CHAPTER II

LITERATURE REVIEWS

1. Skin

Skin covers the entire body and protects it from various types of external stimuli as well as from moisture loss. The surface area of the skin of an adult person is about 1.6 m^2. The thickness of skin varies with age, sex and location. Generally, the skin of men is thicker than that of women. However, women have a thicker subcutaneous fat layer. In general, the skin of the eyelids is the thinnest and that of the soles of the foot is the thickest.

The outer skin is divided into three layers called the epidermis, and the subcutaneous tissue. Various appendages, such as hair, nails, and glands (sweat and sebaceous), are also found in the skin (Figure 1).

The epidermis is composed of several cell layers about 0.1-0.3 mm thick. From the external surface inwards, these layers are called the horny layer (stratum corneum), granular layer, spinous layer, and basal layer. The basal layer is formed of a single layer of columnar cells (basal cells) abutting against the basement membrane which is in contact with the dermis.

The basal cells divide continuously and, the "daughter cells" move in the surface direction to form the spinous layer. The spinous cells have many intracellular connections called desmosomes. As a result, the cell surfaces appear to have spine-like projections. These cells are separated by a very narrow gap through which nutrient-rich lymph flows freely. The spinous layer is several cell layers deep and is the thickest layer in the epidermis. Above the spinous layer, there are two to three layers of granular calls. The granular cells are named after the keratohyalin granules that they contain, giving them a "granular" appearance. The cells in the outermost

horny layer change in a number of ways. A number of organelles including nucleus disappear and the cells are filled with fibrous protein named keratin. The divided basal cells therefore move in sequence to the surface of the epidermis and their from change as described through a continuous and complex process to produce the biologically and chemically resistant horny layer. It is this horny layer which is in direct contact with cosmetics and which reflects good skin condition.

In addition to these keratinizing cells (keratinocytes), the epidermis also contains melanocytes which produce the pigment melanin. The melanocytes are scattered between the basal cells at the basal layer. The epidermis also contains Langerhans cells with immune response functions as a protective mechanism against invasion of foreign materials.

The dermis is composed of connective tissue below the epidermis. The convoluted surface of the dermis is in contact with the epidermis and the areas where the epidermis protrudes downward into the dermis are called epidermal ridges. The areas of the dermis near the epidermal protrusions is called the papillary dermis, and the deeper dermis is called the reticular dermis. Unlike epidermal cells, many of the dermal cells, are not in tight cellular contact with each other, and there are many extracellular spaces. This part of the skin that has a macromolecular network structure is called the extracellular matrix. The dermis also contains mast cells, that produce histamine and serotonin responsible for the immediate allergic response, and fibroblasts, which synthesize and secrete the extracellular matrix. The basic materials comprising the extracellular matrix consist of glycosamino-glycans, or acidic mucopolysaccharides, and fibrous proteins. Glycosaminoglycan has different forms depending on the type of saccharide from which it is formed and depending upon the position of the sulfate group. In skin, however, hyaluronic acid and dermatan sulfate are the most common forms. Glycosaminoglycans exists as proteoglycans, combining protein, and can contain large quantities of water forming a gel. The fibrous proteins are embedded in this gel. The water in the gel transfers nutrients, metabolic products and hormones between the blood vessels and the cell tissues. The fibrous protein is composed of collagen and elastin for constructive purposes as well as fibronectin and

laminin for connective purposes. Collagen is the principal protein of the extracellular matrix and maintains the form of the tissues. Elastic fibers are connected to each other, forming crosslinks to maintain tissue elasticity. As a result of this construction, the dermis plays a large role in the elasticity and tension of the skin. The dermis also contains blood vessels, nerves, hairs, hair-erector muscles, sweat glands, and sebaceous glands. Beneath the dermis, there are subcutaneous tissue which contains many adipose cells in and between the connective tissue. The boundary of the dermis is not very clear. The main role of the subcutaneous fat is to regulate temperature. Subcutaneous fat is generally better developed in woman than in men and in children than in adults.

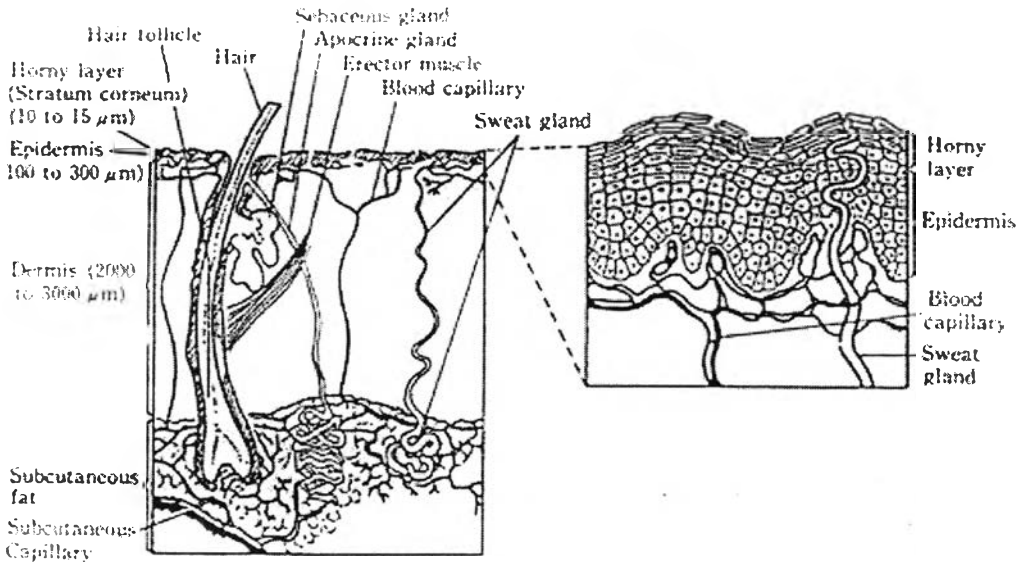

Figure 1. Basic structure of skin (Takeo, 1997)

2. Ultraviolet light (UV)

2.1 Definition of UV radiation

Ultraviolet radiation is part of the electromagnetic radiation spectrum comprising the interval between X-rays and visible light (Figure 2). The classification of electromagnetic radiation into different portions is based on practical considerations with regard to biological effects rather than on physical differences.

Different photobiological effects within the UV region have allowed further subdivision onto three regions termed UVA (320-380 nm); UVB (290-320 nm) and UVC (200-290 nm) . The UVC range does not reach the surface of Earth because of absorption by the ozone layer within the stratosphere.

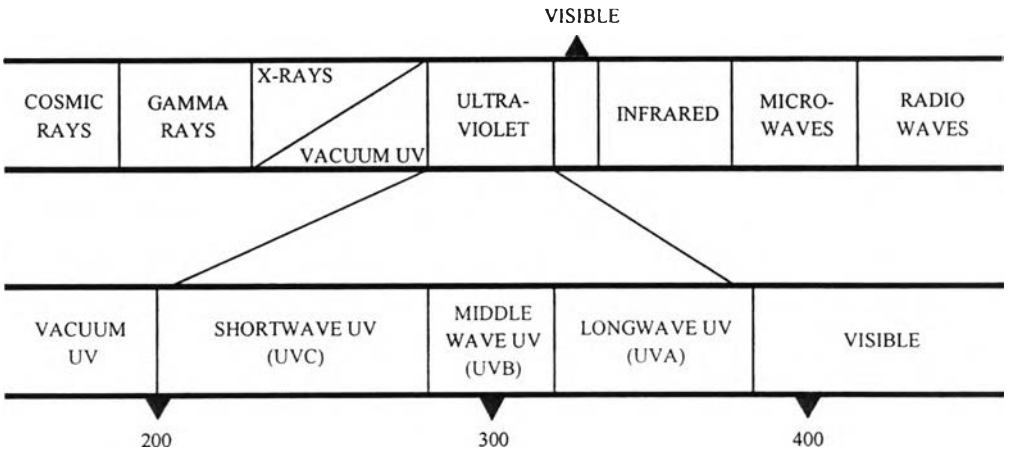

Figure 2. Electromagnetic spectrum with expanded scale of ultraviolet radiation. Wavelength in nanometers (Thody and Hönigsmann, 1986)

The UVB range, also called 'sunburn spectrum', account for a relatively small intensity portion in the solar spectrum and is responsible for the most dramatic reaction in human skin. The UVA range in the solar emission has been considered of minor biological significance in the skin. However, it is now realized that UVA may be more harmful than previously assumed, because it appears to accentuate some effects of UVB. UVB most commonly causes photosensitivity reactions with natural and synthetic photosensitising substances (Thody and Hönigsmann, 1986).

2.2 Radiometric terminology

The basic unit of radiant energy is the joule (J). The exposure dose, which is the radiant energy delivered per unit area of a surface, is equal to the product of irradiance and duration of irradiation and is express in $J \cdot cm^{-2}$ (or in $mJ \cdot cm^{-2}$). For studying photobiological reactions it is necessary to know the irradiance emitted by a UV source as a function of wavelength (spectral irradiance) since equal doses of

different wavelength regions cause effects which differ quite considerably in quality and in quantity (Thody and Hönigsmann, 1986)

2.3 Photobiological principles

The fundamental event in photobiology is the absorption of a photon by a relevant molecule. Only absorbed radiation energy can give rise to photochemical reactions (Grotthus Draper principle). Molecules which are able to absorb radiation are called chromophores. In some common skin reactions several chromophores may be involved. The most critical target (chromophore) for adverse UV effects is deoxyribonucleic acid (DNA) which absorbs maximally at 260 nm (UVC) but it also absorbs well into the UVB region of the solar spectrum (Thody and Hönigsmann, 1986).

3. Effects of UV radiation on skin

3.1 Acute response to ultraviolet light

Immediately after bathing in UV light, the skin begins the immediate darkening phase. This immediate darkening is the result of oxidation of pre-existing melanin pigment, but the darkening appears to return to the original color within several hours. This response is initiated by UVA and visible light.

Several hours after exposure to UV light, the skin begins to become red, reaching a peak after 8 and then gradually diminishing. This phase is called sunburn. When exposed to very large amounts of UV light, blisters develop and the skin feels burnt. The wavelength region causing this sunburn is the short wavelength peak in the UVB band. UV light in the 290-300 nm band is 100 times more effective than at 320 nm (Takeo, 1997). When we consider the distribution of wavelengths in sunlight, most sunburn must be caused by the 300-310 nm wavelength band (erythrema production curve).The cells damaged by the UV light produce an inflammatory mediator, expanding the capillaries and resulting in the appearance of sunburn.

Approximately 3 days after UV exposure, the skin gradually becomes dark. This delayed darkening, or suntan, is produced by an acceleration of the melanocyte function with formation of melanin in large amounts and with movement into the keratinocytes. Although this response is initiated after the reddening caused by UVB, large amounts of UVA have the same effect. Skin that has been suntanned by this mechanism gradually returns to the original color after several months. Simultaneously with this darkening, new skin is regenerated under the damaged skin. The old skin peels off 10-14 days after exposure to the UV light.

The acute response to UV light varies among individuals. The minimum erythema dose (MED) is a value used to indicate the acute sensitivity of individuals to UV light. The minimum erythema dose indicates the minimal amount of UV light required to cause redness when a person is bathed in UV light. In other words, individuals with high sensitivity have a low MED since only a small amount of UV light is required to cause skin redness.

The foregoing describes the response of healthy individuals but some people are oversensitive to UVB while others are oversensitive to UVA or visible light which has no acute serious effect on healthy people. These responses are called photosensitivity and the causes are classified into phototoxic response, photoallergy response and photohypersensitivity response. The former responses result from external materials and UV light, while the latter is thought to have various internal causes (Takeo, 1997).

3.2 Chronic response to ultraviolet light

So-called fisherman's skin and farmer's skin both demonstrate the typical chronic response of skin to UV light. Such skin is dark, feels rough to the touch, and is deeply wrinkled. The nape of the neck, which is constantly exposed to UV light, has characteristic diamond-shaped wrinkles. If this condition worsens, skin cancer may result. Since these types of changes are distinct from natural aging, they are called photoaging or dermatoheliosis. The face is most susceptible to these changes

because it is exposed to sunlight throughout the year. When these photoaging changes are examined histologically, epidermal thickening and overdeveloped melanocytes are observed. The main components of the dermis are collagen fibers and net-like elastic fibers. Photoaged skin has an abnormal increase in the amount of elastic fibers, and the fine dermal blood capillaries are also dilated. These changes are the opposite of what occurs with true aging changes.

It has recently been shown that the immune system is also affected by chronic exposure to UV light. In the future, the effect of UV light on the entire body, and not just the skin, will be clarified (Takeo, 1997).

3.3 UV-induced DNA lesions

UV irradiation from 245 to 290 nm is absorbed maximally by DNA (Tornaletti and Pfeifer., 1996). UV irradiation is able to induce mutagenic photoproducts or lesions in DNA among adjacent pyrimidines in the form of dimers (Ananthaswamy., 1997).These dimers are of two main types: cyclobutane dimers (CPDs) between adjacent thymine (T) or cytosine (C) residues, and pyrimidine (6-4) photoproducts among adjacent pyrimidine residues. CPDs are formed between the C-5 and C-6 carbon atoms of any two adjacent pyrimidines; the double bonds become saturated to produce a four-membered ring (Kanjilal and Ananthaswamy., 1996). Similarly, (6-4) photoproducts are formed between the 5-prime 6 position and the 3-prime 4 position of two adjacent pyrimidines, most often between TC and CC residues (Tornaletti and Pfeifer., 1996). CPDs are produced three times as often overall as (6-4) photoproducts (Tornaletti and Pfeifer., 1996). Both lesions occur most frequently in areas of tandem pyrimidine residues, which are known as `hot pots' of UV-induced mutations (Kanjilal and Ananthaswamy., 1996).

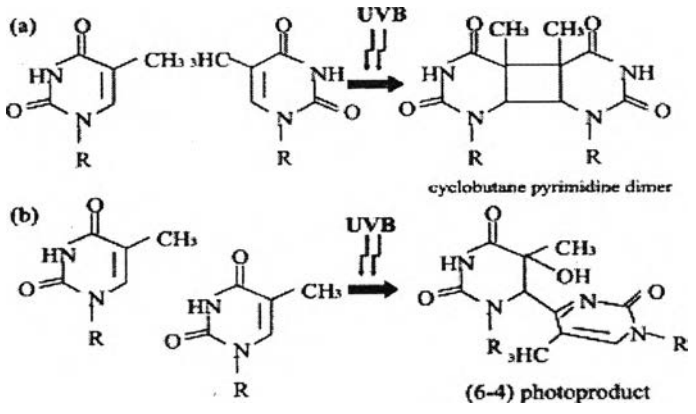

Figure 3. Adjacent pyrimidine bases on the same strand pyrimidine dimer (a) or (6–4) pyrimidine–pyrrimidone photo product (b) alter absorbing UVB light energy (Ichihashi et al ., 2003).

3.4 UV irradiation and reactive oxygen species (ROS)

UV irradiation of skin increases hydrogen peroxides and other reactive oxygen species, and decreases anti-oxidant enzymes. These features are also observed in chronologically aged human skin. In both cases, increased ROS production alters gene and protein structure and function, leading to skin damage (Gail, 2002).

UVA induces the formation of reactive oxygen species that readily react with membrane lipids and amino acids. Membrane damage results in the release of arachidonic acid and leads to activation of secondary cytosolic and nuclear messengers that activate UV-response genes. Human skin exposed daily for 1 month to suberythemal doses of UVA alone demonstrated epidermal hyperplasia, stratum corneum thickening, and dermal inflammatory infiltrates with deposition of lysozymes on the elastic fibers (Lavker *et al.*, 1995).

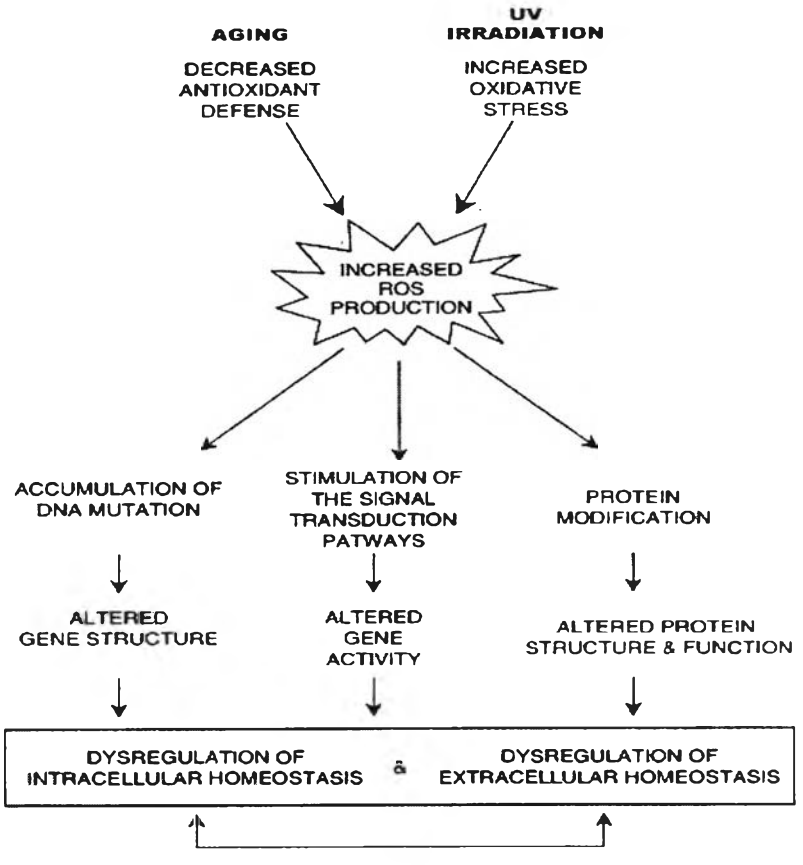

Figure 4. Role of oxidative stress in aging and UV (Rittie *et a*l., 2002).

Besides direct absorption of UVB photons by DNA and subsequent structural changes, generation of ROS following irradiation with UVA and UVB requires the absorption of photons by endogenous photosensitizer molecules. Recently, the identification of the epidermal UVA absorbing chromophore trans-urocanic acid that quantitatively account for the action spectrum of photoaging has been reported (Hanson and Simon., 1998). The excited photosensitizer subsequently reacts with oxygen, resulting in the generation of reactive oxygen species (ROS) including the superoxide anion (O_2-) and singlet oxygen ($^1O_2$). O_2- and $^1O_2$ are also produced by neutrophils that are increased in photodamaged skin and contribute to the overall pro-oxidant state. Superoxide dismutase converts O_2- to hydrogen peroxide (H_2O_2). H_2O_2 is able to cross cell membranes easily and in conjunction with transitional Fe(II) drives the generation of the highly toxic hydroxyl radical (OH.). Both singlet oxygen and OH can initiate lipid peroxidation of cellular membranes (Brenneisen *et al.*, 1998).

3.5 Effects in keratinocytes (Sunburn cells)

Microscopically, so-called 'sunburn cells' appear in the epidermis 8-24 hours after irradiation with UVB and also UVC. They appear scattered high in the epidermis and have shrunken, homogeneous, eosinophilic cytoplasm and a condensed nucleus. They contain melanin granules, clumped intermediate filaments and intact lysosomes. (Thody and Hönigsmann, 1986)

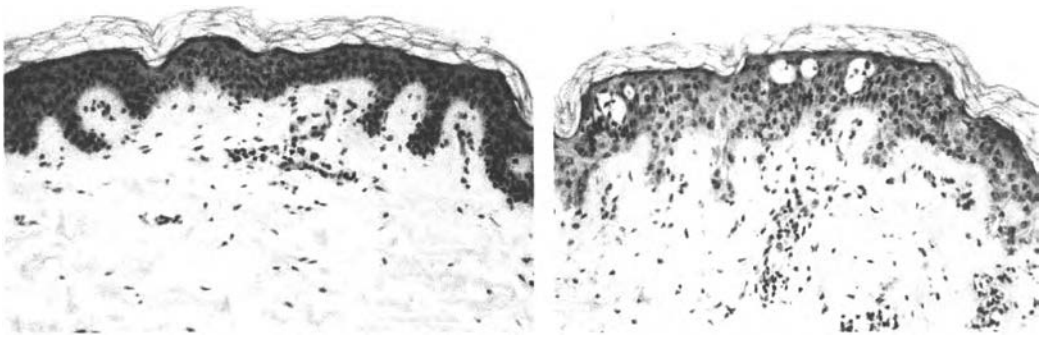

Figure 5. Histologic appearance of skin. A, untreated skin. B, 48 hours after UV exposure (Elmets *et al.*, 2001).

Sunburn cells (SBC) are keratinocytes undergoing apoptosis as a protective mechanism against the carcinogenic effects of ultraviolet-B irradiation. Sunburn cells formation is critically regulated by signalling cascades arising from DNA damage, membrane receptor clustering and generation of reactive oxygen species. The mitochondrion acts as major checkpoint, integrating upstream survival and pro-apoptotic pathways. The final post-mitochondrial apoptotic phase is executed by caspases. Deregulation of signalling cascades controlling SBC formation can ultimately lead to the development of skin cancer (Laethem *et al.*, 2005)

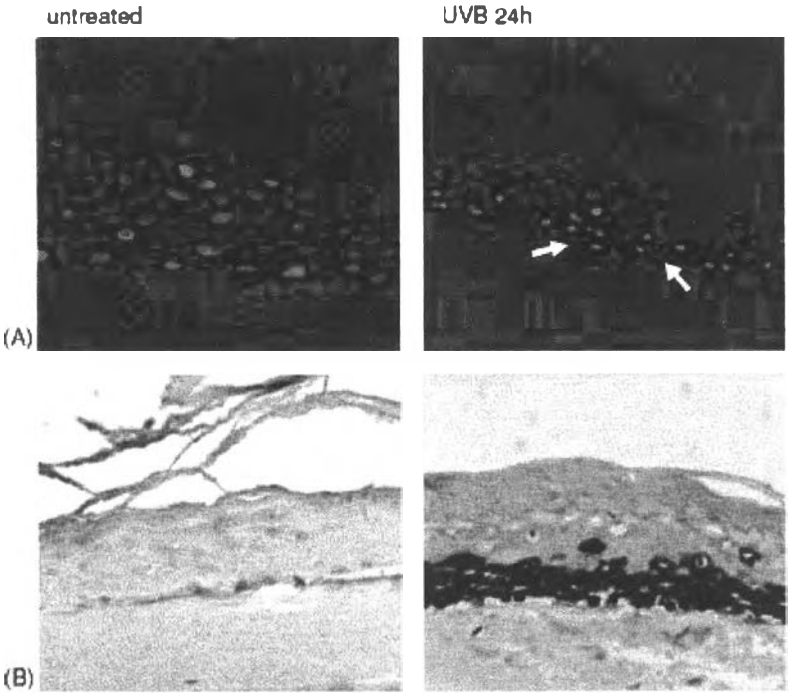

Figure 6. Morphological and biochemical features of SBC formation in UVB irradiated human skin equivalents. (A) Individual scattered SBC showing the typical morphology of apoptotic cells with pyknotic nuclei visualised with DAPI-staining of UVB (100 mJ/cm^2) irradiated human skin equivalents (SE). At 24 h after UVB irradiation, cells with condensed nuclei can be found in the suprabasal layers (right panel, arrows) as compared with an untreated control sample in which no pyknotic nuclei are present (left panel). (B) Correspondingly immunolocalisation of the active caspase-3 cleavage product is only observed in UVB treated SE (right panel) and cane found in the suprabasal layer at 24 h after UVB (100 mJ/cm^2) (Laethem *et al.*, 2005).

4. Protective mechanisms of skin against ultraviolet light

Human skin bears remarkable variety of natural defense mechanisms to survive detrimental effects of the environment. Two different systems have evolved to maintain skin integrity against the constant impact of solar radiation: a protective and a reparative system. The protective system includes a number of structural components capable of scattering (reflecting) and of absorbing UV radiation. The most importance of these are melanin, proteins of the horny layer and of epidermis,

urocanic acid and some other aromatic chromophores. The repair system consists of at lease three different types of mechanisms which are designed for the repair of cellular DNA: excision repair, postreplication repair and photoreactivation (Thody and Hönigsmann, 1986)

4.1 Optical properties of the skin

Relevant photobiological responses can occur only when radiation interacts with the living compartment of the skin. Therefore radiation must penetrate into the skin to reach the absorbing molecule (the chromophore). About 5% of normal incident radiation is reflected at the skin surface due to the difference in refractive index between air and stratum corneum. Penetration of the remaining radiation is determined by two basic phenomena, scattering and absorption. Scattering at structures within the epidermis may result in diffuse outward reflectance (or remittance) and contributes to transmittance because of the fraction of UV that is scattered in a forward direction, downwards into the skin (Anderson and Parrish, 1981).

Absorption of UV, as detailed above, depends on the presence of chromophores; DNA, melanin, urocanic acid and amino acids are the major absorbing molecules. In general, shorter wavelength penetrate less than do longer wavelengths, because various unique chromophores in the stratum corneum and in the upper epidermis provide substantial protection. Although UV-induced hyperplasia of the epidermis clearly has some role in protecting the germinative layer from the shorter UVB region, the most important chromophore is melanin which absorbs over abroad spectrum from UVC to near infrared. Melanin accounts for substantial protection in the UVB region and represents the only filter for UVA radiation (Thody and Hönigsmann, 1986).

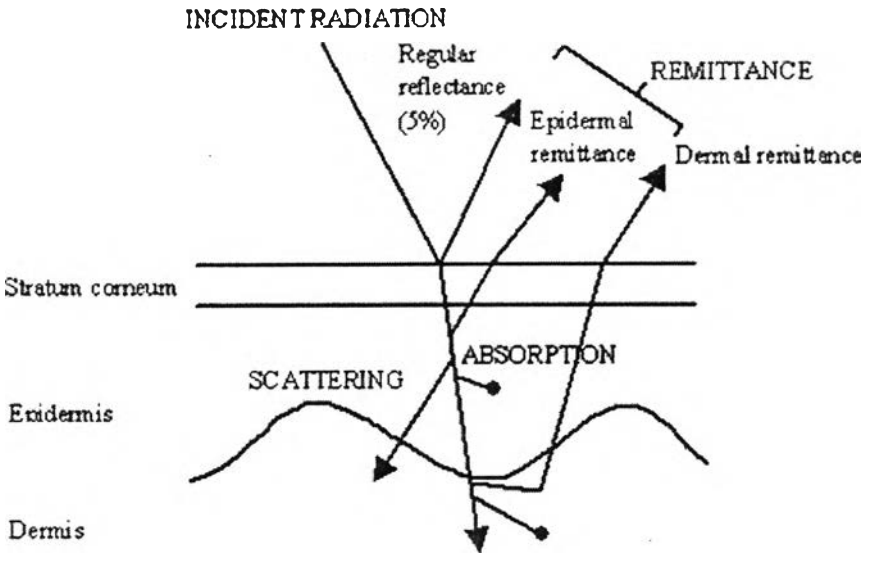

Figure 7. Diagram of optical pathways in human skin. (Thody and Hönigsmann, 1986)

Scattering occurs mostly by collagen fibres, which, in part, leads to remittance of radiation. However, Photobiologically relevant amounts of UV radiation reach important dermal structures such as superfacial nerve endings and blood vessels (Anderson and Parrish 1981). Because of the wide action spectrum for erythema, it appears likely that several different chromophores are involved in this reaction. For wavelengths shorter than 320 nm the DNA absorption spectrum closely parallels the erythema curve, which seems to support the hypothesis that DNA may be the decisive chromophore for erythema production. For wavelength longer than 320 nm the absorption of UV by other chromophores could result in a second step reaction by which energy may be transferred to DNA from these substance.

4.2 Skin tanning

The darkening or tanning of the skin which occurs in response to UV radiation involves two distinct reactions. The first or immediate reaction occurs within minutes of exposure to UVA and visible light and is characterised by the photo-oxidation of preformed melanin. Previous studies have suggested that there is a movement of

melanosomes towards the dendritic processes of the melanocyte and an increase in their numbers in the keratinocytes (Jimbow *et al.*, 1973). This response is most obvious within 1-2 hours of UV exposure but by 4 hours has almost completely disappeared (Quevedo *et al.*, 1974).

The delayed reaction, on the other hand, is much slower in onset and becomes apparent 2-3 days after repeated exposure to sunlight, UVA or UVB. This response involves an increase in the numbers of active melanocytes, an enhanced melanosome production and an increase in melanogenesis. There is also an increase in keratinocyte proliferation and more melanosomes are transferred from the melanocytes into the keratinocytes (Quevedo *et al.*, 1974). The increased numbers of melanocytes result from an enhanced proliferation (Rosdahl & Szabo, 1978; Nordlund et al., 1981). It has been shown that the activation of the precursor melanocytes which follows UV exposure involves the development of the endoplasmic reticulum and the Golgi apparatus and an increase in melanosome formation. Tyrosinase synthesis then occurs and this is followed by the melanisation of the melanosomes (Quevedo *et al.*, 1974; Jimbow and Uesugi, 1982). In the study in which mice were irradiated daily for 30 minutes with UVA, these events were complete by 3-5 days and it was seen melanocyte proliferation occured (Jimbow and Uesugi 1982). Melanosome transfer into the keratinocyte was not obvious until after 7 days of exposure. The capacity to acquire pigmentation appears to reach a maximum in each individual after a certain number of exposures with any kind of UV radiation (Schuler *et al.*, 1982).

4.2.1 Melanocytes

Melanocytes originate from the neutral crest but during embryogenesis are distributed to various sites throughout the body, including the skin, eyes and central nervous system. Those present in the skin are normally situated in the basal layer (Figure 8), in hair follicles and sometimes in the dermis. In the epidermis they are thin, elongated cells with long dendritic processes which ramify among neighboring epidermal cells. This arrangement facilitates the transfer of melanosomes to keratinocytes, and in this way melanin is distributed throughout the epidermis to

provide the protection against UV radiation. In the human epidermis each melanocyte is normally associated with about 36 keratinocytes and together they constitute the epidermal melanin unit.

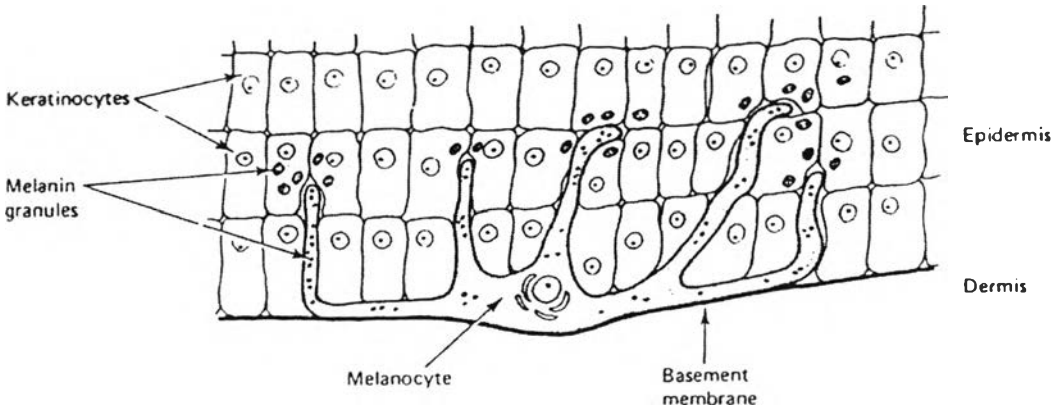

Figure 8. Epidermal melanocytes are situated in the basal layer. Melanin is formed within the melanocyte and then transferred into the neighbouring keratinocytes. In this way the melanin provides protection against UV radiation. In the epidermis each melanocyte is normally associated with 36 keratinocytes and together form the epidermal melanin unit (Thody and Hönigsmann, 1986)

Because of their ability to transfer pigment granules, epidermal melanocytes can be considered to be true secretory cells and like all secretory cells, have a well-developed endoplasmic recticulum and Golgi complex. Dermal melanocytes, which are found in abundance in many mammals such as apes, are unable to secrete their pigmentary granules and for this reason are less important in protecting the skin from the effects of UV. However, by affecting the refraction of light, dermal melanocytes are able to determine skin colour which is important in social and sexual signaling (Thody and Hönigsmann, 1986).

4.2.2 Melanogenesis

The melanin synthesis pathway is believed to as shown in figure 9. In this pathway, tyrosine is oxidized to dopa; the next stage is to dopaquinone due to the action of tyrosinase, and then the reaction progressed by autoxidation though to be accelerated by the enzymes

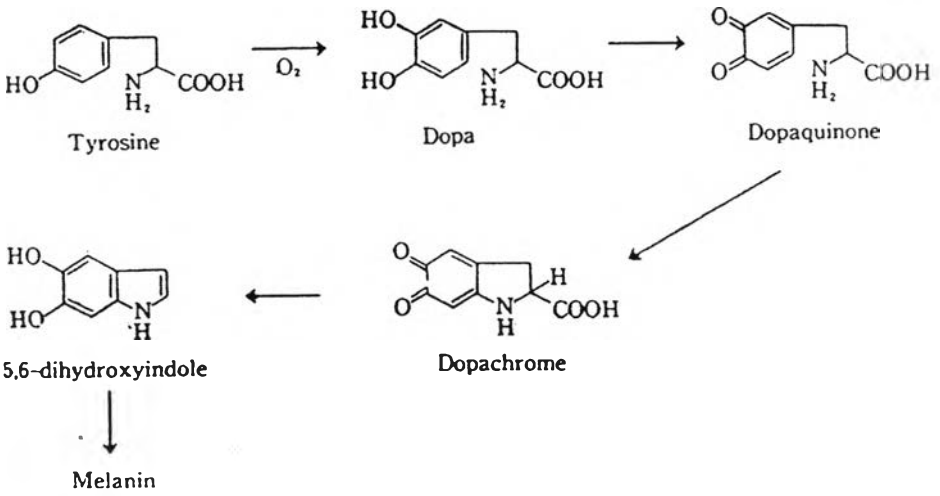

Figure 9. Melanin synthesis pathway (Takeo, 1997).

5. Antioxidant Activity of Flavonoids and Phenolic Acids

Free radicals are produced in the body as part of normal metabolism, for example superoxide, $O_2^{-}\cdot$ and nitric oxide, NO· which have important physiological functions. In general, free radicals are highly reactive and can attack membrane lipids for example, generating a carbon radical which in turn reacts with oxygen to produce a peroxyl radical which may attack adjacent fatty acids to generate new carbon radicals. This process leads to a chain reaction producing lipid peroxidation products (Halliwell, 1994). By this means a single radical may damage many molecules by initiating lipid peroxidation chain reactions. Because of the potential damaging nature of free radicals, the body has a number of antioxidant defence mechanisms which include enzymes such as superoxide dismutase, catalase, copper and iron transport and storage proteins, and both water-soluble and lipid-soluble molecular antioxidants. Oxidative stress may result when antioxidant defences are unable to cope with the

production of free radicals, and may result from the action of certain toxins or by physiological stress (Halliwell, 1994).

Table 1. Some dietary sources of flavonoids and phenolic acids (Croft, K. D., 1999).

Flavonoid	Source
Catechins	Tea, red wine
Flavanones	Citrus fruits
Flavonols (e.g. Quercetin)	Onions, olives, tea, wine, apples
Anthocyanidins	Cherries, strawberries, grapes, coloured fruits
Caffeic acid	Grapes, wine, olives, coffee, apples, tomatoes, plums, cherries

Flavonoids and phenolic acids can act as antioxidants by a number of potential pathways. The most important is likely to be by free radical scavenging in which the polyphenol can break the free radical chain reaction. For a compound to be defined as an antioxidant it must fulfill two conditions: (i) when present at low concentrations compared with the oxidizable substrate it can significantly delay or prevent oxidation of the substrate; (ii) the resulting radical formed on the polyphenol must be stable so as a chain propagating radical (Halliwell *et al.*, 1995). This stabilization is usually through delocalization, intramolecular hydrogen bonding or by further oxidation by reaction with another lipid radical (Shahidi and Wanasundara, 1992). A number of studies have been carried out on the structure-antioxidant activity relationships of the flavonoids (Bors *et al.*, 1990; Chen *et al.*, 1996; Rice-Evans *et al.*, 1996; Van Acker *et al.*, 1996; Cao *et al.*, 1997). The main structural features of flavonoids required for efficient radical scavenging could be summarized as follows:

1. An ortho-dihydroxy (catechol) structure in the B ring, for electron delocalization;
2. A 2,3 double bond in conjugation with a 4-keto function, provides electron delocalization from the B ring;
3. Hydroxyl groups at position 3 and 5, provide hydrogen bonding to the keto group.

These structural features are illustrated in Figure 8.

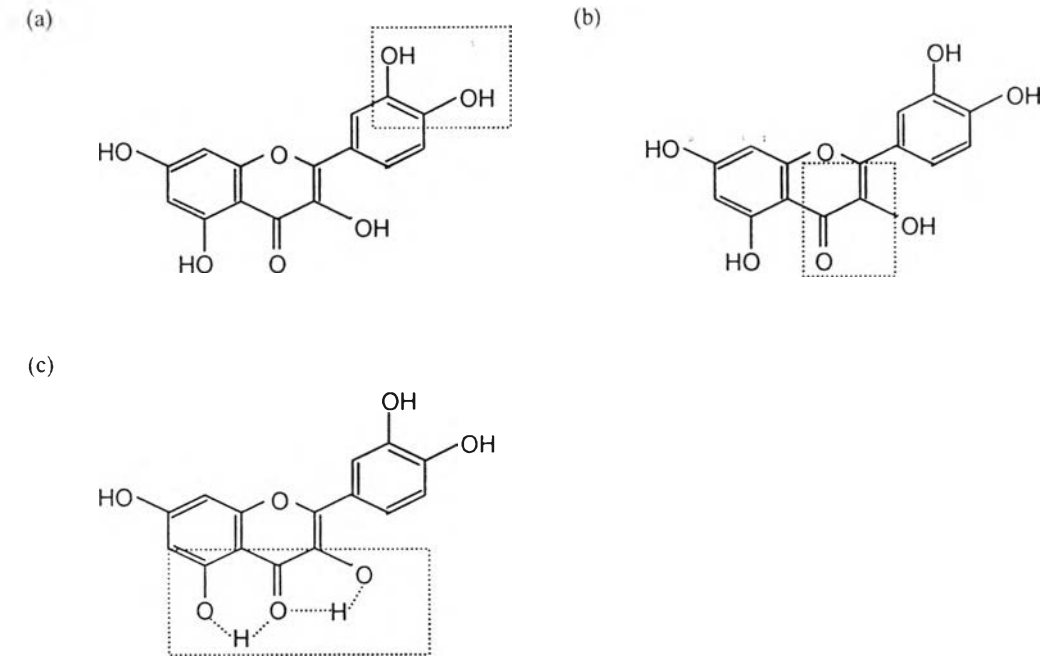

Figure 10. Structural groups for radical scavenging (Croft, 1999).

The phenolic acids may also be good antioxidants, particularly those possessing the catechol-type structure such as caffeic acid (Laranjinha *et al.*,1994; Nardini *et al.*, 1995; Abu-Amsha *et al.*, 1996). Recent studies have indicated that simple cell derived phenolic acids such as 3-hydroxyanthranilic acid may also be efficient co-antioxidants for α-tocopherol, able to inhibit lipoprotein and plasma lipid peroxidation in humans (Thomas *et al.*, 1996). The possible interaction between flavonoids and phenolic acid with other physiological antioxidants such as ascorbate or tocopherol is another possible antioxidant pathway for these compounds.

Another pathway of apparent antioxidant action of the flavonoids, particularly in oxidation systems using transition metal ions such as copper or iron, is chelation of the metal ions. Chelations of catalytic metal ions may prevent their involvement in Fenton-type reactions which can generate highly reactive hydroxyl radicals (Halliwell *et al.*, 1995)

$$H_2O_2 + Cu^+ \rightarrow \cdot OH + OH^- + Cu^{2+}$$
$$Cu^{2+} + O_2^{-}\cdot \rightarrow Cu^+ + O_2$$

The ability of polyphenolics to react with metal ions may also render them pro-oxidant. For example, in a recent study using three different oxidation systems, flavonoids had potent antioxidant activity against peroxyl radicals generated from AAPH and against hydroxyl radicals but were pro-oxidant with Cu^{2+}. Presumably flavonoids can reduce Cu^{2+} to Cu^+ and hence allow the formation of initiating radicals (Cao *et al.*, (1997)

6. Green tea

Tea (the leaves of *Camellia sinensis*) is grown in about 30 countries, and is the most widely consumed beverage in the world. Tea is manufactured as either green, black, or oolong.The basic steps of manufacturing the various forms of tea are similar except in the creation of their aroma and in the fermentation process, which is dependent on the oxidation states of different polyphenolic compounds present in tea leaves. The term green tea refers to the product manufactured from the fresh leaves of the tea plant by steaming and drying at elevated temperatures, with care to avoid oxidation and polymerization of the polyphenolic components. The polyphenolic composition in green tea is approximately similar to that of fresh leaves, including flavanols, flavonoids, and some phenolic acids (Katiyar *et al.*,1996; Katiyar *et al.*,1997).

6.1Green tea polyphenols (catechins)

Most of the polyphenols present in the green tea are flavanols, commonly known as catechins. The major catechins found in green tea are (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin -3-gallate (EGCG).

Green tea polyphenols (GTP_S) in alkali solutions (pH>8) were extremely unstable and degraded almost completely in a few minutes, whereas in acidic solution (pH<4) they were very stable. On the other hand, GTP_S are pH-dependent i.e the lowest the pH the greater the stability. In alkali solution EGCG and EGC are equal unstable but EC and ECG are relatively stable (Zhu *et al.,* 1997). The degradation of EGCG depends on temperature and solution pH. At low temperature (i.e. 4 and 25°C), the degradation appears to be effected primarily by the solution pH while at higher temperature (50°C), the pH effect appears to be enhanced by temperature. The mechanism of degradation of EGCG is closely related to the state of ionization of EGCG. The pKa values of EGCG have been reported to be $pK_1 = 7.55 \pm 0.03$, $pK_2 = 8.74 \pm 0.03$, and $pK_3 = 9.43 \pm 0.06$. Base on pK_1 there is 0.0028 % ionized EGCG present in the pH 3 samples, whereas at pH 5 there is 100 times more ionized EGCG present (Proniuk *et al.*, 2001).

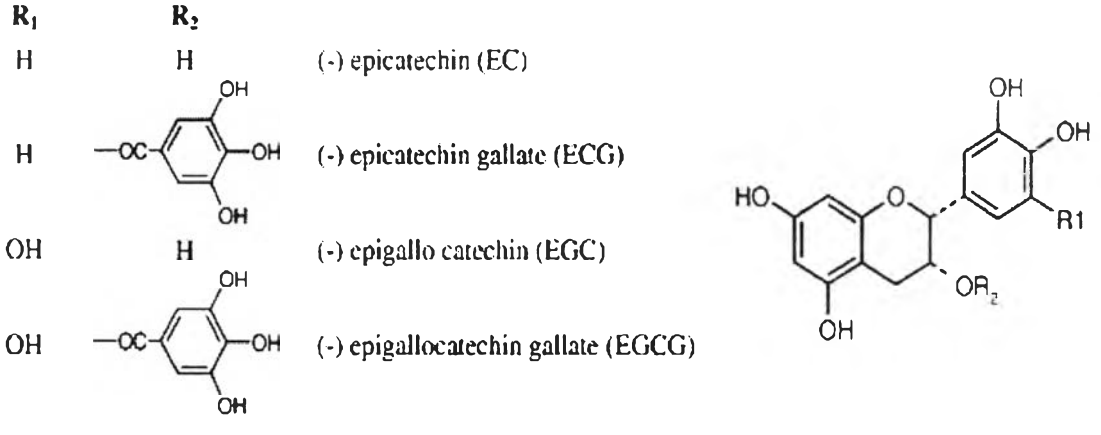

Figure 11. Chemical structures of major catechins (Uzunalic *et al.* 2004.)

6.2 Pharmacological activity green tea

In recent years, there has been an intensive research effort on the study of green tea and its major flavanol constituente, (–)-epicatechin (EC), (–)-epigallocatechin (EGC) , (–)-epi-catechin gallate (ECG) , and (–)-epigallocatechin

gallate (EGCG) for their cancer chemopreventive properties, as has been documented in several reviews (Agarwal and Mukhtar, 1996; Lin *et al.*, 1997; Ahmad *et al.*, 1998; Katiyar and Mukhtar, 1998). Of these 4 major green tea catechin derivatives or polyphenols, EGCG has been shown to be the major component and the most effective chemopreventive agent against cutaneous inflammatory or carcinogenic responses. (Katiyar *et al.*,1997;Ahmad *et al.*,1997). Green tea has shown nonspecific and broad spectrum anticarcinogenic effects in many different animal models. For example, oral administration of green tea inhibited N-nitroso-diethylamine (NDEA)-induced forestomach tumors in mice (Wang *et a*l., 1992b), N-nitroso-methylbenzylamine (NMBzA)-induced esophageal tumors in rats (Xu and Chi, 1990), 7,12-dimethylbenz(a)anthracene (DMBA)-induced tumorigenesis in mouse skin (Wang *et al.*,1991), and azoxymethane (AOM)-induced colon carcinogenesis in rats (Yamane *et al.*, 1997). The mechanism of cancer chemoprevention by EGCG may involve the inhibition of tumor necrosis factor-α (TNF-α) release by blocking the interaction of the tumor promoter to its receptor as well as inhibition of proteolysis of the TNF-α precursor protein (Suganuma *et al.* 1997). EGCG treatment resulted in arrest in the G_0-G_1 phase of the cell cycle and dose-dependent apoptosis in human carcinoma cells (Ahmad *et al.*, 1997).

Several studies suggest that the release of cytokines subsequent to UV-B irradiation plays a significant role in UV-B-induced immunosuppression and thus may be an important factor in the growth and development of immunogenic UV-B-induced skin tumors (Dummer *et al.*,1995; Hodgson *et al.*,2000; Kim *et al.*,1995).

7. Kojic acid

Kojic acid is a γ-pyrone compound with the structure shown in Figure 12. It is produced mainly by microbial fermentation using *aspergillus* and *penicillium* spp. and is important in imparting both color and flavor to miso, soy sauce and Japanese sake. Both in vivo and in vitro experiments have shown that kojic acid inhibits melanin production (Takeo, 1997).

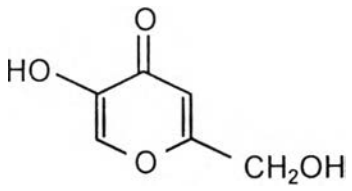

Figure 12. Chemical structures of kojic acid (Takeo, 1997)

In non-cellular *in vitro* test systems, kojic acid inhibited the activity of mushroom tyrosinase, goldfish and the black guinea pig tyrosinase. In the case of mushroom tyrosinase, the activity was inhibited by a non-competitive inhibition mechanism. In studies at the cellular level using B16 melanoma cell cultures, it has been confirmed that addition of kojic acid to the cell culture reduces melanin production and tyrosinase activity (Hatae *et al.*, 1986). In *in vivo* tests, creams containing kojic acid compounds have been reported as effective in preventing pigmentation changes in human skin due to exposure to UVA and UVB (Higa *et al.*1986)

8. Keratinocytes and immunological cytokines

Cytokines are small polypeptide molecules that interact with specific receptors, and are produced by cells locally and transiently in response to exogenous stimulation to mediate cell specific effects. They exert their biologic activities in picomolar concentrations. In general, cytokines mediate events relevant to immune and inflammatory processes. It has become clear that epidermal keratinocytes in the skin also exhibit the capacity to produce a variety of different cytokines (Stoof *et al.* 1994). It is an axiom of cytokine biology that immune and inflammatory cells must be induced or 'activated' to produce cytokines (Kupper 1990). As a sessile cell, however, the keratinocyte differs from the mobile cells noted above by an important criterion; the activating stimulus must come to the keratinocyte (Kupper 1990). Additional features distinguish the keratinocyte from mobile immune and inflammatory cells

with regard to strategies of activation. There can be no question that a major role of the keratinocyte is to provide a physically tough, relatively impermeable barrier between host and environment. This is achieved by differentiation and cornification. Thus, even though not 'activated' with regard to cytokine production, the keratinocyte in the absence of cytokine induction stimuli is growing and terminally, differentiating. Unlike the 'resting' 'T' cell and monocyte, the 'resting keratinocytes' is far from biologically inactive (Kupper 1990).

9. Interleukin-1

Interleukin-1 (IL-1) was originally identified as a product of activated monocytes that could act as a co-mitogen for thymocytes and that augmented. T-lymphocyte response to mitogens and antigens. It was originally named lymphocyte activating factor. However, it is now well established that many cells, including epithelial cells, endothelial cells, fibroblasts and various tumor cells, function as sources of IL-1 (Oppenheim *et al.*, 1986).

It has been shown that the epidermis contains prodigious quantities of biologically active preformed IL-1α at rest (Gahring *et al.*, 1985). As long as the epidermis is intact, IL-1 is eliminated by normal desquamation. After injury, however preformed, as well as newly synthesized, IL-1 is released and may participate in the regulation of local and systemic inflammatory (Gahring *et al.*, 1985). In vitro studies have shown that IL-1α is expressed when keratinocytes are proliferating in low calcium medium whereas this cytokine mRNA is not detectable when keratinocytes terminally differentiate in high calcium medium (Lee *et al.*, 1991). These results can be interpreted to mean that the production of IL-1α is confined to the less differentiated actively proliferating basal keratinocytes in the epidermis (Lee *et al.*, 1991). Upregulation of IL-1 synthesis takes place upon stimulation with physical or thermal injury, ultraviolet irradiation (including UVB), and a variety of cytokines (i.e. colony stimulating factors, GM-CSF, TNF-α, IL-6, TGF-α and IL-1α and IL-β itself (Lee *et al.*, 1991)

Keratinocytes, in addition to producing IL-1, express large amounts of specific IL-1 receptors. IL-1 is a multifunctional cytokine. Keratinocytes can respond to IL-1 in an autocrine or paracrine fashion IL-1 stimulates further release of IL-1 from neighbouring keratinocytes (McKenzie and Sauder, 1990).

In addition to its immunomodulating capabilities, IL-1 also stimulates a variety of other cells including keratinocytes (Stoof *et al.* 1994). Among epidermal cells IL-1 in an autocrine fashion stimulates proliferation of keratinocytes and induces keratinocyte intercellular adhesion molecule-1 expression as well as IL-1, IL-6, and GM-CSF production.