Skin and its role in providing nourishment to the body...

Pungent principles of black pepper, alkaloid piperine, and tetrahydropiperine (THP) as enhancers of nutrient and drug bioavailability

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MAINTAINING GOOD HEALTH: NUTRITION A CENTRAL CONCEPT IN INDO-TIBETAN MEDICINE

For centuries, humans have been aware of the subtle influence of seasonal changes on their physical and emotional well-being. The ancient systems of medicine addressed the specific needs of the human body in its process of adapting to seasonal changes as a means to maintain a state of good health and prevent disease. In the traditional Indian and Tibetan systems of medicine, good health was ensured through appropriate nutritional support which was thoughtfully adjusted to different seasons of the year. The concept of "food as medicine" was thereby exploited to maintain the body in a state of optimal health.

It is logical to assume that continuous care throughout the year for nutritional well-being is essential for overall health. Imbalanced and unsuitable diets impose prolonged periods of stress on the body, weakening its natural immune systems and making it more susceptible to disease. Adjustment of the seasonal menu, on the other hand, aims primarily at preventing or minimizing nutritional errors that may otherwise give rise to pathology and disease.

Traditional Indian and Tibetan diets were devised for compatibility with seasonal changes. They sometimes include a plethora of foods which are "taboo" in the modern nutritional sense; such as saturated fats or ghee (clarified butter), usually associated with high cholesterol levels. However, the diet in totality is a balanced one, providing carefully thought out seasonal nutrition. The proof is manifested in the good health and longevity of people who consume these traditional diets.

By traditional knowledge, selected foods are believed to be beneficial to general health at certain times in the year. Foods with thermogenic properties are more suited to the winter and consumed only in moderation during the warmer months. "Cooling" foods are more compatible with warmer temperatures. At times of the year when there is excess of surface moisture, such as during the monsoon or thaw in early spring, raw foods likely to be contaminated with soil or water borne microbes are best avoided. Traditional practices imposed special restrictions on food intake during these periods. Although these beliefs had religious attributes, the underlying scientific principles are noticeable. These dietary practices are now being validated by science.

One of the emerging concepts in scientific analysis of Indo-Tibetan nutritional practices is that the gastrointestinal tract is considered to be only one system nourishing the body. Skin and the central nervous system (CNS) are seen as equally important in receiving and processing "nutrients" and supporting healthy functioning of the body. This article will discuss skin as a nutrient delivery system to the body and provide examples of traditional herbal ingredients specifically developed for the skin's body nourishing role.

SKIN, THE COMPLEXITY OF A SIMPLE CONCEPT

If we were to ask the question, "what is the basic function of the skin?" most of us would simply answer that it covers and shelters our body. But looking up-close at this very basic organ one may be humbled in discovering how complex this structure really is. Human skin and its appendages, i.e. sweat glands, sebaceous glands (skin lubricant producing glands), hair and hair follicles and nails are referred to as integument. Skin has many layers which translate to two basic components; one directly in touch with the outside environment called the epidermis, and the other made of elastic connective tissue underneath which is known as the dermis. The outermost layer of the epidermis is called the stratum corneum. Made of cornified or keratinized cells, this layer covers skin like a disposable glove ready to be shed off and replaced by the subsequent wave of cells moving up through the multiple layers of the epidermis. The cells of the stratum corneum are a result of maturation of the keratinocytes, cells that originate four steps further down in the bottom (basal) layer of the epidermis called the stratum basale. One step above the stratum basale we find the stratum spinosum which produces "waterproofing" material (mixture of lipids and glycoproteins) which regulates permeability of the skin. Then follows the stratum granulosum which manufactures certain amino acids derived material called keratohyalin. Keratohyalin is believed to be metabolized into eleidin in the stratum lucidum, which is the next cell layer. Eleidin is converted to keratin in the top, stratum corneum.

The epidermis is home to many specialized cells, e.g. Merkel cells which play a role in an early warning system against noxious stimuli to the skin, Langerhans cells which function as immune cells, and melanocytes which provide protection against excess UV light.

Underlying the epidermis we find the dermis made of a cushion-like network of elastic fibers (collagen), capillary blood vessels and lymphatic vessels, fine-touch receptors (Meissener's corpuscles), pressure receptors (Pacinian corpuscles), temperature sensors and pressure receptors (Krause's end bulbs), bundles of smooth muscles attached to hair follicles (when activated giving an appearance of "goose bumps"), eccrin sweat glands (the "sex" glands), wax glands and sebaceous glands. This complex display of specialized cells and structures of the skin shows its ability to communicate with the outside world by allowing certain nutritious stimuli into the body while preventing noxious stimuli from entering the organism, as well as evacuating waste metabolic material (sweat, sebaceous, wax glands). In many ways skin operates like a barrier of the gastrointestinal tract. It works by nourishing the body, protecting it from toxins and cleansing it from the metabolic outcome of nutrition.

NUTRIENT ABSORPTION THROUGH THE SKIN

Skin is the largest organ of our body. It is also one of the oldest structures developed in the evolutionary process to provide the organism with essential information about the outside world. In plant and animal kingdoms skin delineates and establishes the identity of the organism, and effectively works as an intercom between the individual organism and the outside environment by selectively delivering, or regulating, the traffic of information, or variety of "nutrients", to the body.

Information is a nutrient and a nutrient can also become information. In addition to traditional nutrient provided information like minerals, vitamins, essential fatty acids or amino acids, information can be also delivered to the skin in the form of topical drugs and non-conventional nutrients e.g. daily light, temperature of the environment or humidity readings. This myriad of nutrient derived information by the skin is then analyzed and processed in steps comparable to that occurring in the gastrointestinal tract.

A common thinking about nutrient delivery through the skin is that skin acts as a porous organ which, like a sieve, allows a certain size of minerals, fats, proteins or sugars to enter while leaving the larger ones out (1). This type of absorption has now been largely demonstrated, but new research shows a much more complex process of skin nutrient delivery. For example, the role of skin appendages (hair follicles, sweat glands and sebaceous glands) in nutrient absorption has recently been studied comparing absorption of hormonal compounds in normal and appendage-free (scar) skin (2). The levels of estradiol and progesterone applied topically were measured after 30 min., 2 and 6 hrs from time of application and showed to be significantly higher in normal skin than in scar tissue. Therefore only the intact healthy skin can provide optimal absorption, and nourish the appendages and hair follicles. Intact keratinocytes manufacture a master protein, proopiomelanocortin (POMC), from which numerous skin and appendage nourishing neuropeptides are derived. These are hormonal compounds which like corticotropin help preserve hair follicle and stimulate hair growth (3,4).

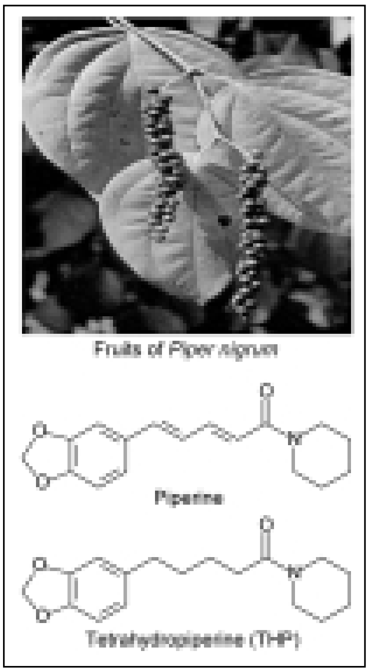

Figure 1 – Chemistry of tetrahydropiperine

Nutrient Delivery Through the Skin, a Different Perspective

Our first contact with the outside world at birth is through the skin, and likely the first impulse reaching skin is light. Light is the first nutrient delivered to the skin. From the very beginning, and throughout life, the nourishing daylight and the darkness of night regulate the organism's biological clock and the entire body physiology. Chronic deprivation of natural light, as it may occur in the polar circle during winter time, is thought to contribute to mental depression in people living within the polar region, e.g. Scandinavian people (5). Also the production of the active form of vitamin D may be diminished since skin contained 7-dehydrocholesterol needs to be cleaved by UV light to form cholecalciferol or pre-vitamin D. Interestingly, northern Europeans (Scandinavian countries) with less exposure to sun have been known to have a higher rate of osteoporosis, a condition in part due to the vitamin D deficiency. Vitamin D supplementation may also be an important treatment for a seasonal affective disorder (depression) or SAD, a condition related to the seasonal decrease in day light (6). It has also been recognized that depression in women is associated with decreased bone mineral density, a typical symptom of osteoporosis and/or vitamin D deficiency (7).

To show the importance of light as a nutrient let us look at our feathery friends – birds. Despite their thick feather covering, birds also require and receive the benefit of sunlight. This occurs in a more complex way than in humans where UV light can penetrate the skin easily. To get around their natural covering, most species of birds have a preen (uropygial) gland which is thought to provide the precursor of vitamin D material. Birds spread this substance on their feathers when they preen, exposing it to the converting action of UV light. When a bird preens again, it ingests the light synthesized product (previtamin D) from its feathers. Interestingly, birds kept

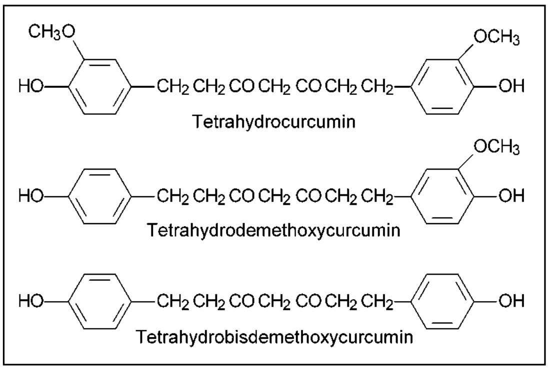

Figure 2 – The tetrahydrocurcuminoids

indoors are less likely to suffer from vitamin D deficiency when exposed regularly to a full range of day light. Exposure to light also coincides with more preening activity thus providing the bird with an improvement in its overall health status.

Day light also nourishes skin through specialized cells called melanocytes, which manufacture the pigment melanin, from the amino acid tyrosine, in response to sunlight. Melanin is thought to provide the body with protection against excessive UVB radiation, but also may participate in regulating the biological clock and psychological functions, e.g. as previously mentioned, mood. It is well recognized that individuals who experience recurrent major depressive episodes in the winter months, with full remission in the summer months, respond well to light therapy, e.g. 10,000 lux light boxes 30 minutes/day, for 2500 lux light boxes 1-2 hours/day. The role of light as a nutrient turning on both psychological as well as metabolic processes can again be examined in birds. It has been established that light information affects birds through two different pathways, the pituitary gland, through melanocytes of the eye, and the pineal gland, through the Harderian gland surrounding the eye. These two pathways, stimulated by day light, help set the rate of many different metabolic processes. Light affects stages of molt (changing feathers), appetite, weight, and posture. Decreased metabolism due to poor light will result in less than optimal nutritional absorption, and less than optimal new feather growth.

Delivery of Sensory Stimuli (Nutrients) Through the Skin

The message of touch is another important aspect of daily nutrient delivery through the skin. This is evident, for example, in the contact between an owner and his pet by the caressing action of the animal. Caressing is an example of sensory nourishment received through the skin and mediated by neuropeptides. Neuropeptides include a group of proteins functioning as neurotransmitters, neuromodulators and neurohormones which participate in sensory nutrient delivery through the skin. These compounds are intricately involved in all physiological functions of the skin ranging from biosynthesis of its components to sensory perceptions, sweat regulation and blood flow regulation. Neuropeptides also affect the functions of our central nervous system and physiology of the entire body.

Neuropeptides together with their neural "highways" and sensory outposts in the skin, e.g. fine-touch receptors (Meissener's corpuscles), pressure receptors (Pacinian corpuscles) and temperature sensors and pressure receptors (Krause's end bulbs), play an essential role in receiving sensory nutrients through the skin. In this process skin provides nutrients and also receives nutrients in return.

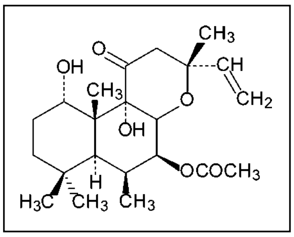

Figure 3 – Forskolin

The sensation experienced through tickling is another example of an important sensory nutrient delivered through the skin. It is well known that you cannot tickle yourself. The reason is that when tickling activities are self-produced, they are predictable. This predictability is read by specialized brain centers, like the cerebellum, as "non-threatening" which results in attenuation of the ticklish sensation (8). However, when such activity is generated by somebody else, this is read by the brain as unfamiliar or "threatening" and the ticklish effect, which in essence is a body defensive reaction, is experienced. In early stages of evolution the unfamiliar meant threatening, such as the touch of a predatory animal. An outside threat produced various forms of defensive reactions, and the ticklish response is thought to be a remnant of a basic defensive reaction of revulsion from a perceived threat.

Delivery of Immunological Nutrient (Stimuli) Through the Skin

As previously mentioned, skin establishes the identity and integrity of the organism and that is why it is closely linked with the immune system. The role of the immune system, in principle, is to distinguish self from non-self. Skin is the outermost outpost in this surveillance role of the immune system. Skin is constantly in touch with multitudes of information or nutrients, e.g. chemicals in the air, water and skin treatments. Each substance is being screened by the skin for its property as a nutrient or anti-nutrient, for representing self or non-self. This screening process is a basic immunological reaction in protecting the identity and integrity of the organism.

Langerhans cells, which are located in the stratum spinosum of the epidermis, play an important role in the immune system, especially in the initial phase of the self and non-self recognition process (9,10). Langerhans cells specialize in examining the potential nutrient and then translating that obtained information to the next line of the immune cells or lymphocytes, especially the so called memory cells (a subtype of T lymphocytes).

This recently discovered immune function of Langerhans cells has now been tested in one of the first attempts of skin delivery of certain vaccines and immune drugs, like interferon (both examples of immunological information or nutrients) (11,12).

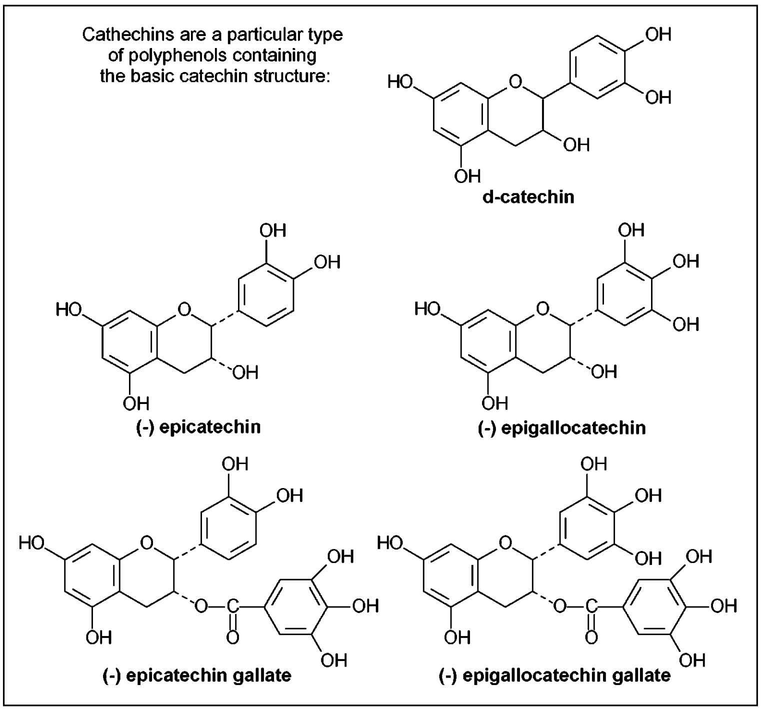

Figure 4 – The catechins in green tea

REGULATION OF SKIN NUTRIENT DELIVERY

The abundance of skin nourishment indicates the important role of regulating various mechanisms of nutrient delivery through the skin. Effective topical delivery of essential nutrients could provide affordable means of disease prevention and sustaining good health.

Selective nutrient absorption is an important physiologic property of the skin. This selective process starts with the outermost layer of the skin, stratum corneum, which provides an outstanding barrier against the external environment (13). The function of this barrier is related to the unique composition of the lipid moiety in the epidermis (14-17). These keratinocyte manufactured lipids are released to the intercellular spaces where they undergo enzymatic processing to produce a lipid mixture consisting of ceramides, cholesterol and fatty acids. The intercellular lipids become organized into a complex structure that fills most of the intercellular space of the stratum corneum, sealing it. The intercellular lipids mediate transdermal delivery of both lipophilic and hydrophilic molecules (18).

Fatty acids play a central role among the epidermal lipids in regulating nutrient bioavailability because they are important components of cell membranes and form the hydro-lipid skin surface film (19). Topical application of certain fatty acids can lead to changes in the bioavailability of nutrients through the skin. On the one hand, fatty acids may restore a damaged stratum corneum barrier. Fatty acids are also able to enhance nutrient and drug transport through the skin by increasing cell membrane fluidity. The fluidity of cell membranes in the epidermis translates to better accommodation of the absorbed molecule. Hyaluronic acid (HA), a high molecular weight glycosaminoglycan of the extracellular skin matrix involved in growth, inflammation and wound healing, also contributes to the hydration and elastic properties of the skin.

Research shows that regulating the composition of intracellular lipids in the skin can increase or decrease the bioavailability of nutrients. For example, skin's sealant can initially be diminished (skin barrier becomes more porous and more permeable) by a diet deficient in essential fatty acids, topical organic solvents, or prolonged topical application of agents interfering with lipid synthesis, e.g. lovastatin, fluvastatin, or cholesterol

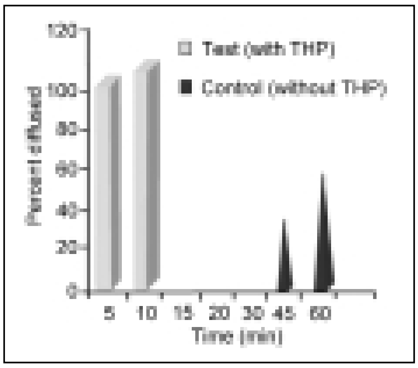

Figure 5 – Transdermal absorption study with betamethasone dipropionate. Reference: Research Report, Sami Labs Ltd. (April 2000)

sulfate (16). This decrease, however, would lead to an over-compensation mechanism increasing the synthesis of lipids, DNA and the number of epidermal cells. These are defensive mechanisms which are the means to restore the integrity of the skin barrier and its function. Interestingly, the artificial barrier repair, e.g. applying topical sealant in the form of latex, prevents the above mentioned skin-repair mechanisms, probably by exerting an epidermal sealing effect (16).

Besides the modification of skin lipid composition there are several strategies to improve topical nutrient bioavailability (20). Improvement can be accomplished by supersaturation of the delivered ingredient. The delivery formulation may also contain ingredients which decrease the diffusional (electrostatic) resistance of the lipid bi-layer to the passing molecule. Topical liposome preparations are effective penetration enhancers for the delivery of certain co-applied drugs and biological compounds, (e.g. interferon) probably due to their role in increasing cell membrane fluidity (21,22). In addition, an increase in blood supply to the skin can enhance absorption of delivered nutrients (20). All above mentioned methods can be combined for a synergistic effect.

Novel Method of Enhancing Nutrients Absorption Through the Skin with a Natural Compound Derived from Black Pepper Fruits

Our previous discussion points to two goals in optimizing skin delivery of nutrients:

1. to keep skin healthy and selectively receptive to various forms of nutrients; and
2. to actively and safely enhance absorption of nutrients through the skin.

It is obvious that certain nutrients, like UV light, can best be provided through well maintained, healthy skin, i.e. skin being able to benefit from UV action while also able to phase out the excess of the pro-oxidant (free-radical forming) action of sun rays. However, the delivery of more conventional nutrients should be based on the principle of enhancing skin physiological events leading to nutrient delivery, or ways with least interference in human physiology. For example, use of organic solvents or inhibitors of lipid synthesis for increased skin permeability may in fact be counterproductive. By decreasing the lipid barrier to increase absorption of a particular nutrient, other aspects of nutrient delivery and overall skin health may be compromised.

One of the possible bioavailability enhancers has been derived from fruits of black pepper, an herb that enjoys thousands of years history of use as a food condiment. Tetrahydropiperine or THP, a derivative of pungent alkaloid piperine, naturally occurs in the fruits of the black and long peppers (Figure 1). THP has recently been developed under the trade name Cosmoperine®. Based on initial research, THP has the property to enhance natural abilities of the skin to absorb nutrients for local and systemic utilization.

As previously mentioned, the necessary characteristic of any skin

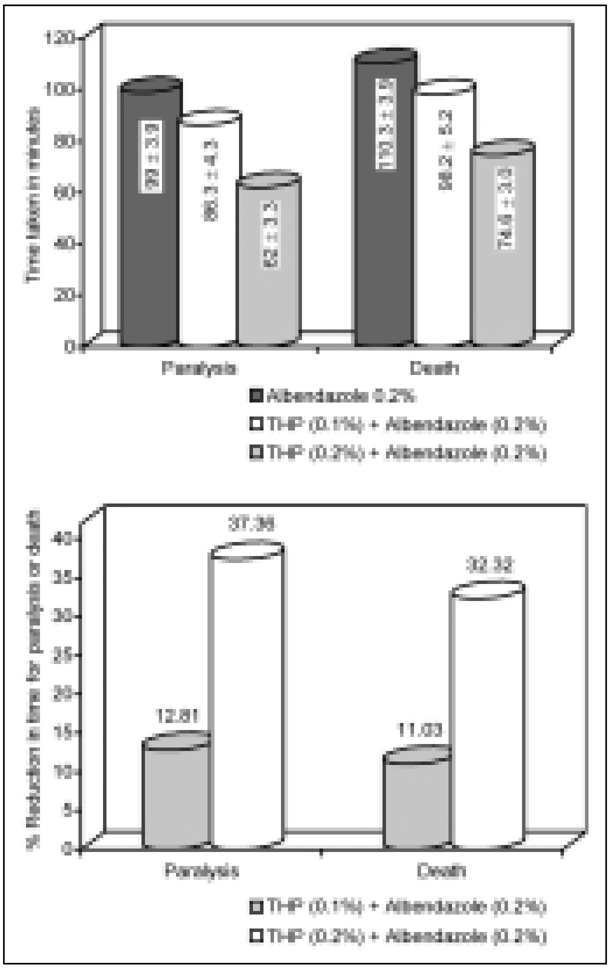

Figure 6 – Activity of albendazole and THP against earthworms.
Reference: Research Report, Sami Labs Ltd. (May 2000)

A	**Conc** (mg/mL)	1 x 10^{-2}	1 x 10^{-3}	1 x 10^{-4}
	Inhibition (%)	55.5	10.2	1.0
B	**Conc** (mg/mL)	1 x 10^{-2}	1 x 10^{-3}	1 x 10^{-4}
	Inhibition (%)	8.1	3.3	3.2
C	**Conc** (mg/mL)	1 x 10^{-2} for each	1 x 10^{-3} for each	1 x 10^{-4} for each
	Inhibition (%)	66.2	16.5	5.8

Sample A: Tetrahydrocurcumin (THC)
Sample B: THP
Sample C: 1:1 mixture of Sample A and Sample B
DPPH at 100 µM Optical density at 517 was used

Figure 7 – Enhancement of antioxidant properties of tetrahydrocurcumin (THC) in presence of THP. Reference: Research Report, Sabinsa (April 2000)

delivery system should be safety. Although THP is based on a pungent principle, it is non-irritant and it interacts with the skin quantitatively and qualitatively in a different way than a pungent principle like, for example, capsaicin from cayenne pepper. Capsaicin is recognized by the US FDA as an OTC topical pain reliever in a dose of 0.025%. However, besides the pain relieving action this dose provides, it often causes skin reddening due to vascular engorgement as well as a slight skin tingling sensation. This reaction to capsaicin can occur within minutes, or a few hours after topical application, and usually lasts from half an hour to several hours from the moment it occurs. Interestingly this reaction tends to subside with regular, sustained use of topical capsaicin in a pain relieving dose.

Recently a study was conducted to determine whether THP, at a level of 0.01% and 0.1%, which is considered an effective dose range for the compound, would produce symptoms of topical irritation. A skin patch test using Cosmoperine in a petrolatum vehicle was conducted on 50 healthy volunteers for 48 h with reading of the results after 48 h and 72 h. Neither dose caused skin irritation at the time of clinical evaluation of the study subjects. The irritation score was reported by the supervising physician, a practicing dermatologist, as 0. This study was conducted by the US FDA accredited BioScreen Testing Inc. laboratory. These results indicate that Cosmoperine does not act as a skin irritant at a dose range considered effective for topical nutrient delivery (Sabinsa Report, December 2000).

The bioenhancing potential of THP was also evaluated in experiments with the steroidal anti-inflammatory drug Betamethasone dipropionate, or BMDP, antihelmintic drugs, a topical anti-oxidant compound derived from turmeric root (*Curcuma longa*, Fam. Zingiberaceae), tetrahydrocurcuminoids or THC (Figure 2), diterpene forskolin derived from *Coleus forskohlii* roots (fam. Labiateae) (Figure 3), and tea catechins obtained from green tea (Figure 4).

In the experiment involving BMDP, the skin preparation was mounted in a Franz Diffusion Cell which resulted in two compartments; "donor" and "receptor". The drug (100 µg/ml) was applied with 0.1% (active sample) or without (control sample) Cosmoperine in the donor compartment. Subsequently the absorbances of the fluid in the receptor compartment for the presence of BMDP and THP were measured in time intervals of 5, 10, 15, 20, 30, 45 and 60 minutes (Figure 5). The active sample resulted in

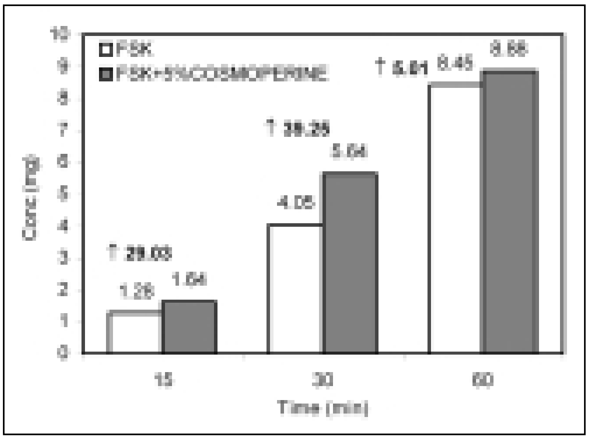

Figure 8 – Effect of THP on the dermal penetration of forskolin

100% diffusion of the BMDP within the first 10 minutes. The control sample resulted in 29% diffusion of BMDP after 45 minutes and only 54% diffusion after 60 minutes (Sabinsa Report, December 2000). Experiments to see how tetrahydropiperine enhances the permeability of anthelmintic drugs such as albendazole on earthworms and Fenvalerate (synthetic pyrethroid) on cut worms were conducted. THP in concentration 0.1% to 0.5% was shown to enhance penetration of Fenvalerate through the cut worm and albendazole through the earthworms (Figure 6) (Sabinsa Report, December 2000).

In another experiment, the bioenhancing potential of Cosmoperine on the free-radical scavenging properties of topically applied THC was evaluated (Figure 7). In this *in vitro* DPPH radical scavenging method, the ability of an anti-oxidant to bind and inactivate the 1,1-diphenyl-2-picrylhydrazyl radical, or DPPH, was measured. DPPH is considered an example of a very stable free radical. The control sample contained 0.01 mg/mL of THC while active samples contained 0.01 mg/mL of THC with Cosmoperine concentrations ranging from 0.01 mg/mL-0.0001 mg/mL. Additionally, controls containing various concentrations of Cosmoperine alone were also tested for DPPH binding. While Cosmoperine by itself did not show any significant anti-oxidant properties, together with THC it was shown to enhance the anti-oxidant properties of THC by up to 30% as compared to when THC was used alone. Even in its highest dilution of 0.0001 mg/mL Cosmoperine still displayed some beneficial THC bioenhancing activity (Sabinsa Report, December 2000).

In the Franz Diffusion Cell model, permeation of forskolin was enhanced when the concentration of THP was 5% of Forskolin concentration (Figure 8). However, when the concentration of tetrahydropiperine was 2% of forskolin concentration, enhanced permeation was not observed. In a similar *in vitro* model, permeation of green tea extracted polyphenols (3 mg/ml with 0.2% DMSO) was enhanced with 1% THP in 0.2% DMSO (Figure 9) (Sabinsa Report, December 2000).

At present, there is insufficient experimental data to postulate a bioenhancing mechanism of THP. However, there are experiments done both *in vitro* and *in vivo* with the parent compound piperine which indicate that THP may operate by increasing either or two of the events: 1. membrane fluidity,

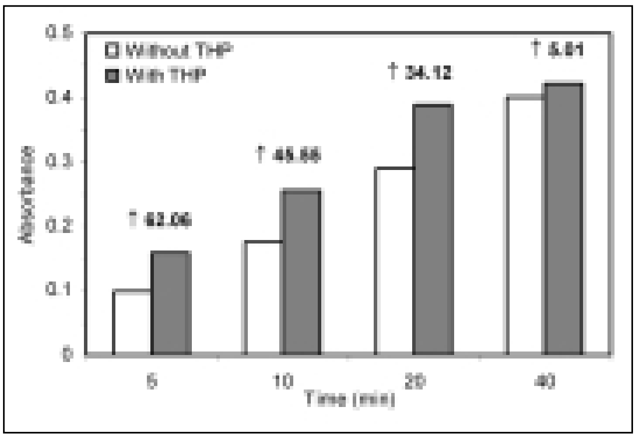

Figure 9 – Effect of THP on penetration of green tea polyphenols across egg membrane.
THP tested using Egg Membrane in Franz Diffusion System

and 2. affinity of nutrient/drug to the cell membrane (23). It should be also considered that THP which is a lipophylic compound may increase solubilization of the intracellular lipid moiety in the skin, making it more permeable to the applied nutrient/drug.

Regulation of skin nutrient delivery to the target cells (bioavailability) becomes an important aspect of skin care and skin health. Tetrahydropiperine is emerging as a new class of topical bioavailability enhancers and based on clinical experimentation with its parent compound piperine, holds real promise as a versatile ajuvant in nutrient delivery through the skin. Piperine has been previously evaluated in oral dosages for its potential to enhance the gastrointestinal absorption of drugs and nutrients in animals and humans. Compounds successfuly studied include drugs such as vasicine, pyrazinamide, rifampicin, isoniazid, propranolol, theophylline and phenytoin, and nutrients such as fat soluble beta carotene, water soluble vitamin B6, vitamin C, coenzyme Q10 and the mineral selenium in the form of L-selenomethionine (23).

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