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Skin ageing

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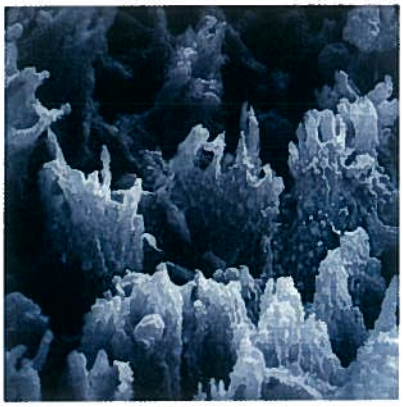
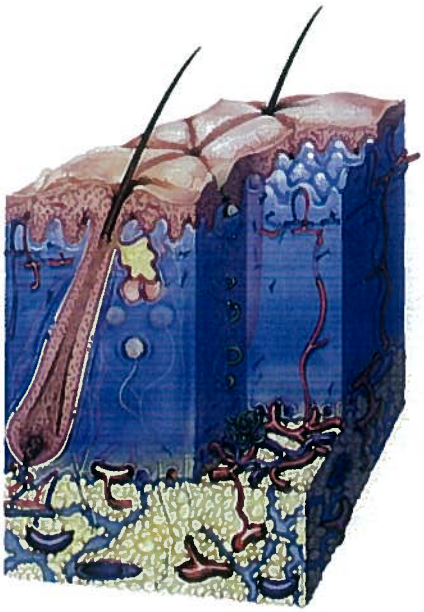
SKIN AGEING





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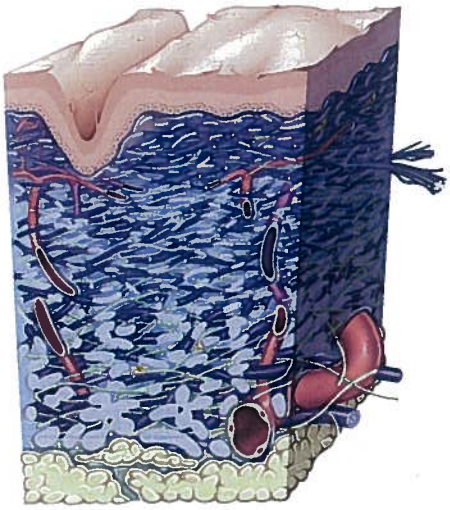

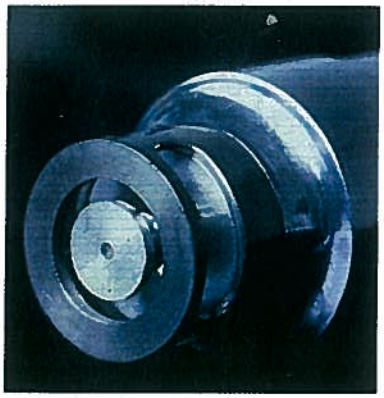

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Beardsley. Private collection

Senescence is not a slope that everyone descends at the same speed. It is a series of irregular steps which some tumble down more quickly than others.
Howell quoted by Simone de Beauvoir
[S. de Beauvoir. *La Vieillesse.* Gallimard, Paris, 1970]

SKIN AGEING : A SPECIAL CASE

Ageing is a physiological phenomenon which develops slowly, inescapably and irreversibly. It leaves its mark by modifying anatomical and histological structures and altering the correct functioning of various organs and tissues.

The theories of ageing

Several theories have been proposed to explain the origin of this process. Genetic theories put forward the existence of a limited number of genes the activation or repression of which regulates cellular ageing ; the error catastrophe theory maintains that a first error infiltrated into the cellular machinery and not repaired will be the starting point for a succession of errors ; the environmental theory incriminates the harmful role of free radicals on the integrity of cellular constituents (proteins, DNA, lipids) while finally, the mitotic clock theory suggest that the progressive loss of the extremities of the chromosomes (telomeres) acts as a stop signal for the cell cycle. None of cited theories is absolute and all remain paths to be explored in order to find the cellular targets and the means of preventing, slowing or treating the signs of ageing in an era where, at least in economically developed countries, life expectancy is continually increasing.

Age and the appearance of age

Cutaneous ageing, from a cosmetic and dermatologic point of view, is particularly interesting because of some unique caracteristics of the skin : it covers the whole body and is the interface between the external environment and the organism. It is also subject to two types of ageing : intrinsic or chronological ageing due to age and genetic factors and extrinsic ageing due to the environment and its main factor, the sun.

This means that age and the appearance of age are separate entities and that significant disparity may be observed between the two. Chronological ageing affects all parts of the body but it is best assessed on covered areas not exposed to the sun. Apparent age results from the superimposition of chronological ageing and photo-ageing and can easily be appreciated in areas exposed to the sun such as the face, neck or arms.

While clinical signs related to chronological ageing become apparent late in life, signs caused by ultraviolet rays appear early and leave the often spectacular mark of photo-ageing. The passing of time and the environment not only affect the appearance of the skin but also its function. The skin is not a simple envelope : it is subjected to mechanical strains which it withstands due to its flexibility. With time, its properties of elasticity and tension are lost, its protective, metabolic and sensory functions as well as its capacity to adapt diminish.

The link between clinical aspects and molecular biology

Because skin ageing is not only a visible phenomenom but also a series of physiological events, both the clinician and the molecular biologist have reason to be implicated and active in skin ageing research. For example, the deep wrinkles observed in skin exposed repeatedly to the sun are related to a disorganisation of the network of elastic fibres in the dermis. Cutaneous ageing is studied not only in vivo, in man, but also in vitro in reconstructed skin models. Subjected to ultraviolet rays, skin models reproduce the effects of UVA and of UVB observed in vivo and, thus serve as essential tools for evaluating sunscreens.

The study of cutaneous ageing also calls on biophysical methods to characterise the skin in its natural state and quantify structural and functional modifications to it. Evaluations are carried out with more and more precise measurement instruments and non-invasive imaging techniques which allow the skin to be explored « in depth ».

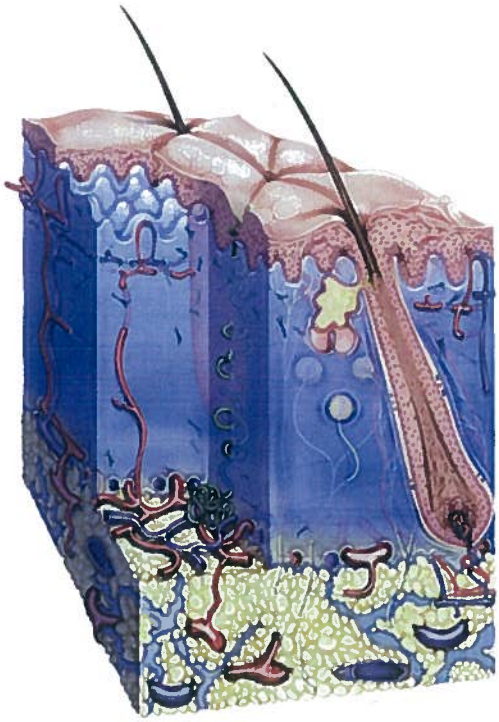


Diagram of the skin showing its three compartments : the epidermis, the dermis and the hypodermis.

STRUCTURE OF THE SKIN

Anatomically, the skin is an organ composed of three compartments : the epidermis, the dermis and the hypodermis. In an adult, the skin weighs between 3.5 and 4.5 kilograms and its total surface area can be up to two square metres. Its thickness varies between 1.5 and 4 mm depending on the region of the body considered, being thick on the soles of the feet or on the palms of the hands, thin on the eyelids. This envelope, combining properties of suppleness and strength, not only protects the organism from external attack but also represents a site for exchange between the inside of the body and the exterior.

The epidermis : a continually renewed protective envelope

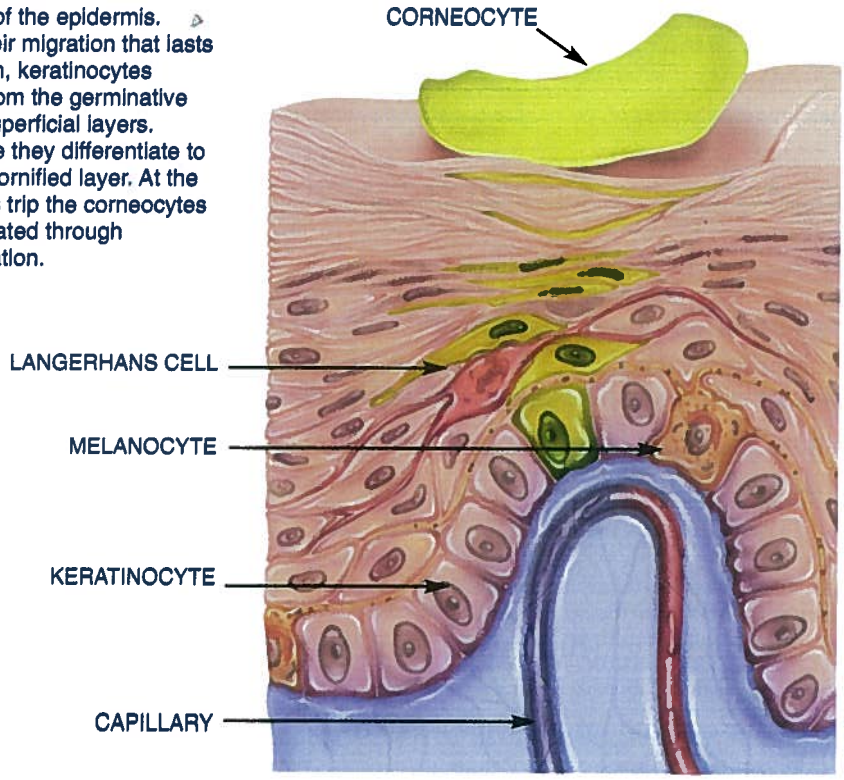
The epidermis (from the Greek *epi*, above and *dermis*, the skin) forms the outer compartment of the skin, in direct contact with the external environment. Its keratinised multi-stratified structure is composed of a number of layers of keratinocytes, cells representing nearly 85 % of the epidermal cellular population. Renewal of the epidermis, a process requiring approximately 30 days, begins in the basal layer, also called the germinal layer, as it is here that the keratinocytes multiply. Each keratinocyte gives rise to two identical daughter cells : one remains in place

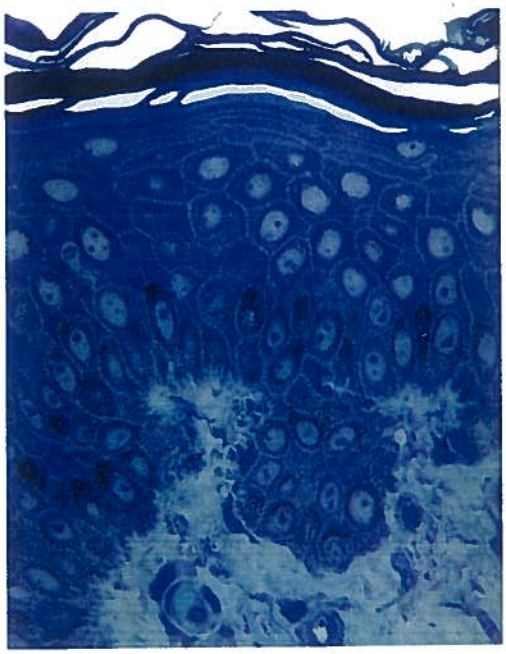
to divide again while the other loses its ability to divide and progressively rises into the upper layers where it begins its programme of epidermal differentiation.

The migrating keratinocytes are transformed through successive stages. They increase in size, their shape changes to become more and more fusiform and flattened in the superficial layers. The cellular structure is modified, the size of the nucleus decreases and the cytoplasm becomes filled with filaments of keratin. The cornified layer, the most superficial layer composed of corneocytes which are anucleate, metabolically inactive keratinocytes almost completely composed of keratin. The corneocytes are strongly linked one to another by a lipid cement and cohesive proteins which eventually are degraded through enzyme actionuntil they lose their cohesion through programmed enzyme action. The corneocytes, as a result, detach one by one in the process of desquamation.

Keratins represent 95 % of epidermal proteins and due to their rigidity and their insolubility confer on the skin its protective function. The lipid cement linking the corneocytes and the hydrolipidic film

Renewal of the epidermis. During their migration that lasts one month, keratinocytes migrate from the germinative layer to superficial layers. Meanwhile they differentiate to form the cornified layer. At the end of this trip the corneocytes are eliminated through desquamation.





Fairly thin section of black skin stained with toluidine blue. In an apical position within the supra-basal keratinocytes, clumps of melanosomes cover the top of the nuclei.

composed of sweat, sebum and lipids covering the surface of the skin participate in the latter's barrier selective function.

Cells specialised in skin pigmentation and in immune defence

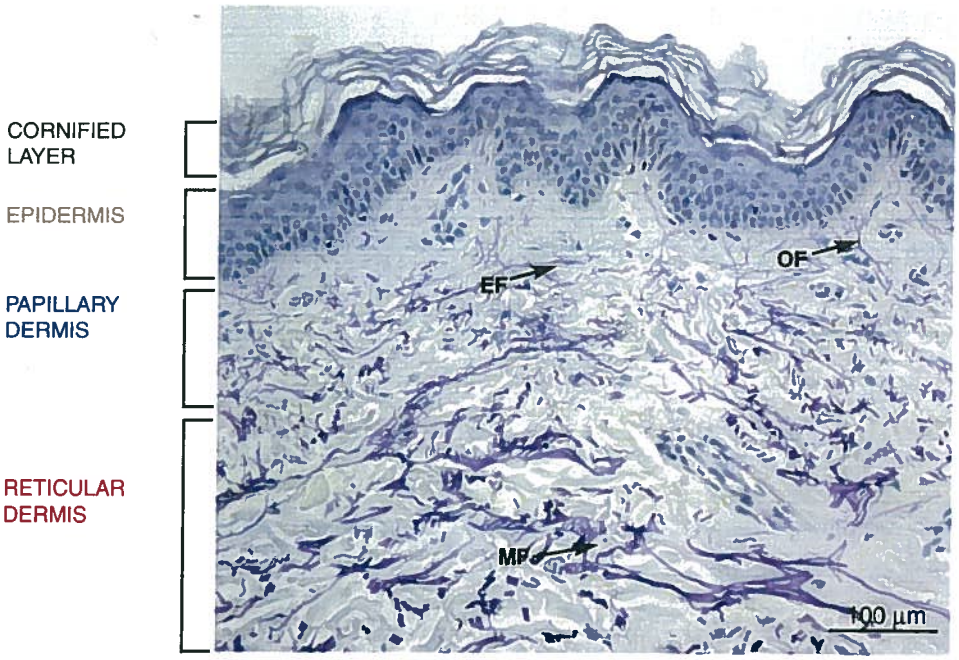
Besides the keratinocytes, the epidermis has other highly specialised cells :

- the melanocytes, located in the basal layer, are dendritic cells which synthesise melanin, the pigment which plays a role in skin colour. Melanins are produced and stored in small sacks called melanosomes, which are then transferred via the dendrites to neighbouring keratinocytes. One melanocyte is capable of supplying about 40 keratinocytes which, as they migrate upwards, will disperse the pigment through the epidermis. In general these pigments do not reach the superficial layers since the melanosome membranes and the melanin are progressively digested by enzymes. Two types of melanin are produced : eumelanin (brown/black) and phaeomelanin (red/yellow). Their synthesis is determined genetically : black people synthesize essentially eumelanin and red-headed people only synthesize phaeomelanin. The mixture of these two pigments in different proportions gives all the varieties of skin and hair colour.

- the Langerhans cells, located in the suprabasal layer, are also dendritic cells. They play an essential role in the epidermal immune response as they detect foreign bodies (antigens) which have penetrated into the epidermis and present them to lymphocytes of the dermal lymphatic ganglia which eliminate them.

The dermis : a network structure

Skin thickness is largely determined by the dermis. The dermis is the supporting tissue of the skin, compressible, extensible and elastic. Its properties are the results of an architecture which itself depends upon interactions between the constituents of the extracellular matrix and fibroblasts which both synthesise and break down the former. Thus, collagen and elastin fibres form a fibrous web which is bathed in a gel of macromolecules (proteoglycans and glycosaminoglycans) while struc-



Histological section of young skin : Luna stain (coloring elastic fibres). OF = oxytalan fibres, EF = elaunin fibres in the papillary dermis and MF = mature elastic fibres of the reticular dermis. The fine elastic fibres or oxytalan fibres, orientated perpendicularly to the surface of the skin, form a candelabra-shaped network in the hollows of the dermal papillae. Elaunins, thicker fibres orientated horizontally, complete the elastic network of the papillary dermis. The mature fibres are found in the reticular dermis.

tural glycoproteins provide the interface between the fibroblasts and the extracellular matrix which surrounds them.

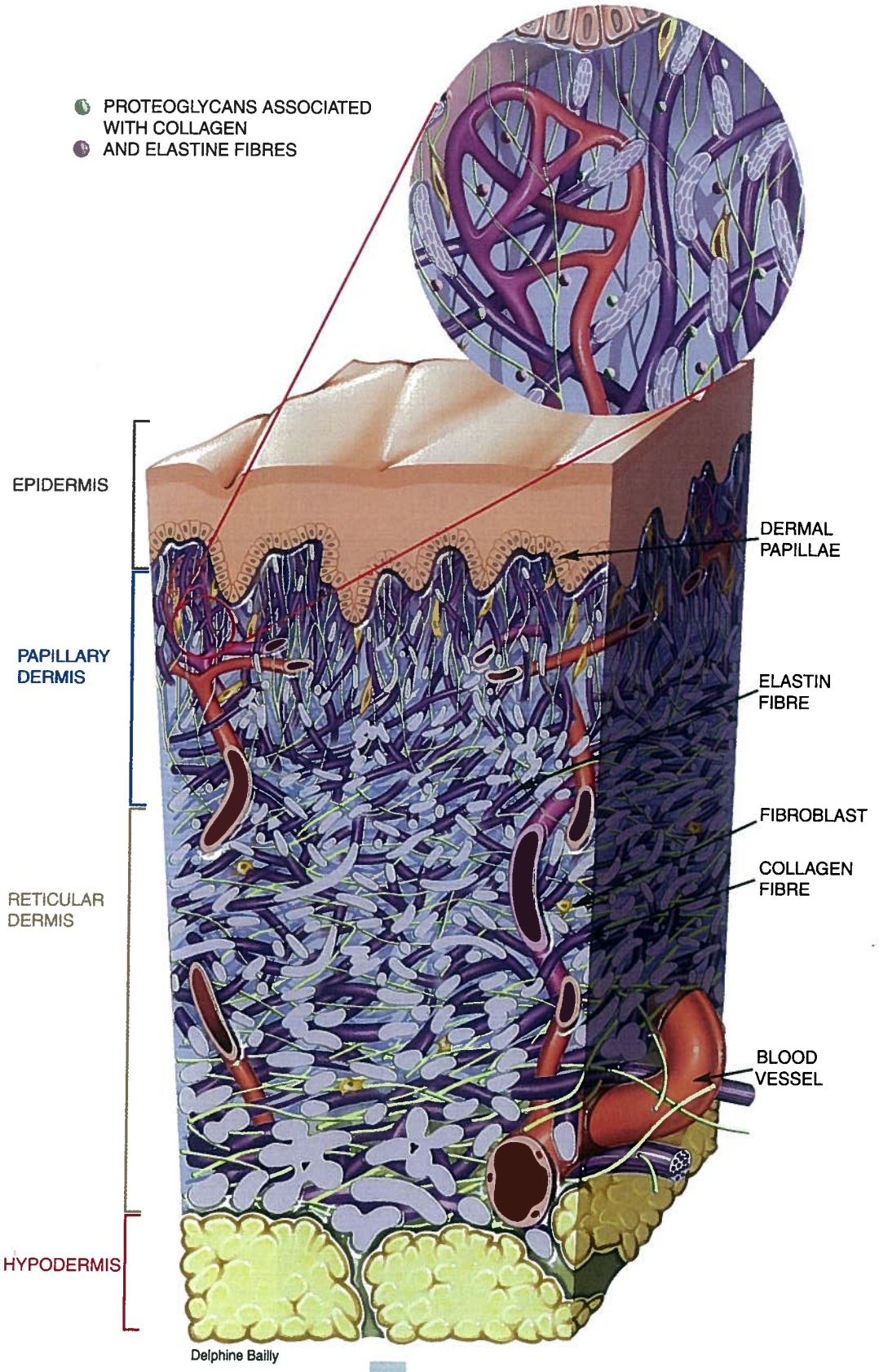
The dermis is subdivided into two areas : a narrow superficial area or papillary dermis and the deeper reticular dermis which makes up more than 4/5 of the total thickness of the dermis.

The papillary dermis in young skin is characterised by the presence of involuting dermal papillae. It is a loose connective tissue formed of collagen fibres organised in bundles and elastic fibres composed of oxytalan and elaunin fibres. The oxytalan fibres, anchored to the dermo-epidermal junction and vertically orientated relative to the surface of the skin, give the papillary dermis its elasticity. It is in this area irrigated by blood and lymphatic capillaries that nutrient exchange takes place with the non-vascularised epidermis.

The reticular dermis is composed of collagen fibres arranged in waves which criss-cross each other in all directions but always remain parallel to the skin surface. The collagen associates with elastin fibres in a thicker fibre network which is more and more compact with increasing depth. It also contains arterioles, venules, small nerves, pilo-sebaceous follicles and the excretory channels of the sweat glands.

A unique and complex structure maintains the cohesion between the epidermis and the dermis. This is the dermo-epidermal junction which defines area between the basal cells of the epidermis and the most superficial layers of the dermis. On the epidermal side, it provides anchorage for the keratinocytes to the basal membrane via attachment structures called hemi-desmosomes, and on the dermal side, it provides solid attachment of the dermis to the basal membrane via the anchoring fibres. The basic network providing mechanical stability is composed of the assembly of collagen IV molecules linked to a second network formed of laminin molecules.

Diagram of the structure of the dermis of young skin.
The papillary dermis is formed from loose connective tissue enclosing fine isolated collagen fibres (usually orientated perpendicularly or obliquely relative to the plane of the basal membrane), terminal ramifications of the elastic network, terminal capillary loops of the vascular network and nerve endings.
The reticular dermis, much thicker than the papillary dermis, consists of dense connective tissue where the thicker collagen bundles and the elastic fibres criss-cross parallel to the skin's surface. The most numerous cells of the papillary dermis form two populations : a fixed population composed of fibroblasts and a mobile population of haemopoietic origin : macrophages, dermal dendritic cells, mast cells and in small numbers plasma cells, lymphocytes etc.



Delphine Bailly

The hypodermis : an energy store

The hypodermis is the innermost and thickest layer of the skin. It serves as a source of energy store for the organism, is responsible for the body's silhouette and plays a part in thermoregulation because of the insulation properties of fat. It protects subjacent tissues from trauma by acting as a shock absorber. The hypodermis is essentially formed from adipocytes, cells which are specialised in the accumulation and storage of fats. These cells are grouped into lobules separated by connective tissue. They are capable of changing their volume depending on the food intake, age and physical activity of the person.

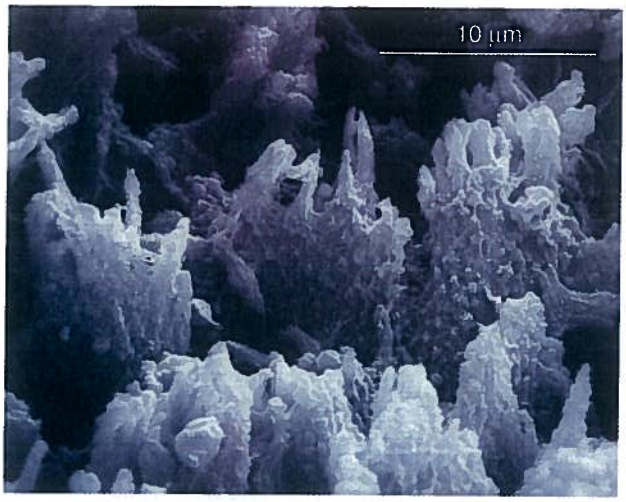
Skin appendages and glands

The skin also has appendages : hair, eye brows, eye lashes and nails, which are epidermal prolongations rooted in the dermis or even in the hypodermis, and sweat glands (eccrine and apocrine) and sebaceous glands, the former playing a role in thermoregulation and the secretion of pheromones and the latter in producing sebum.

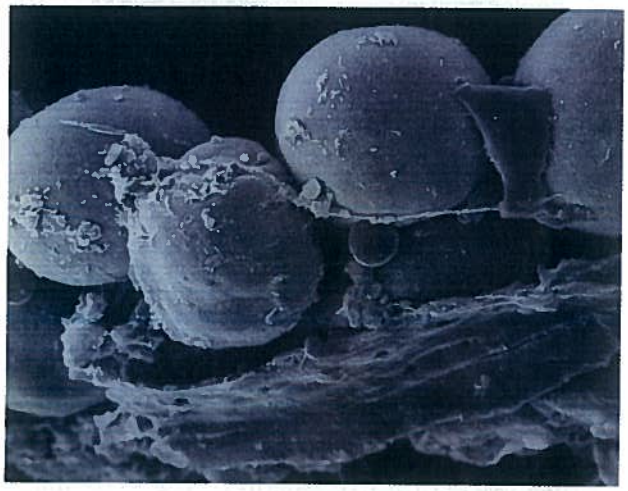
A sensory organ

The skin has a battery of sensory receptors which respond to touch, contact, temperature variation and pain. These stimuli are treated by the sensory nervous system of the skin and are then sent in the form of nerve discharges through a network of fibres to the brain. There they are translated into messages which produce a sensation. The density of these receptors varies greatly from one skin area to another. The face and the ends of the limbs are very richly innervated ; on the finger tips there are at least 2,500 receptors per cm^2. Cutaneous receptors are in the form of encapsulated nerve endings such as Meissner, Pacini and Ruffini corpuscles or free nerve endings some of which, associated with Merkel cells, go as far as the epidermis.

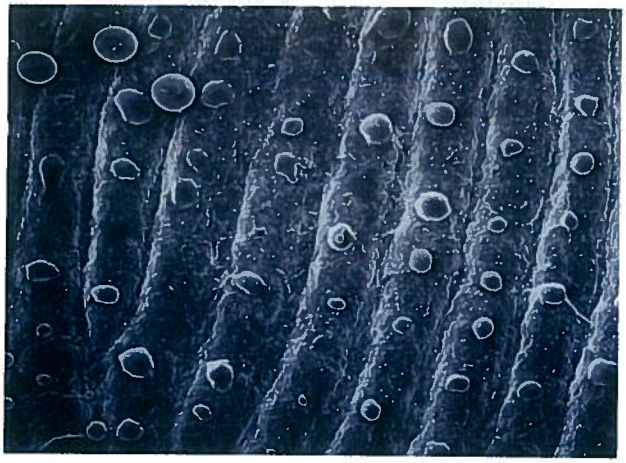
Area of epidermal crests. At the junction between the dermis and the epidermis is a particular structure, 75nm thick, called the dermo-epidermal junction. On the epidermal side, this structure permits anchoring of the epidermal keratinocytes to the papillary dermis and on the dermal side the anchoring fibres interact with the basal membrane forming a network which traps the collagen fibres of the upper part of the dermis.



Adipocytes seen under a scanning electron microscope. Diameter of an adipocyte : 85 µm



Replica of a fingerprint with sweat secretion



Hairs seen through a microscope, a white hair on the right.

THE CLINICAL SIGNS OF SKIN AGEING

Skin ageing depends on several closely linked factors including the individual age of age and the environmental aggression (sun, pollution, wind etc.) he or she is subjected to. Skin ageing has been defined by characteristic clinical features.

Chronological ageing : moderate signs

Modifications of skin protected from the sun are relatively moderate : thinning, fine lines, dryness of the skin, laxness or loss of firmness, benign epithelial proliferation and senile angiomas.

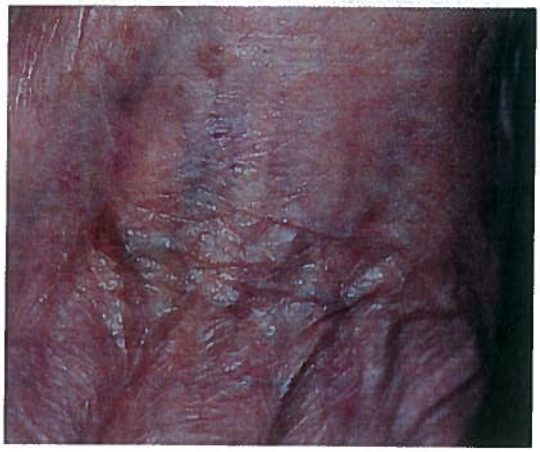
Wrinkles or expression lines are the permanent external signs of the concertina-like action of the skin and the normal furrows of the face constantly produced by the action of the platysma muscles. The sagging folds follow from the loss of tone of the skin's muscles related to the combined action of dermo-hypodermal alterations and gravity, responsible for the pull downwards of the subcutaneous tissue and in particular of fat tissue. The consequence is the appearance of heavy cheeks, a double chin, pouches under the eyes and ptosis of the eyelids.

Seborrhoeic keratoses are epidermal thickenings of melanin-laden keratinocytes. These lesions can occur at any age but are frequent above all in elderly people on the trunk.

Senile angiomas or cherry angiomas result from the dilation of capillaries of the dermal papillae which become convoluted and appear as small bright red lesions which do not disappear on pressure.

Skin dryness : diminished activity of the sweat and sebaceous glands is partly responsible for the disappearance of the hydro-lipid film on the surface of the skin. Desquamation of the cornified layer increases and becomes irregular. The cornified cells clump together giving a feeling of roughness to the skin.

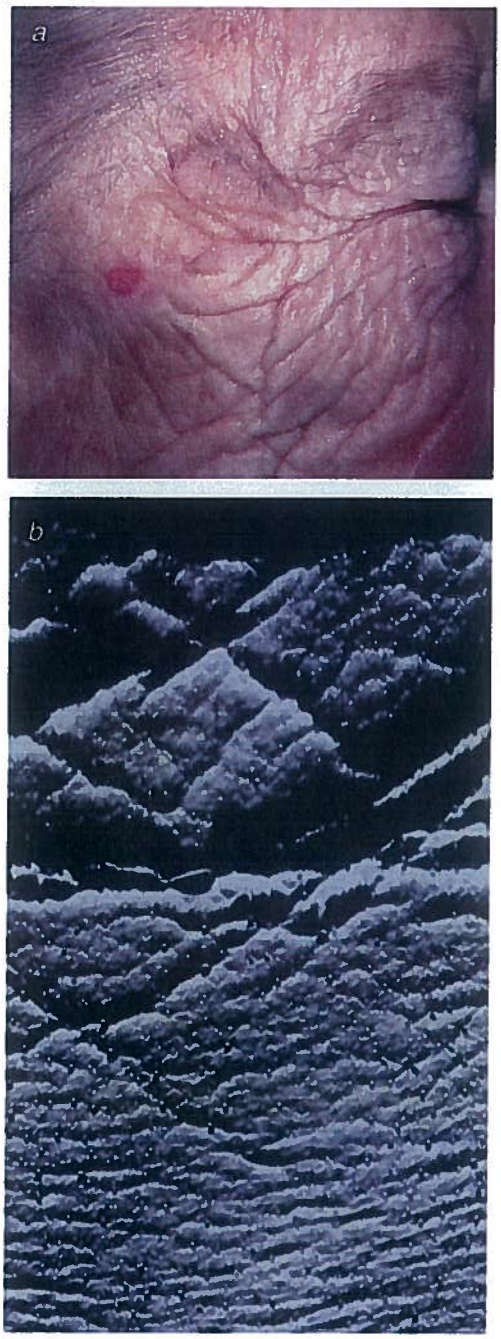
Skin appearance ressembling parchment or cigarette paper : disorganisation of the structure of the dermo-epidermal junction leads to a loss of cohesion between the epidermis and the dermis. One can note the presence of « live spots » due to clumping of melanocytes in the basal layer of the epidermis.



The reduction in quality of the hydro-lipid film gives the skin a grey hue and the regression of micro-vascularisation makes it yellowish.

Photo-ageing : roughness, deep skin furrows and pigmented marks

Modifications in skin exposed to the sun add to the changes previously described and are characterised by two essential symptoms : actinic elastosis and the appearance of marks. Elastosis appears as a coarse skin which is rough and yellowish (citrine skin). The skin is loose, covered with wrinkles and furrows. The rhomboid appearance (from the Greek rhombos, lozenge) of the skin of the nape of the neck of people who have worked all their lives outside (sailors, farmers, builders etc.)



Solar elastosis and wrinkles *(a)*. Micro-cutaneous relief print of the rhomboid area of the neck *(b)*. The upper part corresponds to exposed area, the lower part to the protected area. The lozenge shaped furrows are absent from the protected area.

is a striking illustration of this The skin is marked by deep furrows which intersect and outli ne lozenges of variable size.

When the nape of the neck is not protected from externa attack (sun, wind, cold), the skir eventually forms deep furrows These lozenge shaped furrows are absent from the protected area Irregular hypo or hyperpigmenta tion gives the skin a speckled appearance.

Hyperpigmented lesions can arise either from an excess o melanin in the keratinocytes (senile lentigos and sun blemis hes) or from a proliferation o melanocytes (solar lentigos).

To these signs of photo ageing can be added benign cuta neous tumours such as senile sebaceous adenomas, or malignan ones such as basal cell and spino cellular carcinomas and malignan melanomas.

The phototype plays ar important role in the clinical signs of ageing induced by the sun alterations are more marked anc occur earlier in pale skins than ir darker skins.

The skin appendages are also affected by senescence :

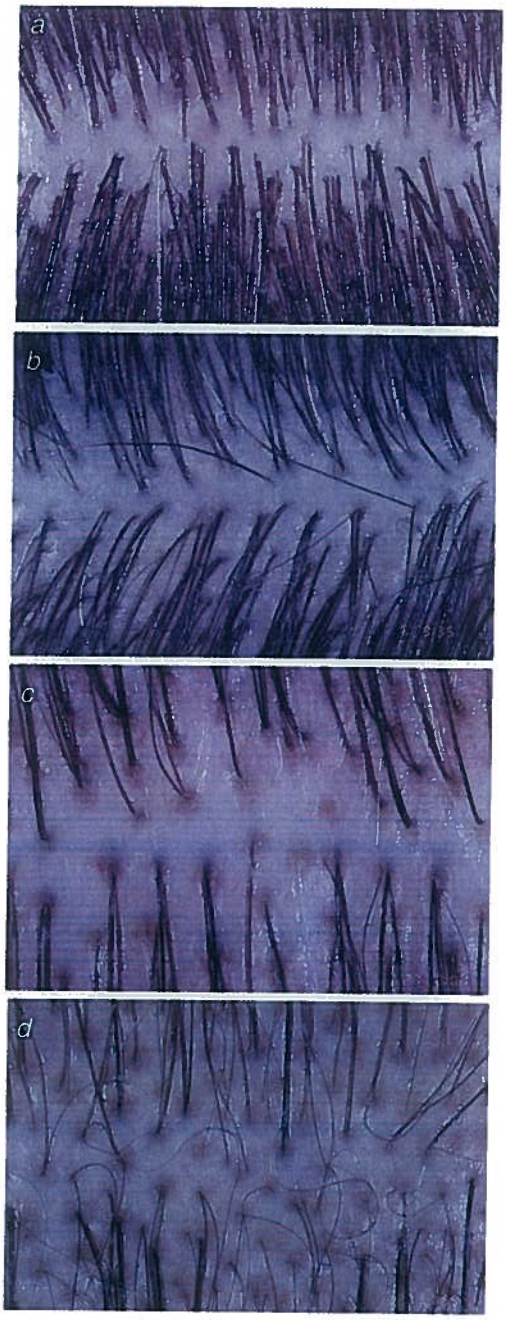
With ageing, the hair becomes sparser, the diameter of each hair decreases and hair color becomes greyish then whitens. In post menopausal women androgens, which are no longer counterbalanced by the oestrogens and progesterone are sometimes responsible for hirsutism of the chin and of the upper lip and an androgenic alopecia.

In men, the number of hairs in the ears and the nose increases, the eyebrows become thicker and stiffer.

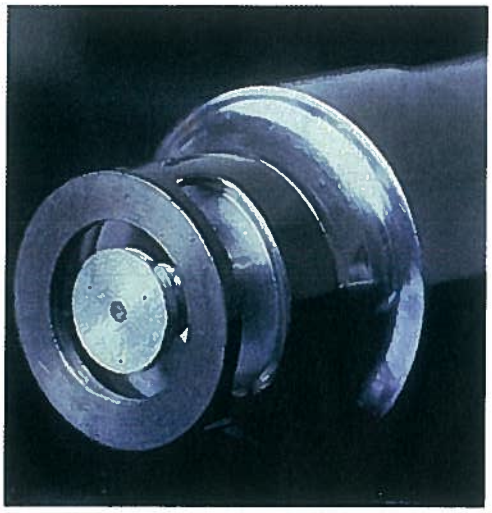
The nails grow more slowly, they are thicker and harder and break more easily ; they are dull with an increase in longitudinal striation and disappearance of the "half-moon".

The glands do not escape the ageing process : the size of the sebaceous glands increases with age but, paradoxically, production of sebum decreases.

The decrease in the number of eccrine glands is accompanied by a reduction in sweat secre tion.



Macrophotograph of a healthy scalp *(a)* ; Macrophotograph of a scalp showing variation oh hair diameter *(b)* ; Macrophotograph of a scalp showing peripilar signs *(c)* ; Macrophotograph of a scalp associating the two parameters (variation in diameter and peripilar signs *(d)*.



The twistometer measures the mechanica properties of the skin. It twists the skin in a zone bounded by the ring and the resultin deformation of the skin (dependent on the elasticity) is measured relative to time.

BIOPHYSICAL METHODS OF EVALUATION

The properties of the skin can be measured *in vivo* using non invasive biophysical methods. Some methods characterise the skin in it natural state, measuring the colour, the level of hydration or the cutaneous topography while others record the skin's response when subjected to external physical or chemical stimuli. Comparison of the measurements obtained from the same subject on an area exposed to the sun and a protected area enables precise evaluation of the impact of the sun's rays on a given parameter. Measurements made on the same non exposed area, repeated over time, provide indications concerning modifications related to chronological ageing.

Only a few of these methods are touched upon in this chapter

The micro-relief of the skin and wrinkle

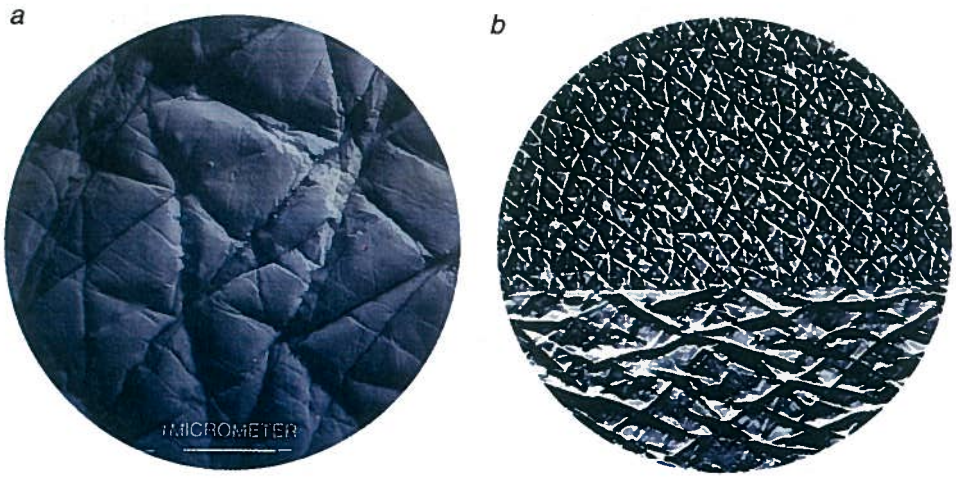
The skin's surface has a certain number of more or less straigh sided depressions that can be classified by their depth and the magnif cation necessary for easily describing them. The most visible are the fold around the articulations and the expression lines of the face which hav a depth of between 100 µm and several mm depending on age and env ronmental factors.

At low magnification (x10), the skin's surface appears as bein organised into a network where parallel furrows intersect forming geome

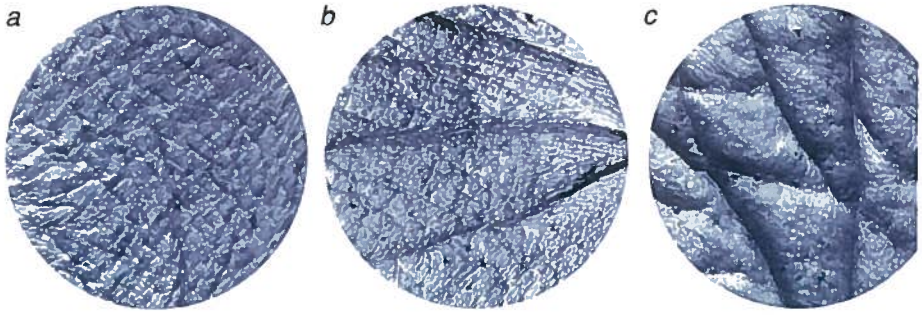
tric shapes. Four groups of lines can be distinguished : the primary lines are wide and between 20 µm to 100 µm deep depending on the site and the age. Secondary lines are finer (5 to 40 µm) : they are extensions of the primary lines and trace diagonals to the previous lines. At higher magnification (x 100), the tertiary lines, which are the edges of the corneocytes and the quaternary lines which cross the corneocytes, can be seen.

For practical reasons such as the presence of a translucent cornified layer which disrupts optical observations (reflection, diffraction), almost all studies are carried out on replicas of the skin moulded in silicones. The global three-dimensional study of skin topography is based on recording shadows and their degree of greyness formed on the imprint of the skin by an incident light detected by an image analyser : with skimming lighting, the shadows created behind the crests (i.e. the skin folds) can be selected by thresholding and measured. Thus the main directions of skin folds can be defined and for each direction the density of lines and their mean depth determined. Using this technique the evolution of the micro-topography can be studied from exact replicas of a precise area of the skin over time and/or before and after the application of products.

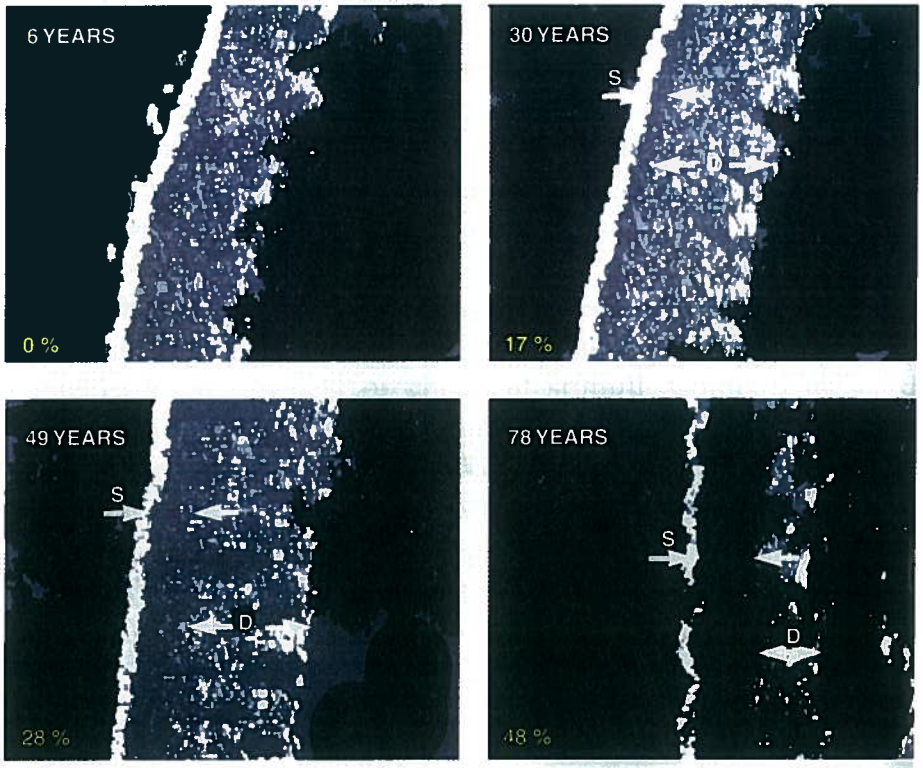
Each skin site adapts and changes depending on local tensions which, repeated throughout life, lead to remodelling the skin texture.



Replica of the surface of normal skin *(a)*. Comparison of the micro-topography of the skin of the forearm of a seven-year-old child and that of a 79-year-old woman. The fine star-shaped network of childhood is replaced over the years by a broad anarchic texture resulting from the underlying disorganisation of the papillary dermis and its junction with the epidermis *(b)*.



Imprint of crow's foot lines. Accentuation of the lines of the crow's foot with age and effect of the environment, here, the rays of the sun. *(a)* 25 years, *(b)* 50 years, *(c)* 50 years or more.



Echograms of the skin of the forearm : the points seen below the line representing the surface of the skin indicate the echostructure of the skin. Each of these « echos » corresponds to the reflection of ultrasounds from the interface between two components of the structure of the skin.
The sub-epidermal black band which appears progressively with age corresponds to a non-echogenic band, the Seneb *(Sub-epidermal non-echogenic band).*
The reduction in echogenicity of the skin indicates a decrease in heterogeneity of the medium and forms a marker of chronological cutaneous ageing and photo-ageing since it is more marked in areas exposed to the sun.
S : surface of the epidermis. D : Dermis.

Skin color

Skin color can be evaluated using a chromameter which measures three parameters of light reflected by the skin during a brief flash on a small area (L* = indicator of the white/black component of the skin, a* = indicator of the red component, b* = indicator of the yellow component). This apparatus is a useful tool for quantifying colour in a given area (e.g. a pigmented area) but is of limited use for overall clinical observation. The differences found between adults and very old people are only significant for the value L which is generally lower in the latter (darker skin).

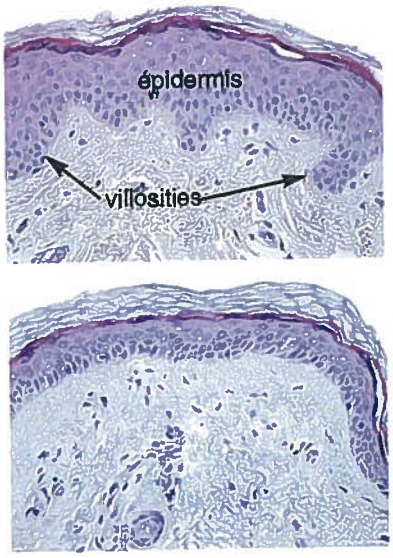
The echostructure of the skin

Echography is a process producing images in real time. A probe emits ultrasounds which are reflected differentially depending on the tissue encountered. The same probe receives the signals returned and transforms them into images. This process is used to measure the echo structure of the skin and obtain information on the thickness : the thickness of the skin is measured by determining the time taken for ultrasounds to be transmitted between the surface of the skin and the interface between the dermis and the hypodermis. The thickness of the skin decreases slowly but progressively with age and it is only from the age of 70 that thinning becomes significant.

The biomechanical function of the skin :

The twistometer, an apparatus developed within L'Oréal laboratories, measures the biomechanical properties of the skin. As its name implies it twists the skin and either the resulting deformation of the skin or the elastic recovery after deformation can be measured relative to time.

The results obtained by this type of measurement show that elderly skin is more rigid, more difficult to deform or stretch and that elastic recovery after deformation is reduced whatever the skin site considered. This reduction can be up to 30 % on the forearm.



Histological sections of young and old skin : Thinning of the epidermis and disapearance of dermal papillae (villosities).

HISTOLOGICAL AND ULTRA-STRUCTURAL SIGNS OF SKIN AGEING

While chronological ageing leaves its mark essentially on the epidermis and the dermo-epidermal junction, with photo-ageing the most severe effects are seen in the dermis.

Epidermal cell « fatigue »

With age, the epidermis becomes thinner : the keratinocytes proliferate less and the number of cellular layers decreases. The cornified layer keeps roughly the same thickness whatever the age but its barrier properties are affected by the lack of cohesion between the corneocytes. The number of Langerhans cells, involved in the skin's immune response, decreases... by up to 50 % in areas exposed to the sun. As for the melanocytes, their number decreases after the age of 30 by 6 to 8 % per decade and their function becomes compromised : their capacity to proliferate and to interact with keratinocytes decreases. These alterations, by adversely affecting the pigmentation process, lead to a decline in the natural protection against the sun's rays. Moreover, by forming clumps, melanocytes are at the origin of lentigos or « liver spots » seen mainly in areas exposed to the sun.

Exposure of the skin to the sun leads to early visible reactions such as sunburn and tanning and effects only visible later such as photo

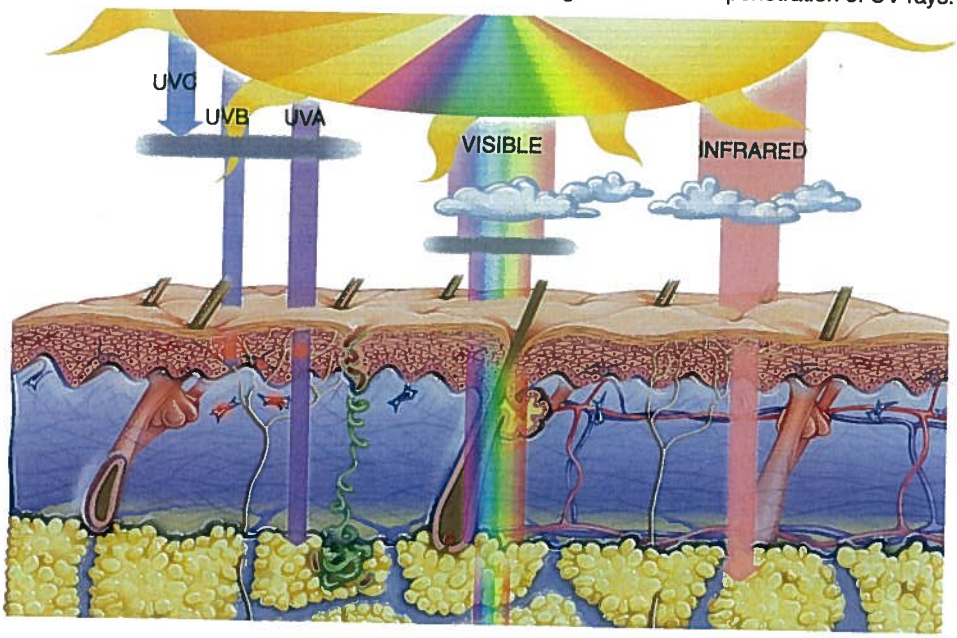
ageing and skin cancers. In the short term, the epidermis reacts to UV rays by activating a natural photoprotection system which involves the synthesis of melanin, thickening of the epidermis and the repair of damage inflicted on the DNA.

The pigmentation of the skin induced by the sun's rays results from melanogenesis and forms a defence reaction and protection against the dangers of the sun : the melanin granules synthesised by the melanocytes and transferred to the neighbouring keratinocytes collect above the nucleus of the keratinocytes and form a screen which protects the DNA against UV radiation. This protective effect must however be qualified, since only eumelanin (brown/black) has the property of absorbing UV rays ; phaeomelanin (yellow/red melanin synthesised by red-haired individuals) does not provide protection and produces free radicals when exposed to UV radiation.

Cell damage caused by UVB radiation causes the appearance of abnormal cells 24 hours after sunburn. These cells, called "sunburn cells" are keratinocytes which have begun a process of cell death.

DNA, the genetic code of the cell, is also a prime target of UV rays, the effects of which may result in lesions known as genetic mutations. One of these is the formation of « pyrimidine dimers » which result from abnormal bounding of two neighbouring pyrimidine molecules

Diagram of the skin penetration of UV rays.



Delphine Bailly

(pyrimidines are nucleotide bases forming the DNA chain). Gene mutations are normally eliminated by the cellular repair systems but sometimes these systems are not efficient enough, in particular when exposure to the sun is intense and repeated. Thus, genes promoting cellular multiplication (the oncogenes) may be activated or on the contrary, genes suppressing tumours may be inactivated. The abnormal cells multiply without hindrance and become tumours.

The majority of malignant skin tumours are induced by the sun and affect keratinocytes (basal cell and spinocellular carcinomas) and melanocytes (malignant melanomas). The incidence of melanomas varies depending on the phototype and the risk of developing such a cancer is thus greater in people with fair skin, blond or ginger hair, who tan with difficulty and burn easily in the sun.

Flattening of the dermo-epithelial junction

With time, reduction in the production of type VI and VII collagen disorganises the structure of the dermo-epidermal junction. It becomes less folded and loses its adhesion with the oxytalan fibres of the superficial dermis supporting the network of collagen fibres. The disappearance of the dermal papillae leads to a significant decrease in the area for exchanges between the two compartments and the weakening of the dermis/papillary epidermis junction network, which results in a loss of elasticity and helps form wrinkles.

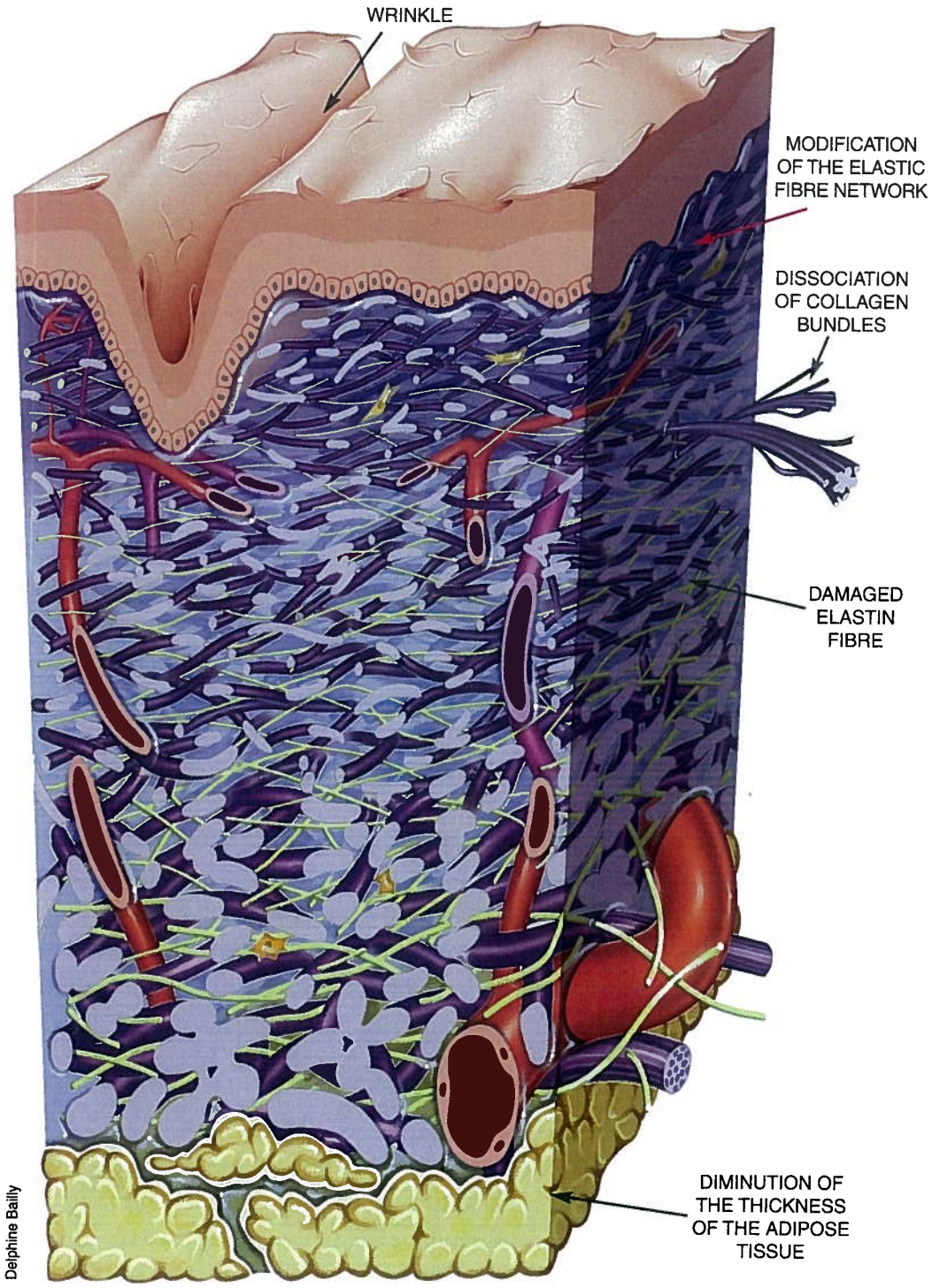
Dermal fibroblasts lose their reactivity

The superficial or papillary dermis is the dermal area most affected by skin aging. Fibroblasts become rarer and their capacity to produce collagen, elastin and proteoglycans decreases.

The ageing of fibroblasts studied *in vitro* indicates that these cells, cultured in dishes, are capable of dividing between 50 and 100 times, the number of divisions depending on the age of the donor. As the fibroblasts get older, their frequency of division slows during ageing.

The extracellular matrix is altered. Collagen bundles dissociate. Disorganised and fragmented, they have a "dishevelled" appearance. The collagen I/III ratio is modified in favour of type III collagen, the finer fibrils of which may explain the thinning of collagen fibres with age. The proteolytic activity of enzymes involved in the degradation of collagen I, III is increased.

STRUCTURE OF THE AGED SKIN



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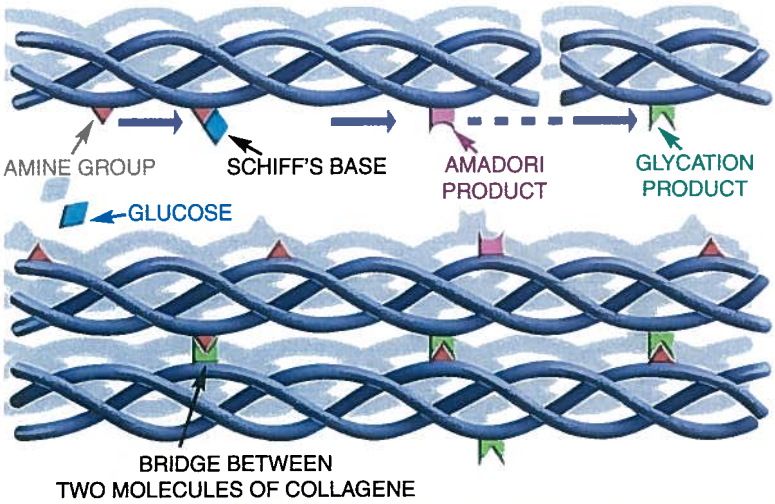


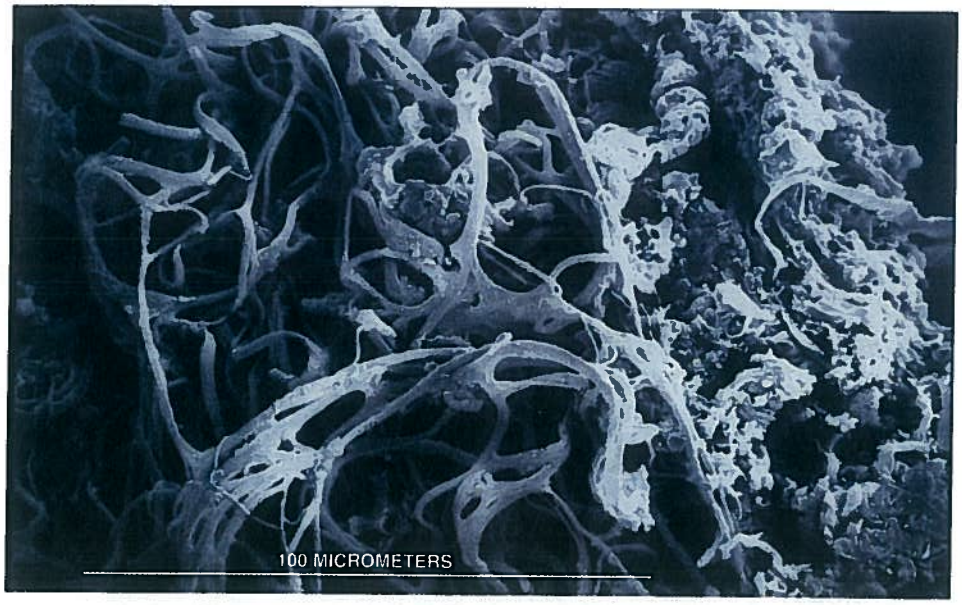
Cross-bridging of collagen fibres by Maillard reaction. Glycation is a slow non-enzymatic reaction between free amine groups on amino acids, making up proteins and circulating sugars such as glucose. The reaction, also called Maillard reaction, leads to the formation of products called AGE (Advanced Glycation End Products) responsible for the formation of bridges between the chains of macromolecules.

Modified properties of collagen include a decrease in its solubility and its resistance to breaking, an increase in its resistance to stretch and to digestion by the collagenases. Chemical bridging between collagen fibres resulting from the glycation reaction leads to cross-linking of the collagen and to rigidity of the fibres, which makes them more resistant to digestion by enzymes.

Modifications of the elastic fibre network are responsible for the lax, sagging appearance of the skin and for the fine wrinkles characteristic of chronological ageing. In the papillary dermis, the vertically orientated candelabra-like network of fine oxytalan fibres, mounting towards the dermo-epidermal junction gradually disappears. This causes flattening of the dermal papillae resulting in a loss of epidermal tonicity.

The proteoglycan and glycosaminoglycan content (particularly hyaluronic acid) decreases considerably in the ageing dermis. This loss contributes to a decrease of hydration and, in turn, the aged appearance of the skin.

During ageing, fibronectin is subject to the phenomenon of glycation which makes it less functional. Synthesised by the fibroblasts, this structural glycoprotein, composed of two arms - one attached to the cell membrane, the other to components of the extracellular matrix - plays the role of a biological adhesive gluing the cells to the surrounding matrix

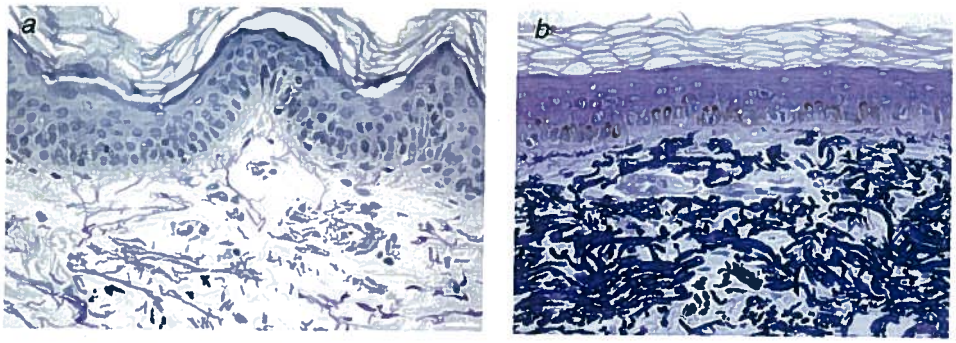


Three-dimensional view of the network of elastic fibres in the superficial dermis.

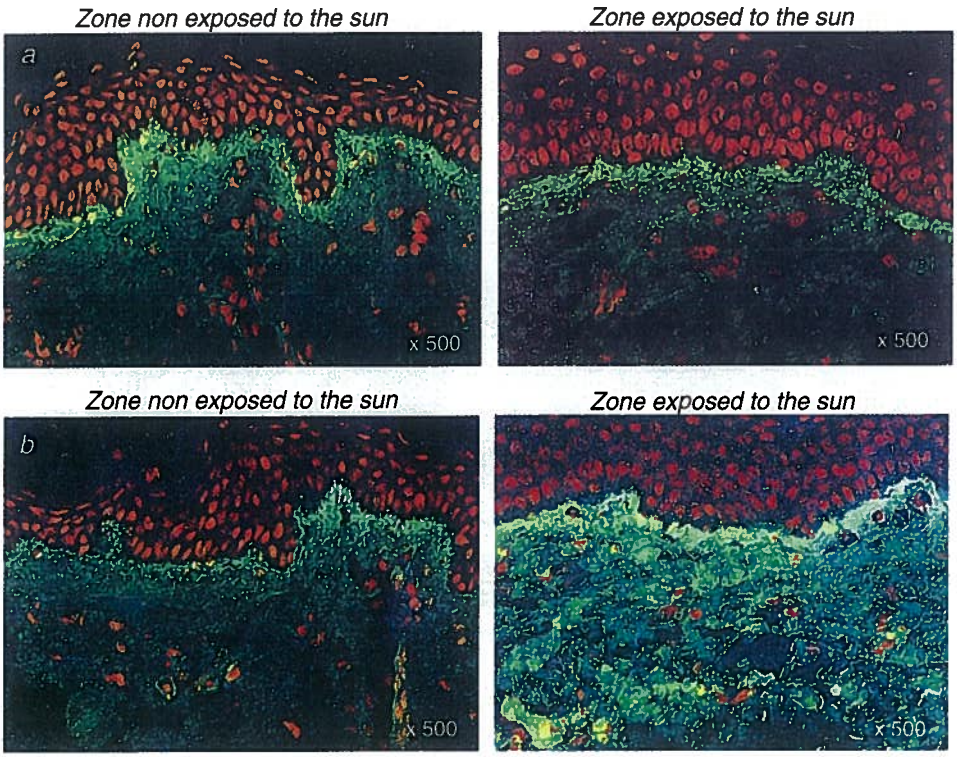
The blood vessels become more fragile and more rigid, the terminal capillaries reaching the dermo-epidermal junction lessen in number reducing the supply of nutrients to the epidermis.

The main target of UVA rays in the dermis : elastin

The proportion of UVA rays emitted by the sun and reaching the earth's surface is 20 times higher than that of UVB radiation. These rays are not attenuated by the ozone layer and pass through clouds and glass. Emitted throughout the day they are capable of penetrating the skin as deep as the reticular dermis. They play a decisive role in photo-ageing, damage to DNA, the response of the immune system, and various photo-dermatoses.



Histological sections of young skin not exposed *(a)* and an aged skin exposed to the sun *(b)*.



(a) Decrease in the expression of type I pro-collagen labelled in green just below the dermal-epidermal junction in the zone non exposed to the sun.
(b) Increase in the expression of type III pro-collagen labelled in green in the zone exposed to the sun. Cells nuclei appear in red after coloration by propidium iodide).

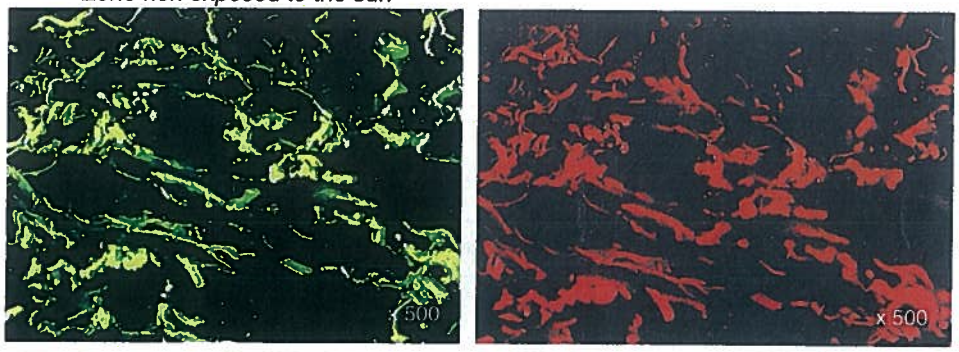
Accumulated damage from UV radiation and particularly from UVA rays results in the formation of free radicals. Free radicals are molecules which have lost an electron. For this reason, they are no longer in equilibrium and become unstable and hyper-reactive. To try to compensate for this lack, the free radicals « recover » an electron from other molecules and a chain reaction occurs.

There are many targets for free radicals : DNA of damaged cells, peroxidated membrane lipids, damaged collagen fibres, oxidised proteins. Free radicals are also responsible for increasing the synthesis of collagenases which breaks down collagen.

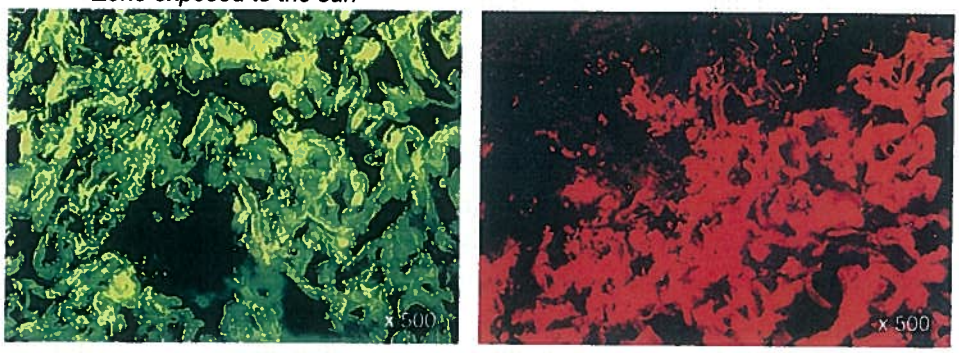
Solar elastosis : the histological sign of photo-ageing

Analysis of a photo-aged skin shows major modifications in the dermis : deterioration of the connective tissue, decrease and disorientation in the collagen content and accumulation of degenerated elastic fibres known as « solar elastosis ». The reduced expression of type VII

Zone non exposed to the sun



Zone exposed to the sun



« Cutis rhomboidalis » nuchae : deposits of lysozyme on the elastic fibres. Correlation of the intensity of the deposits with the importance of sun damage. Left in yellow-green, labelling of the elastic fibers. Right in red, labelling of the lysozyme. Both labellings were realised on the same histological section of skin.

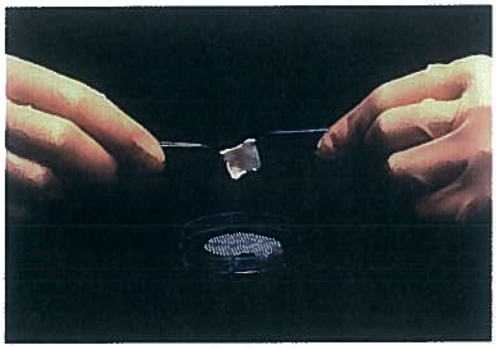
collagen, a major component of dermo-epidermal junction anchoring fibrils, contributes to the formation of wrinkles and to the fragility of the photo-aged skin.

Deposits of lysozyme on the elastic fibres

L'Oréal scientists have identified proteins which can serve as early markers of damage induced by UVA rays. Among them, lysozyme, an enzyme secreted by monocytes, macrophages and by certain epithelial cells that fixes to the damaged elastic fibres. This bonding prevents the proteolytic breakdown of degenerated elastic fibres and plays a role in their accumulation in photo-aged skin.

Ageing of the hypodermis

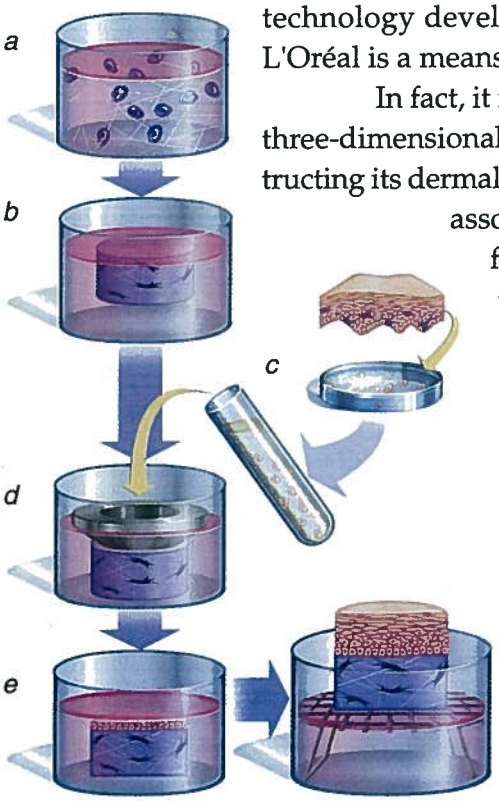
With age, the decrease in the thickness of the adipose tissue results in withering of the skin. When the connective tissue surrounding the fatty lobules becomes flaccid, these lobules appear in the form of cellulite.



IN VITRO MODELS FOR STUDYING AGEING

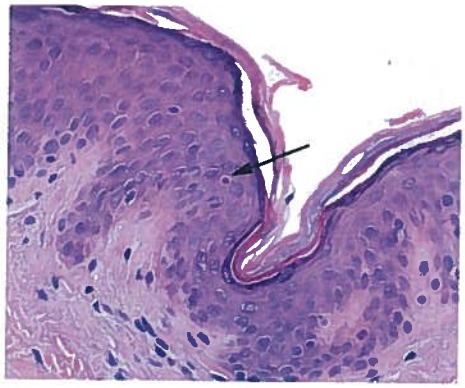
For technical and ethical reasons it is often difficult to carry out studies *in vivo* requiring skin biopsies taken from volunteers. Moreover, classic cultures of cells reproduce neither the cellular interactions nor the tissue architecture which exists *in vivo*. Nowadays reconstructed skin technology developed over more than 20 years by L'Oréal is a means of overcoming these limitations.

In fact, it is possible to reproduce *in vitro* the three-dimensional architecture of the skin by reconstructing its dermal and epidermal compartments. The association of fibroblasts and collagen forms a "dermal equivalent". Contraction and concentration of collagen fibres by the fibroblasts, serves as a support for the seeding of kera-



Model of reconstructed skin (Asselineau) epidermis on a living dermis. Fibroblasts treated with trypsin are mixed with collagen I *(a)*. Organisation of the fibroblasts causes a contraction of the gel which eventually becomes stable *(b)*. A ring is placed on this dermal support *(d)* inside which keratinocytes in culture are seeded *(c)*. The keratinocytes proliferate for seven days in an immersion phase in the culture medium *(e)*, then the unit is placed on a grid. The keratinocytes exposed in the emersion phase for seven days will form an epidermis perfectly differentiated up to the cornified layer.

Tissue lesions appear in the epidermis 24 hours after sunburn, and lead to the formation of characteristic cells called "sunburn cells": these are keratinocytes with an abnormal morphology where the cytoplasm is filled with vacuoles and the nucleus is retracted.



tinocytes. The cultures are kept immersed for seven days and are then placed on a grid at the air-liquid interface for a further seven days, during which the keratinocytes, in contact with the air, differentiate as far as the corneocyte stage.

By exposing this model of reconstructed skin to UVB or UVA rays or to both at the same time, it has been possible to carry out detailed studies of the effects of UV radiation on the skin and to identify biological markers specifically modulated by UVB and UVA radiation.

UVB rays : epidermal targets

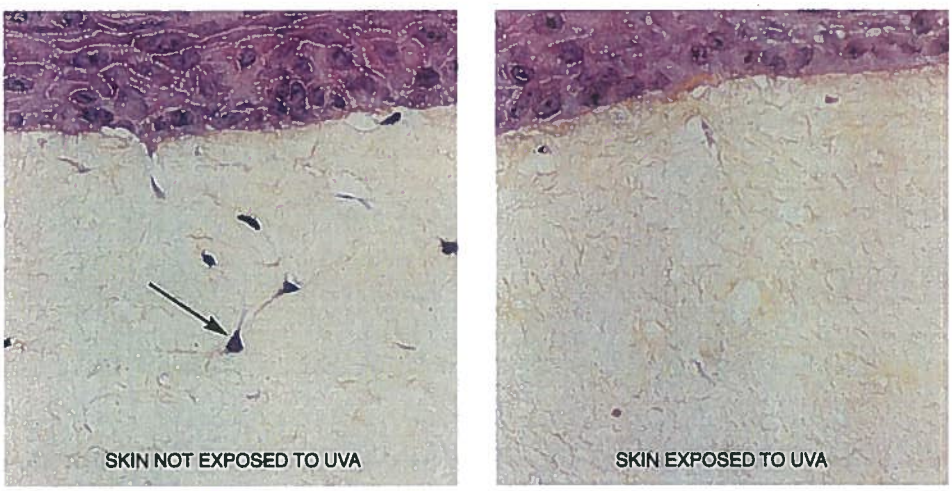
Exposure of these reconstructed skins to UVB radiation (290 to 320 nm) shows that the early damage caused to the epidermis is comparable with that observed in vivo : it consists of the appearance of apoptotic cells characteristic of sunburn and mutations in the DNA formed from pyrimidine dimers.

UVA rays : dermal damage

Irradiation with UVA rays (320 to 400nm) does not induce the same alterations as irradiation with UVB rays. The effects of UVA rays occur essentially in the dermis and can be seen as the disappearance of fibroblasts from the superficial dermis 48 hours after irradiation and by the activation of collagenase I.

After two weeks, tissue regeneration can be observed : fibroblasts from deep within the dermis recolonise the superficial dermis and synthesise pro-collagen I and fibronectin, components of the extracellular matrix.

By reproducing numerous effects of UV rays observed in vivo, these models have proved to be tools not only for acquiring knowledge



Disappearance of fibroblasts (arrow) from the dermis after irradiation with UVA rays.

about the biological mechanism induced by UV rays but also for testing the efficacy of sunscreen products containing UVB and/or UVA filters such as Mexoryl SX and XL.

Modelling chronological ageing :

In order to model chronological ageing of the skin, L'Oréal scientists have produced a reconstructed skin modified by preglycating with ribose the collagen used in the production of the dermal support described previously.

The morphology of the skin modified by glycation of the collagen is close to that usually observed except that the fibrillar structure of the dermis is altered. The formation of glycosylation products in the dermis of the reconstructed skin is verified using antibodies which specifically recognise advanced glycation end products (AGE) including carboxymethyl lysine. AGE cause biological modifications involving increased synthesis of extracellular matrix macromolecules (proteoglycans etc.) and cytokines and the activation of enzymes breaking down the matrix (collagenases), which results in the reorganisation of the surrounding matrix. Certain glycosylation products can be photolysed by UV radiation and generate reactive forms of oxygen in the surrounding matrix which is responsible for further damage.

This model is thus both a research tool and a system for studying the effect of anti-glycation molecules, which are potential anti-ageing candidates.

Cover. Noble chinese lady holding a mirror in the left hand and a comb in the right hand. The hairstyle is held by two hairpins. The lady does hair by looking in the mirror ; she wears a long dress with a shawl covering both shoulders and her left hand. Time Tang (618-907 A.C). Photography of Monique Vincent.

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