proved

its significant superiority over placebo for efficacy and tolerability, while soy isoflavones can improve prostate diseases as reported by Mark W. Empie of ADM Nutraceuticals from UK. These compounds have been shown to interact with a number of biological systems inhibiting also prostate-cancer's cell development. The isoflavone-aglycones seem to be the most effective of all phytoestrogens playing an important role in the prevention of sclerotic cardiovascular diseases according to Dr. Hideaki Hiranoi from NICHEM Corporation of Japan.

A correct diet is fundamental for a good living. Prof. Thomas Tallberg from the Institute of Bio-immunotheraphy of Helsinki, pointed out that the uncommon cases of spontaneous cures reported, support the notion that our body harbors a naturally ability to re-transform malignant cells in normal healthy transcription without apoptosis or lisys, as demonstrated in both human and animal experiments.

At this purpose, oxidation and reduction biomolecules such as radical oxygen or nitrogen intermediates are considered to be "signals" in certain instances and are utilized for the maintenance of cellular homeostasis. Further understanding of the complex chemistry of nitrogen or oxygen oxides may help develop new therapies and/or new diet supplements based on augmenting what seems to be an host defense system.

This is one of the message expressed during this very interesting meeting that underlined also the importance of a correct diet for our better living.

NEW CYTOKINES AS POTENTIAL DRUGS

By SK. Narula and R. Coffman

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Cytokines, specific subsets of cell in response to a local stimulus, are produced in very small quantities and their functions are always modulated by specific receptors on the cell's surface. Even if tremendous amount of work has been done to elucidate the structure-function of cytokines and cytokines antagonists, a lot is the work to be done in order to design small molecule drugs that could be easily ingested having little or no side effects.

In order to produce these drugs it's necessary to deepen the knowledge on all the relevant functions that cytokines have demonstrated to have.

In chapter 8 of tbis interesting book are reported the final experiments carried out by several research groups on cytokines recently identified and their antagonists using also the sequence identification discovered by the Human Genome Sciences database.

As it's well known eosinophils accumulate in some inflamed tissue, such as the lungs of patients with asthma, the nasal activity of patients with allergic rhinitis, and the skin of patients with atopic dermatitis.

By the way the predominant help cell involved in allergic diseases is the TH2 subtype, based on the secretion of IL-4, IL-5 and IL-10.

While IL-4 is recognized as the essential cytokine for generating and maintaining a TH2 response, IL-5 has a pivotal functional effect on eosinophils and as such is considered particularly suitable as a therapeutic target.

In fact when eosinophils are activated, they synthesize and release inflammatory cytokines, such as IL-5, and secrete a set of potent cytotoxic enzymes and proteins that can lyse epithelial tissues. Thus, inhibition of IL-5, by reducing its systemic concentration, blocking its receptor association, or inhibiting downstream cell signaling, could provide a novel therapy.

Even if it should be possible to identify small-molecule inhibitors of IL-5 biosynthesis, as it happens, for example, for glucocorticosteroids and cyclosporin, the most practical and specific therapeutic agent at present time is its neutralization by monoclonal antibodies.

As matter of fact, they are extremely selective, have a very high affinity for their target protein, and if properly engineered, can be used in buman therapeutics.

IL-10 is an immuno suppressive molecule down-regulating the antigen preventing and accessory cell functions of monocytes/macrophages and blocking the production of proinflammatory cytokines.

For these activities IL-10 is considered an attractive therapeutic agent for the treatment of the major chronic Inflammatory Bowel Disease (IBD).

On the basis of its safety profile and its back not immunogenicity IL-10 can be used in long term

treatment regimens, representing also a promising alternative to steroids.
All the data on the scientific rationale for therapy in humans with IL-5 and/or IL-10 are reported in chapters 1 and 2.
IL-16 is synthesized by a variety of immune cells as well as by array epithelium and fibroblasts.
It is a pro-inflammatory cytokine generated as immunomodulatory cytokine generated as a 631 aminoacid precursor molecule which is enzymatically cleaved at a serine residue following stimulation with T cell mitogens resulting in the secretion of a 17KDa peptide.
Functionally, bioactivity of IL-16 appears to reside in the hydrophilic region located in the carboxyl end of the secreted molecule. For its ability to increase the infiltrating CDh+ cell membranes at sites of inflammation, IL-16 can be considered as a pro-inflammatory cytokine.
Therefore IL-16 inhibitors may have therapeutic implications in suppressing T cell-mediated inflammation.
The anti-viral effects of IL-16 on HIV-1 replication with its growth factor capabilities for CDh+ cells suggests also another potential therapeutic application for IL-16.
In fact these two complimentary activities are both required for immuno-reconstitution in individuals with HIV-1.
On the contrary very little data have accumulated concerning the role of IL-17 in diseases, even if it seems to have a pro-inflammatory function.
IL-17 seems to induce secretion of prostaglandin-E2, which helps the attracted cells to cross the blood vessels and thus to access the site of injury; production of nitric oxide by articular chondrocytes in response to IL-17 could represent a more direct mechanism to destroy microbes; finally the ubiquitous distribution of IL-17 receptor support the hypothesis of its involvement in signaling the initiation of an immune response.
However, the importance of its role in alloimmunity, rheumatoid arthritis and psoriasis remains to be established by testing the molecules, which specifically block IL-17 or its receptor in the animal models pertinent to these diseases.
IL-18, with a molecular mass of 18,3 KDa is a cytokine with pleiotropic effects on various types of immunocompetent cells. It acts on NK cells to induce IFN-g production and to augment their lytic activity without reqniring any costimulatory signals.
IL-18 enhances also the cytotoxic activity of NK cells, the citotoxic T cells (CTL) functions and seems to have positive effects against some fungal, bacterial and viral infections.
Attempting to better understand the molecular mechanism of IL-18 its receptor was identified (IL-18R). This receptor is capable of binding IL-18 and of transducing also signals, including the activation of the transcription factor NFkB. The available data today obtained suggest that IL-18 play important roles in the adaptive immune responses as well as in the innate immune response exemplified by the activation of NK cells.
Moreover the finding that this interleukine induces the production of proinflammatory cytokines such as TNFa, IL-1b and IL-8 raise the possibility that antibodies of IL-18 or antagonists to its receptor may be therapeutically applicable as soon as possible.
IL-16, IL-17 and IL-18 are described respectively in the chapters 3, 4 and 5.
Hepatopoiesis, i.e. the generation process of blood cells, is controlled in part by interactions of cytokines with their receptors. These interactions can deliver stimulatory or inhibitory signals to cells bearing appropriate receptors. In chapter 6 are summarized all the biological properties of the cytokine FLT3 and its potential clinical applications.
First of all FLT3 seems to have potent effects on myeloid and lymphoid differentiation and therefore

it has the potential for therapeutic use in a number of hematological as well as immunological areas.
The keratinocyte growth factor family (KGF) is treated in chapter 7.
Growth factors are a group of hormone-like polypeptides playing a central role in the process of tissue repair. KGFs, KGF-1 and KGF-2 seem to be the key regulators of keratinocyte proliferation and migration playing an important role in the complex phases of wound repair.
While KGF-1 mainly stimulates re-epithelialization, KGF-2 promotes both re-epithelialization by a direct effect on keratinocytes via proliferation and migration, and may stimulate granulation tissue formation by a direct chemotactic effect on fibroblasts, playing also an important role in the epidermal-dermal interactions during wound repair. For all these reasons the exact determination of the role of KGF-2 and FGF family members in the process of tissue repair may be important in improving the development of a new therapeutic strategies for wound healing and tissue repair.
The last chapter tries to clarify the real role played by chemokines.
At present we know little about why different chemokins send different signals through different receptors. The immune system is comprised of many cell types, which constantly migrate. This characteristic is essential for the defense of the organism against foreign agents and for reparative processes. Insufficient recruitment and activation of leukocytes leads to inefficient clearance of pathogens. Conversely, their over-activation or unregulated recruitment leads to pathological damage.
Understanding mechanisms of chemokine presentation and clearance for interfering with unregulated trafficking and activation of leukocytes may offer new opportunities in drug development.
Moreover understanding the role of chemokine ligand molecules encoded by viruses may give novel chemokine-based molecules to treat infections disease inflammation and autoimmune disease.
This interesting book provides the reader with a lot of news for better understand the human immune response, both at a cellular and molecular level.
All the new cytokines recently discovered are well described and biochemically identified in a way to better explain their effective therapeutic utility.
Therefore, it is very useful for all the doctors in medicine and specialized medical doctors such as dermatologists and/or oncologists but it's furthermore useful for all who are interested in examine the topic cytokines and growth factors both for research interests and for personal information.
I deem it useful also for all the people interested in understanding the topic activity performed by the cosmetic products especially all if defined "cosmeceuticals".

P. MORGANTI
Editor-in-Chief

HIGH THROUGHOUT SCREENING FOR NOVEL ANTI-INFLAMMATORIES

By Michael Kahn

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2000. 272 pages. Hardcover
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This interesting book represents the state of the art on the integration of Combinatorial Chemistry and High Throughout Screening (HTS) used for the discovery of new anti-inflammatory agents. The use of these new techniques will make possible the discovery of new active drugs useful to cure many pathologies, the inflammatory processes in this case.

The book by means of its 12 chapters gives an update and exhausting overview of what was done and still has to be done in order to examine the immunitary mechanisms that our body activates to defend itself from the continuous environmental aggressions, and how the new therapeutic devices set up by the modern pharmacology will be developed.

The first chapter illustrates some of the chemistries involved in the preparation of compounds used in anti-inflammatory research, based essentially on polymer-supported synthesis. As a matter of fact, this innovative solid phase synthesis has become an indispensable tool for preparing large numbers of compounds thanks to the relative ease manipulation and amenability to automation. Examples are reported which describe solid phase synthesis to prepare libraries of 4-thiazolidinone for Cox-1 cyclo-oxygenase (Cox-1) inhibition or N-carboxyalkyl peptides as inhibitors of human stromelysin (MMP-3), and neutrophil collagenase (MMP-8) etc.

In the second chapter big room is given to the proteasome, multifunctional protealytic complex responsible for non-lysosomal protein degradation. Located in both the cytosol and nucleus of eukariotic cells and predominant as cellular component, it plays a major role in the degradation of misfolded, damaged or abnormal proteins, as well as in the modulation of levels of key cells signaling regulators.

Many researchers are attempting to develop inhibitors for this enzyme, since any deregulation on proteasome activity has the potential to lead to many pathological conditions. In fact, the proteasome is involved in the processing of intracellular antigens, which are presented via the MHC-1 complex.

Among the important proteasome substrates are IxB, the inhibitor of the transcription factor NF-kB, the cyclin-dependent trinase inhibitors p21 and p27, and the cell cycle regulator p53.

For all these reasons the ability to intervene in these processes with a proteasome inhibitor could have important therapeutic implications for the treatment of inflammatory disease, cancer, and a variety of other conditions.

Unfortunately despite the importance of the proteasome, little work has been done on the design and development of small molecule inhibitors. Except for lactacystin analogs peptide-based aldehyde

and boronic acid containing inhibitors, few small organic molecules have been studied up today.
The caspase intercellular cysteine proteases with a critical role in cytokine maturation and apoptosis are treated in chapter 3.
Pharmacological modulation of caspase activity in vivo may be effective in inflammatory diseases such as rheumatoid arthritis and septic shock or, in any conditions where inappropriate apoptosis contributes to pathological conditions. This is the opinion of the author of the chapter, who reviews all the biological roles and properties of the caspases and the progress in the discovery of caspase-specific inhibitory drugs.
Thus, caspase-1 directly and indirectly regulates production of IL-1 b, IL-18, 1L-1a , and IFNg and therefore it might be effective as anti-inflammatory drugs.
Biochemical evidence, confirmed by the crystal structure, identified Cys 285 as the active site nucleophile of caspase-1.
Based on understanding of the catalytic and structural properties of this enzyme, great step ahead has been made in the design of caspase-1 inhibitors. With further work, caspase inhibitors, efficacious in a variety of chemical indications, will certainly be produced. With this hope this interesting chapter ends.
The fourth chapter is entirely dedicated to the elastase referred to endogenous proteinases capable of hydrolyzing and degrading structural matrix proteins, such as elastin.
This enzyme is one of the key components of inflammatory response system. In mammalians elastase provides homeostasis for tissue repair and normal degradation of damaged tissue. As a matter of fact, it assists neutrophils in their migration to the site of inflammation and participates in the degradation of invading microorganisms. When the balance of elastase and endogenous inhibitors is upset, as during an acute inflammatory response, the balance must be restored to impede the destruction of healthy elastic fibers.
In fact, neutrophil elastase has been shown to degrade fibronectin, laminin, collagen and proteoglycans. For these reasons the development of exogenous elastase inhibitors as therapeutic agents is a formidable challenge in drug discovery. Subsequently are widely described different chemical classes of reversible and irreversible elastase inhibitors known today, such as peptidic electrophilic ketones, acylating and alkylating agents, and heterocyclic and nonbeterocyclic agents. Big room is given also to the combinatorial chemical methods developed to create new pharmacophore-based library of elastase inhibitors.
Finally the last studies on the matrix metalloproteinases (MMPs) and matrix metalloelastase (MME or MMP-12) are reported. These enzymes are involved in the normal turnover and maintenance of extracellular matrix (ECM) proteins in connective tissue and basement membranes such as elastin. ECM degradation is necessary for normal tissue remodeling and repair associated with development. For these reasons inhibitors of MMPs could be useful for the treatment of a range of inflammatory disorders.
The chapters 5, 6 and 7 are dedicated to the tumor necrosis, factor-a (TNFa implicated in cancer and inflammatory diseases, to human tryptase mediator of several mast cells related allergic and inflammatory conditions, and to the specific role played by lectins in important biological functions.
Demonstrated the pathological role of TNF in arthritis and Crohn's disease, started an intense study to find small molecule TNF- antagonists at its multiple contact points, thanks also to the new methodology used, the scintillation proximity assay (SPA).
As a matter of fact, this new technology permitted rapid progress in the study since it allows to control in a fast way the activity performed by TNF antagonists without separating, for example, the

product from the substrate.
Clarified and understood the implication of tryptase as key regulatory enzyme in the development and progression of both chronic and acute allergic inflammatory and immunological diseases and the critical tryptase-inhibitor interactions, has been possible to obtain significant advances for therapeutic intervention by the use of new tryptase-inihibitors such as, for example, zinc-mediated compounds or heparin antagonists.
Lectins through their carbohydrate recognition domains (CRDs), recognize carbohydrate chains present on invading bacteria, viruses and parasites.
CRDs in each group of lectins contain highly conserved aminoacids at well-defined sites, a characteristic that has enabled their classification into C-type, P-type lectins and galectins. The "adhesion" of leukocytes to the activated endothelium is the critical step that initiates the host defense as well as the progression of the inflammatory response, adhesion or "locking on" in mediated through L, E and P-selectin. Therefore it seems that modulation of any of the steps during the "adhesion-firm adhesion-migration" could help control acute and/or chronic inflammatory problems. Selectin antagonism has been considered as one of the most efficient ways to develop novel therapeutics that could be used to control adhesion-related disorders at every early stage of the process.
With a clear understanding that carboxhydrate ligants are capable of inhibiting adhesion in appropriate in vitro assays, synthesis of the sulfated trisaccharide, the sialic acid containing tetrasaccharide and larger oligosaccharides, were carried out. Moreover, screening studies had indicated that the carboxylic acid function of sialic acid and the three contiguous hydroxyl groups of l-fucose are essential for recognition of these complex carbohydrate ligants by selectins.
These observations formed the basis for synthesis and screening of next generation of compounds in which sialic acid was substituted for acetic, lactic, mandelic or phenyl acetic acid, and scaffold was also replaced with lactose and l-fucose with a glyceril residue.
In chapter 8 are focused the antagonists of those integrins known to mediate the leukocyte-endothelial interactions i.e. the CD18 and the CD49d integrins. The leukocyte integrins are transmembrane receptors expressed on circulating white blood cells that regulate their adhesion to other cells, the extracellular matrix, soluble proteins, and pathogens.
The capacity to modulate integrin adhesiveness, regulated in response to various cellular stimuli, prevents cells from non-specific attachment in the absence of specific inflammatory signals, and allows them to detach from the blood vessel wall and migrate into the tissue.
Integrins transduce also informations into the interior of the cell regulating processes such as anchorage-dependent cell-growth, intracellular pH, proliferation and protein phosphorylation. A successful anti-inflammatory drug could antagonize leukocyte's adhesion, migration or the leukocyte's functional properties. Therefore, integrins are clearly desirable pharmaceutical targets because of their pivotal role in early every step in the inflammatory process and their cell type specificity.
Many molecules integrin-anatagonist are reported and described.
The mitogen-activated protein Kinase (MAPK) family is treated in chapter 9. MAPK impacts many cellular processes such as proliferation, oncogenesis, development and differentiation, cell cycle and cell death. Selective inhibition of MAPK's signal transduction processes may be an effective way for treating a large number of diseases.
An interesting reported review of new selected compounds demonstrates that protein kinase at multiple levels in the MAPK cascade are fruitful targets for small molecule drugs that have the potential to treat a variety of different diseases.
NF-kB plays a central role in various responses leading to host defense and immune function, acti-

vating a program of gene expression. This is described in chapter 10. NF-kB can be activated in cells by a wide variety of stimuli associated with stress, injury and inflammation. Potent inducers of NF-kB include: cytokines such as interleukin 1b (IL-1b) and tumor necrosis factor a (TNFa).
NF-kB exists in the cytoplasm in an inactive form associated with inhibitory proteins IKB's. IKB phosphorylation serves as a molecular tag leading to rapid ubiquitination and degradation of IKB by components of the ubiquitin-proteasome system.
The basic drug discovery strategy is to prevent the activation of NF-kB, blocking cell surface receptors, interfering with intracellular targets or mobilization, modulating key kinases and preventing localization of proteins.
In such a way, a wide variety of compounds have been shown to block NF-kB activation, including several different classes of antioxidants.
The volume ends after having largely treated other two interesting topics the IL-1 antagonist discovery and the phosphodiesterase inhibitors, both described with precise data and novelties useful also at therapeutic level.
This interesting book shows in an irrefutable way how combinatorial chemistry in conjunction with HTS can be undoubtedly considered a revolutioning process to study and discovery new drugs in a more simple and fast way.
This book reports the present state-of-the-art on the study of new anti-inflammatory products and can be surely be very useful for the people devoted to research and also for the medical class involved in the different problems linked to the anti-inflammatory and-or skin regenerative processes.
The reading of this short fluent book can attract also dermatologists, immunologists, allergologists, cosmetic chemists and who want to learn more about the charming field of Cosmetic Dermatology.

P. MORGANTI
Editor-in-Chief

FREE RADICALS AND INFLAMMATION

By Winyard P.G./Blake D.R.
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This 17th chapter book is focused on the role of both oxygen-and nitrogen-centered free radicals in inflammatory diseases.
Inflammation, as a physiological protective response which lead to repair the tissue injury, plays a central role in mediating immune mechanism of host defense and wound healing.
Description of this defense response forms the basis of the pathology of many diseases. The immune complexes, recognized as antibody/antigen interactions, induce also all cells such as polymorphonuclear leukocytes (PMN) and macrophages to secrete lysosomal enzymes capable of degrading connective tissue components.
Moreover, the interaction of T lymphocytes with antigen or mitogen working as an antigen-induced inflammatory response increases DNA synthesis, mitosis and the synthesis of lymphokines, cytokines and many other proinflammatory factors.
All these products provide a system of communication between cells by means of molecular signals. Many are the "key players" which mediate the inflammatory process, such as plasma enzyme cascades, arachidonic acid products, platelet-derived mediators etc. However one significant feature of many of these inflammatory mediators, generated from different kind of cells, is that reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be involved in their mechanism of action.
Such capacity infers a potential for cells to utilize ROS/RNS as a means of rapid antimicrobial defense and/or to employ them as efficient signaling molecules in the communication and relay of messages, indispensable also to self-limiting the inflammatory response.
As matter of fact, recent studies show also that the signaling capacity of many forms of ROS are not only associated with toxicity and pathology but have controlling influences in many physiological processes such as the acute inflammatory response.
What are reactive oxygen and nitrogen species?
Oxygen is the major biological-oxidizing agent used from all aerobic life forms through the stepwise enzymatic reductions of this molecule. The reduction generates a number of ROS with unpaired electrons such as superoxide, and hydroxyl radical. As matter of fact oxygen, having two electrons orbiting alone in separate orbitals with parallel spin, makes it well suited for receiving electrons, one at a time, in an ordered fashion.
In the same way exist also nitrogen based reactive species (RNS), such as nitric oxide (NO) and peroxynitrile.
In this way seems that low levels of ROS may act as signaling molecules that initiate reparative responses leading to resolution of the inflammatory response and re-establishment of tissue integrity. Damage and response can than occur almost simultaneously. Moreover radical gases, such as O.2-,

as well as hydrogen peroxide, can influence all these connected processes as redox-based mediators. Potential toxicity of ROS is controlled by an extensive and physiological range of antioxidant systems intrinsic to aerobic cells, such as superoxide dismutase, vitamin E and vitamin C. A significant increase in ROS generation or a decrease in cellular antioxidant/scavenger defenses, disrupting this balance, results in a state of oxidative stress.

All these problems are well described in the chapter 1, 2 and 3.

Neutrophil-generated ROS have been reported to cause damage in a number of inflammatory diseases. In fact neutrophils are attracted to infection sites by chemio-attractants secreted by the excited cells of the immune system.

Through the receptors (placed on their plasma membranes) neutrophils may initiate the complex series of events which prepare their cytocidal role.

All the mechanism involved are described in an extensive way in chapter 4.

The 5th chapter introduces the reader on the enzymatic synthesis of nitric oxide (NO), focusing the discussion on the important role that it has in defense mechanism of mammals, meanwhile its activity in inflammatory diseases and bone destruction has been well documented in chapters 11 and 12.

NO is a small molecule with similar chemical and physical characteristics to molecular oxygen and its synthesis from l-arginine seems to affect a large range of hiochemical and physiological processes of body systems.

As an example, NO synthesis increases during many diseases, particularly those involving infection and inflammation.

What is interesting to remember is that nitrate of our diet, when swallowed, is rapidly absorbed and at least 25% is concentrated in the salivary glands where it has reduced to nitrate ($NO.2^-$). Saliva containing large amount of nitrite is then acidified in the normal stomach to produce nitrous acid, which could potentially nitrosate amines to form N-nitrosamines, which experimentally are powerful carcinogens. But in general it was found no relationship or no inverse relationship between nitrate intake and cancer in humans.

So those individuals who had a high nitrate intake had a lower rate of cancer, and in animal studies it has been impossible to demonstrate an increased risk of cancer (or any other adverse effect) when nitrate intake is increased. Therefore it is clear that acidified nitrite is produced on mucosal surfaces, and that this combination is effective in killing a variety of human gut and skin pathogens, but we have no definite evidence as yet this mechanism is truly protective in humans exposed to a contaminated environment. We don't yet completely understand the mechanism by which NO and other nitrogen oxides provide selective toxicity towards pathogens.

Further understanding of the complex chemistry of nitrogen oxides may help develop new antimicrobial therapies based on augmenting what seems to be a single and effective host defense system.

As matter of fact the most likely physiological role of L-arginine/NO pathway is in mediating the effects of mechanical stress on skeleton. Therefore evidence that NO is generated in inflammed joints has raised hopes of improving treatment by modulating the intrarticular activity of NO by use, for example, of NOS inhibitors.

In fact if NO can be manipulated to modulate chondrocyte metabolism in the appropriate manner, this would have for reaching consequences for the treatment of osteoarthritis (OH) as well as enhancing the repair of cartilage and other intrarticular tissues.

Other way, it was also demonstrated that estrogens provide an important stimulus to production of NO in the vascular system and this may explain the protective effects of hormone replacement therapy (HRT) on cardiovascular disease in women. Evidence that xantbine oxide reductase (XOR) is

capable of reducing nitrate to NO adds a new dimension also to the potential role of this enzyme in the inflammatory process as reported in chapter 6.
As matter of fact has been demonstrated that XOR is a generator of ROS in inflammation signal transduction.
Alternatively XOR could have a cellular role as an NADH oxidase, able to form superoxide radicals and direct them into the phagocytic vacuole. Moreover XOR, widely distributed in mammary endothelium, has been also identified as the source of destructive oxygen radicals in ischemia-reperfusion injury.
Now it's well known how the co-ordinated inflammatory response is linked to the increased transcription of a defined set of genes. Many of these "inflammatory genes" are target genes for NF-kB, capable not only to mediate apoptosis, but also to block apoptotic cell death in response to a variety of stimuli.
In fact, the cellular signaling events leading to NF-kB activation may be initiated by a wide variety of receptor-stimulus interactions. The environment associated with a particular cell type may also influence the nature of the signaling cascades that precede NF-kB activation, where the ultimate event is IkB phosphorylation.
Moreover the reducing systems that exist within the cell are presumably as important in redox-regulated transcriptional events as those contributing to oxidative stress. Therefore the redox balance of the cell, playing a key role in events which both precede and follow the association of IkB from NF-kB, needs to be delicately balanced so as not to perturb the physiological function of the transcription factor: Activator protein - 1 (AP-1).
It is to remember that treatment with antioxidants seems to activate AP-1 through a specific DNA site.
For these reasons oxidation and reduction of biomolecules are considered to be "signals" in certain instances and are utilized for the maintenance of cellular homeostasis. At this purpose since NF-kB regulates expression of cell adhesion molecules (CAMs), it is considered to play a fundamental role in inflammation and hematogenic cancer cell metastasis.
How the cell defends itself?
The first line defense system is to reduce peroxide by GSH in the presence of gluthatione peroxidase.
The second line defense is to repair the oxidized macromolecules by thioredoxin (Trx). Therefore, it appears that while GSH is directly involved in scavenging the radical oxygen intermediates (ROI), Trx appears to participate in the cascade by repairing the oxidized proteins through its reducing activity.
Moreover involvement of ROI is suggested at least in one of the essential steps of the NF-kB activation pathway, since the signaling seems to be efficiently blocked by pretreatment of the cells with antioxidants such as N-acetyl-l-cysteine (NAC) or a-lipoic acid.
For these reasons anti-oxidants are considered to be effective NF-kB inhibitors.
This topic is well explained in chapter 9.
Many chronic joint inflammatory processes, such as rheumatoid arthritis (RA), are associated with the sustained over production of NO., O.2- and possibly other radical species. Many recent studies reported in chapter 8 suggest that reduced oxygen species may initiate and/or perpetuate chronic joint inflammation by activating the transcription of a variety of different genes known to be important in the inflammation response. For example, the cytokine-receptor interaction may activate NF-kB. Once activated, NF-kB translocates to the nucleus of the cell where it binds to its consensus se-

quence on the promoter - enhancer region of different genes, thereby activating the transcription of genes known to be important in the immune and inflammatory responses.
Moreover NO. seems to be very effective to modulate leukocyte-endothelial cell interactions, even if its mechanism of action has not been clearly delineated.
What is sure is that the enhanced leukocyte infiltrate observed in human synovitis and RA, seems to be mediated by the interaction between leukocytes and specific endothelial cell adhesion molecules (ECAMs).
At this purpose eukaryotic cells contain many specialized enzymes (superoxide dismutase and catalase) devoted to the removal of ROS. In addition, many small molecules such as vitamins C and E have the ability to neutralize oxidants.
Hence, deficiencies of antioxidants or increased formation of ROS can produce oxidative stress. Therefore, an antioxidant therapy may help slow down the degenerative changes observed in the inflammed joints establishing a new approach to treat, for example, RA (Rheumatoid Arthritis).
Although there is overwhelming evidence that the oxidative stress occurs in human inflammatory diseases, and the probability exists that there are as many types of "inflammation" as there are agents that provide it, chronic inflammation and its persistence is an intellectual conundrum.
Who or what signaled death for the T cell and where did it happen?
What "weapon" was used, cytokines and/or ROS?
By the way NO, for example, seems to exert both protective and damaging actions in various cell types; No has also been shown to directly induce apoptosis in various cell lines and the mechanism of the proaptotic signal of NO is not clear until today.
The relation, also, between vitamin E and vitamin C in vitro is well documented, although how these two vitamins interact in vivo is not so clear.
It is likely however, that no antioxidant works in isolation, and until we have a better understanding of the "working relationships" between various antioxidants, it will be difficult to design clinical trials effectively.
In addition, there must exist a balance between antioxidant defense and repair systems and pro-oxidant mechanism of tissue destruction that we have to better understand.
This way ends this interesting book that explain in a very simple and efficacious way the role of radicals within the contest of all stages of inflammation.
Through this book the reader receives all the answers about the role played by free radicals and some of the solutions to better treat the inflammatory stage typical of several relevant pathologies. The book can be useful for the whole medical class and for who is involved in studies that require a deeper knoledge in those complex mechanisms regulating our cell lifetime like biologists, physiologists but also cosmetic chemists devoted to the formulation of "active" cosmetic products. In fact, fundamental rule always remains "primum non nocere" and to avoid any damage is necessary to know.

P. MORGANTI
Editor-in-Chief

SCARLESS WOUND HEALING

By H.G. Garg and M.T.Longoker
2000. 335 pages Hardcover
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Even if excellent progress has been made in wound healing, still today it is not possible to prevent repair defects.

This interesting book, in 15 chapters provides an overview of all the research developed in this fundamental biological area, leading from the alterations in the composition and organization of the matrix in the scar tissues, through the role of different macromolecules in wound repair, to artificial covering materials used for wounds.

Following an injury, the repair process of the skin entails removal of damaged tissue and laying down of a new extracellular matrix (ECM) over which epidermal continuity can be reestablished. During this process, known as scar formation and maturation, proteoglycans play an important role in the organization of an aberrant skin architecture during injury repair.

It has been found that the solfonation of proteoglycans (GAGs) increases in different scars to different degrees, and that chlorate ions eliminate sulfation to various degrees depending on the concentration of chlorate ions. The fibroblasts present in the healed skin tissue are the tissue elements responsible for the biosynthesis of the matrix structural components and different are the structural changes that occur in different types of scar formation following an injury.

Therefore, both the size of the GAGs side chains and the protein core change; an alteration in the degree and location of sulfation, and differences in the proportions of the different species of dermaton sulfate proteoglycans, biglycan (PG-I) and decorin (PG-II) occur.

In fact GAGs, key matrix components in scarring after an injury, serve as the functional "business ends" while the core proteins serve to direct transport and channeling for the biosynthesis, placement, and maintenance in appropriate locations.

However it is well documented that dermatan/chondroitin sulfate proteoglycans are important factors in healing, and that their structures can be modified greatly under conditions of low sulfate availability. Eventual modifications of proteoglycans, sulfonation by ingestion of dietary sulphydryl-containing aminoacids, could be a potential remedy for scarring of individuals not capable of synthesizing sufficient sulfate.

The chemistry of scarring and the potential effects of the sulfate depletion are well described in chapter 1 and 2.

The repair of injury is orchestrated by a variety of soluble and insoluble effectors such as cytokines, grow factors, extracellular matrix components, etc., but the cell surface proteoglycans (PGs) named syndecans, seem to control the wound repair process.

In fact syndecan-1 binds cell via its heparan sulfate chains to a variety of extracellular matrix components, but it also acts as a dominant negative inhibitor of cell proliferation during wound repair.

Genome organization development and regulation of syndecans during tissue injury are all descri-

bed in chapter 3.
Normal wound healing processes can be temporally categorized into three major groups overlapping in time: inflammation, tissue formation, and tissue remodelling.
Immediately after skin injury, a temporary repair is achieved in the form of a clot that plugs the defect. Inflammatory cells and their fibroblasts and capillaries invade the clot to form a contractile granulation tissue that draws the wound margins together.
At this purpose molecular and cellular activities include cell proliferation, cell adhesion, cell migration, extracellular matrix (ECM) production and reorganization, and cell apoptosis.
All these activities require special signals that include integrins, cytokines grow factors and matrix components.
The integrins seem to play the role of critical players, as they are involved in all phases of wound healing after an injury.
The understanding of integrin regulation will lead to the comprehension of fundamental wound healing knowledge and the development of therapeutic approaches.
Chapter 4 is focused on this theme.
The major protein component of granulation tissue and dermis is collagen.
In dermis the collagen fiber bundles are arranged in a basketweave pattern; in granulation tissue, they are randomly organized; meanwhile in normal scar they are arranged in parallel arrays.
Therefore, the cellular organization of collagen fibrils into fiber bundles is important for the integrity of skin and scar in terms of volume, stability and strength. Knowledge of how the collagen fiber bundles are organized within the wound site may point out a strategy for controlling scarring and promoting scarless repair in the injured adult.
In chapter 5th the role of collagen in scarring and regenerative repair it's reviewed.
Evidence suggests that interaction and signaling between the cell and the extracellular matrix (ECM) is critical to homeostasis following injury.
The restoration of tissue structure and function following injury involves a balance between repair that is suitable for continued normal function and repair in which the deposition of scar modifies the normal architecture of the tissue. The ability to modify this balance in favor of a normal architecture would represent an important advance in tissue engineering and restoration of tissue integrity.
At this purpose the molecular weight of hyaluronan (HA) appears to determine the type of scarring, exerting direct effects on cells and on the extracellular matrix. For instance, high concentrations of high-molecular weight HA inhibit white cell functions, while lower molecular weight of HA enhances them.
The interaction of HA with its cellular-binding proteins, the hyalodherins, appear to be key to tissue response-to-injury.
Therefore, the interference with the binding HA to its receptors holds promise as furthers novel targets to modify the response to injury and potentially reduce scar formation.
As matter of fact, some studies indicate that, under appropriate conditions using suitable hyaluronan, it appears that healing is enhanced and scarring reduced or eliminated.
But how is hyaluronan composed?
HA is made of repeating dimers of b-glucuronic acid and N-acetylglucosamine which form linear, unbranched polyanionic HA chains.
In the skin it has protective shock-absorbing and structural stabilizing role in the extracellular matrix (ECM).
The dermis contains more than half of the total HA in the body filling the space between collagen

and elastin fibrils and maintaining the separation between fibers. Its water-retaining capacity suggests also its role in facilitating the transport of metabolites, and preserving tissue hydration. Finally HA provides a beneficial effect with regard to the quality of tissue repair scar formation, playing a key structural role in the organization of the fibrin matrix.
These and other interesting goal are well described in chapter 6 and 7.
In order to understand defects in the repair process after an injury, it is important to know the molecular and cellular biology of fibroproliferative disorders, such as keloids.
Keloids, characterized by an overabundant ECM, represent a pathological response to cutaneous injury creating disfiguring scars with no known satisfactory treatment.
Compared with normal dermal fibroblasts, keloid fibroblasts show an aberrant response to metabolic modulators implicating their possible role in the pathogenesis of keloids. The exact understanding of molecular mechanism of keloid scar formation will provide a basis for the development of more effective keloid treatments.
Hypertrophic scarring (HSc) is another dermal fibroproliferative disorder characterized by excess ECM deposition in the dermis and subcutaneous tissues. The key differences are that HSc remains within the confines of the wound margin and eventually undergoes partial resolution spontaneously whereas keloids invade surrounding tissue and rarely regress spontaneously.
Cellular biology of these fibroproliferative disorders and the emerging therapies are reported on chapter 8 and 9.
New and advanced studies in the molecular and cellular biology of fetal wound healing have opened new ways in understanding/controlling scarring:
Therefore, the role of growth factors in wound healing process has been studied and controlled extensively.
At this purpose, transforming growtb factor-beta (TGF-b) appears to play a central role even if its exact mechanism of activation is still not well understood.
What it's sure is that TGF-b is a potent chemoattractant of macrophages, neutrophils, and fibroblasts, and that these cells in turn secrete TGF-b when activated.
In fact, while TGF-b appears to play a key role in tissue repair, its excessive or abnormal production can be detrimental because TGF-b appears to be responsible for excess extracellular matrix deposition. The role of TGF-b in cutaneous scarring is described in chapter 10.
Mammalian embryos can heal wounds without apparent scar formation and in absence of an inflammatory response. On the contrary, adult wound is characterized by the formation of granulation tissues stimulated by the cytokines and growth factors of the acute inflammatory response.
Comparing how embryonic and adult tissues heal will supply us with possible therapeutic strategies for preventing excessive fibrosis and scarring in clinical medicine.
The current knowledge of the intrinsic properties of the fetal skin and wound healing are reported in chapter 11 and 12.
Adult mammalian dermis does not regenerate spontaneously but this regeneration may be induced by use of appropriate agents, under appropriate conditions.
Several are the approaches to solve this problem by the use of new advances in tissue engineering skin substitutes.
Most skin substitute available today addresses issues of epidermal and dermal regeneration with a goal of providing safe wound coverage and durable reconstruction of the integument.
The continuing evolution of biotechnologies will create new generation of innovative products more and more active and safe. The techniques used up today and the new ones set up are widely descri-

bed in the 13th and 14th chapters.
Chapter 15 is focused on the usefulness of hyaluronan-based membrane, SeprafilmÒ for the prevention of post-surgical adhesions.
This bioresorbable membrane is composed of a matrix of sodium hyaluronate (HA) and carboxymethyl cellulose (CMC) that has been chemically modified with 1 - (3 dimethylaminopropyl) - 3 - ethyl carbodiimide (EDC).
When chemically modified HA/CMC form a water insoluble complex that can be formed into a membrane suitable for placement on injured tissue areas. This membrane that bioresorbs in vivo within 28 days after implantation; significantly reduces the incidence and severity of post surgical adhesions following a variety of surgical trauma.
This text contains precious updated news on the wound repair process and a clear description of the innovative means used to prevent the formation of keloids and/or unpleasant scars. So that, it's a useful tool for plastic surgeons, dermatologists and/or biologists aimed to know more about this delicate and severe field.
Furthermore the book could be interesting also for cosmetic chemists and all the people involved in the aesthetic field.

P. MORGANTI
Editor-in-Chief

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