Cosmetic Science and Technology Series/Volume 23

Cosmeceuticals

Drugs vs. Cosmetics

edited by
Peter Elsner
Howard I. Maibach

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Drugs vs. Cosmetics

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About the Series

The Cosmetic Science and Technology series was conceived to permit discussion of a broad range of current knowledge and theories of cosmetic science and technology. The series is composed of both books written by a single author and edited volumes with a number of contributors. Authorities from industry, academia, and the government participate in writing these books.

The aim of the series is to cover the many facets of cosmetic science and technology. Topics are drawn from a wide spectrum of disciplines ranging from chemistry, physics, biochemistry, and analytical and consumer evaluations to safety, efficacy, toxicity, and regulatory questions. Organic, inorganic, physical and polymer chemistry, emulsion and lipid technology, microbiology, dermatology, and toxicology all play important roles in cosmetic science.

There is little commonality in the scientific methods, processes, and formulations required for the wide variety of cosmetics and toiletries in the market. Products range from preparations for hair, oral, and skin care to lipsticks, nail polishes and extenders, deodorants, body powders and aerosols, to quasi-pharmaceutical over-the-counter products such as antiperspirants, dandruff shampoos, antimicrobial soaps, and acne and sun screen products.

Cosmetics and toiletries represent a highly diversified field involving many subsections of

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science and "art." Even in these days of high technology, art and intuition continue to play an important part in the development of formulations, their evaluation, selection of raw materials, and, perhaps most importantly, the successful marketing of new products. The application of more sophisticated scientific methodologies that gained steam in the 1980s has increased in such areas as claim substantiation, safety testing, product testing, and chemical analysis and has led to a better understanding of the properties of skin and hair. Molecular modeling techniques are beginning to be applied to data obtained in skin sensory studies.

Emphasis in the Cosmetic Science and Technology series is placed on reporting the current status of cosmetic technology and science and changing regulatory climates and presenting historical reviews. The series has now grown to 26 books dealing with the constantly changing technologies and trends in the cosmetic industry, including globalization. Several of the volumes have been translated into Japanese and Chinese. Contributions range from highly sophisticated and scientific treatises to primers and presentations of practical applications. Authors are encouraged to present their own concepts as well as established theories. Contributors have been asked not to shy away from fields that are in a state of transition, nor to hesitate to present detailed discussions of their own work. Altogether, we intend to develop in this series a collection of critical surveys and ideas covering diverse phases of the cosmetic industry.

The 13 chapters in *Multifunctional Cosmetics* cover multifunctional products for hair, nail, oral, and skin care, as well as products with enhanced sunscreen and antimicrobial properties Several chapters deal with the development of claim support data, the role of packaging, and consumer research on the perception of multifunctional cosmetic products. The authors keep in mind that

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in the case of cosmetics, it is not only the physical effects that can be measured on the skin or hair, but also the sensory effects that have to be taken into account. Cosmetics can have a psychological and social impact that cannot be underestimated.

I want to thank all the contributors for participating in this project and particularly the editors, Perry Romanowski and Randy Schueller, for conceiving, organizing, and coordinating this book. It is the second book that they have contributed to this series and we appreciate their efforts. Special thanks are due to Sandra Beberman and Erin Nihill of the editorial and production staff at Marcel Dekker, Inc. Finally, I would like to thank my wife, Eva, without whose constant support and editorial help I would not have undertaken this project.

Eric Jungermann, Ph.D.

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ADDITIONAL VOLUMES IN PREPARATION

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Preface

The term ‘‘cosmeceuticals,’’ coined by Albert Kligman 20 years ago, has rightfully provoked thought and discussion among scientists, industry, and regulating authorities. Basically, the controversy revolves around the question of whether there are any substances applied to the skin that do *not* modify its structure and function. Since scientific evidence shows that even purportedly ‘‘inert’’ substances such as water may profoundly change the structure and function of the skin, this does not seem helpful in distinguishing cosmetics from drugs. Indeed, there is a legal problem with the definition of cosmetics in the United States, but not in other major countries such as Europe and Japan.

In Europe, the Council Directive 76/768/EEC of 27 July 1976, as amended by six Directives, defines cosmetic products in Article 1:

> A cosmetic product means any substance or preparation intended for placing into contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and mucous membranes of the oral cavity with a view exclusively or principally to cleaning them, perfuming them or protecting them, in order to keep them in good condition, change their appearance or correct body odours.

Thus, a cosmetic is defined by its mode of application and by the intention with which it is used. While cosmetics are used on normal or nearly normal skin, drugs are defined as preparations to be used for the treatment of diseased skin. Obviously, there remains a gray zone between what is considered ‘‘normal’’ as opposed to ‘‘diseased’’ skin. This may vary depending on the individual, the society, and over time. In this situation, Article 2 of the Council Directive is helpful.

> A cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use.

Therefore, consumer safety is of utmost importance in cosmetics, while it is a relative issue in drugs, where a balanced benefit–risk assessment has to be made de-

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pending on the severity of the disease. This is reflected by a recent decision of the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) of the European Commission regarding the use of the antifungal ketoconazole in cosmetics. Following a thorough review of the safety profile of the substance, the SCCNFP decided that there were no safety concerns in using up to 2% ketoconazole in cosmetic products. Obviously, this only refers to the cosmetic use of ketaconazole-containing products (e.g., as antidandruff preparations); when used to treat fungal skin disease, they would be considered drugs from a regulatory point of view.

The Council of Europe is an intergovernmental institution that fosters cooperation between European countries. Members are not only the European Union member states, but also nearly all other countries on the European Continent. Its Public Health Committee/Committee of Experts on Cosmetic Products states in a recent document (Comparative Study on Borderline Products and Borderline Situations, RD 4-1/32/1999) that a cosmetic product has to fulfill the four criteria of *function* (Art. 1 as above); *presentation* (i.e., the claims made for the product); *mode of application* (i.e., externally or on the mucous membranes of the oral cavity); and *composition* (i.e., not containing a prohibited substance or too much of a regulated substance). The Committee has analyzed these criteria for a number of cosmetic designations and substances and found that remarkable differences exist in the regulatory approach between countries. For example, in Switzerland, products containing up to 10% α hydroxyacids are considered cosmetics, whereas concentrations above 10% lead to the classification of pharmaceutical. In Austria, the concentration limit is 30%, while there is no limit in Belgium, Finland, Germany, the Netherlands, and the U.K. (RD 4-1/32/1999). Since different classification of the same products hinders free movement of goods and has a negative effect on the establishment and functioning of a common market, the Committee rightfully proposes to start a harmonization process regarding these borderline products, and states

> Such a harmonisation process may come to the benefit of all parties involved—including the consumer.

We hope that this book will contribute to a sincere discussion of the status of ''cosmeceuticals,'' products that are intended for cosmetic use but contain active substances. Since people worldwide are getting older, becoming more aware of their skin health and appearance, and more committed to use safe and effective products to achieve this goal, this debate is a timely one.

Finally, we would like to take the opportunity to thank the contributors to this book, all experts in their fields, who devoted time and effort to their chapters. We are also indebted to Sandra Beberman and Elyce Misher of Marcel Dekker, Publishers, who were more than helpful in the editorial process.

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Cosmeceuticals: Do We Need a New Category?

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I introduced the term cosmeceuticals almost 20 years ago at a meeting of the Society of Cosmetic Chemists. I thought this neologism was both timely and useful, since it would reconcile archaic legal statutes with modern science.

I anticipated immediate endorsement of a concept whose time had come. Instead, the response was immediate disapproval and denouncement. My colleagues in the industry branded me a troublemaker, unfaithful to those who had supported my research. Since then, the cosmeceutical concept has generated a huge amount of controversy. Along the way, the term has acquired political, economic, and legal connotations that have further obscured the intended purpose of the idea.

Whether one is pro or con, the term cosmeceutical has permanently entered the vocabulary of skin care science. For some, the term has been transformed into a marketing tool, touting the benefits of skin care products. Others see it as a provocation for unwanted, costly, regulatory actions. The most benign view is that the category is superfluous and has no raison d'etre. Cosmeceuticals seem to have a certain semantic resonance, as witnessed by similar sounding neologisms; for example, neutraceuticals (foods with health benefits) and neoceuticals (over-the-counter drugs with cosmetic effects).

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In any case, the term is here to stay and has provoked lively debates that, in the end, may strengthen our understanding of the science of cosmetics. I think it will be informative to review the history of this curious case.

I present the unfolding story from the viewpoint of an investigative dermatologist who appreciates the tremendous technical strides made by the cosmetic industry in recent times.

Interest in cosmeceuticals has rushed forward at an impressive pace. Seminars entitled ''Cosmeceuticals'' are being staged annually. These forums are well attended by groups having widely different backgrounds and interests (regulators, basic scientists, physicians, manufacturers, publishers, merchandisers, lawyers, toxicologists, pharmacologists, and industry watchers). Papers and books have been written covering every aspect of the subject, and these provide a rich source of information (1).

Cosmeceuticals are a hot topic on an international scale. The literature has expanded rapidly, presenting a great variety of views dictated by special interests. A number of forces have converged to power this surge of interest. It would seem that some merchandisers have realized the potential for increasing the sale of products that go well beyond the traditional view of cosmetics as merely decorative or camouflaging. Skin care products can now be viewed as active they do something useful and beneficial. They contain ''bioactives'' which, though not medicinal, are endowed with functional and measurable attributes. Alternative terms for cosmeceuticals have sprung up (performance cosmetics, functional cosmetics, dermoceuticals, active cosmetics). All these imply value added desirable attributes, the touchstone for success in a wildly competitive marketplace.

This is a marketer's playground, which makes it possible to incorporate in skin care products an unlimited number of active substances from natural sources (plants, the sea, the earth). The list of beckoning substances, including those synthesized by chemists, is staggering, including vitamins, antioxidants, anti-inflammatories, mood-influencing fragrances (aromatherapy), and even such exotica as placenta, amniotic fluid serum, and hormones ad infinitum. The choices range from the preposterous to the persuasive, and cover the spectrum from the irrational to the rational.

The natural and green movements also provide a background for understanding the robust interest in cosmeceuticals. For many uninformed consumers, natural is good and synthetic is bad; green protects the environment and prevents cruelty to animals. The phrases, ''not tested on animals'' and ''cruelty-free'' have become a marketing ploy that is often hypocritical and false. Marketers understand these lofty impulses of consumers and are quite willing to cater to the widespread prejudices of a chemophobic population. However, cosmeceuticals are here to stay because they serve the multiple needs of manufacturers and consumers.

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THE 1938 FOOD, DRUG, AND COSMETIC ACT: THE BEGINNING OF TROUBLE

In 1938, the U.S. Congress enacted a statue that officially defined cosmetics and drugs in detailed terms, setting up formal criteria for classifying a product as either a drug or a cosmetic. No intermediate category exists, although it was appreciated that a topical could be both a cosmetic and a drug at the same time. This remains the law to this very day.

The 1938 act came into being as a corrective reaction against the ludicrous number of elixirs and patent medicines—some dangerous—which promised cures for all human ailments. It defined a cosmetic, in pertinent part, as an ''article intended for beautifying and promoting attractiveness.'' In contrast, a drug was defined as a substance for use in the diagnosis, cure, treatment, or prevention of disease, *intended to affect the structure and function of the body*. This last clause legally determines whether a formulation is a drug or a cosmetic. It is this narrow phrase that prompted me to coin the term cosmeceutical.

It is important to note that it is not the ingredients in a product, but the claims in labeling or advertising, that determine whether the substance will be classified as a cosmetic or a drug. Congress also declared that the ''intended'' use would determine a product's classification. Thus, if the intended use relates to the diagnosis and treatment of a disease, the substance is a drug; if its intended use is described in advertisements as promoting attractiveness, the substance is a cosmetic. Thus, in reality, you are what you claim you are.

ENTER COSMECEUTICALS

When the 1938 law was written, the science of cosmetology was primitive and crude, steeped in folklore and unsupported claims. The 1938 definition of a drug is now completely archaic and, in fact, an oxymoron. With the great advances in our understanding of skin physiology, it is impossible to think of a single substance that cannot, under some circumstances, alter the structure and function of skin. The most compelling example is water, the milieu in which all vital processes occur which is considered innocuous. However, when a water-moistened cotton pad is sealed to human skin for 2 days, proinflammatory substances such as interleukins are released from the dead stratum corneum. These incite a series of cytotoxic changes in the viable epidermis below (2). In another few days, an inflammatory reaction is provoked in the dermis. This is the basis for the adverse clinical events associated with prolonged exposure to water, for example, in bartenders, housewives, canners, etc. Thus water can be beneficial in emulsions that hydrate xerotic skin or harmful under intensive exposure.

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Another traditional substance considered inert is petrolatum. However, various studies show that petrolatum promotes healing of wounds and prevents ultraviolet-induced tumors, even though it is not a sunscreen (3). These are clearly medicinal effects that affect the structure and function of skin, yet no rational person would want petrolatum to be reclassified as a drug. From these and many other examples, it is apparent that nearly all cosmetic articles would have be be reclassified as drugs, if a strict interpretation of the ''structure and function'' proviso of the 1938 act were used.

Most skin care products lie somewhere in between drugs and cosmetics. They comprise a continuous spectrum of substances intermediate between the two polar categories defined by Congress. Some traditional cosmetics are more druglike in their beneficial effects and some drugs impact principally on appearance. It is this intermediate, broad-spectrum range of substances that consists of both drugs and cosmetics which justifies the fusion term cosmeceuticals. This is simply a biological concept that recognizes the new realities of skin care products.

This acceptance of biological reality does not mean that we need new laws that officially define in statutory terms the category of cosmeceuticals. The FDA has always had the authority to determine from advertising claims and labeling whether a product promoted as a cosmetic has crossed the line and requires reclassification as a drug.

Cosmeceutical is a pragmatic term that enables us to state without pretense the benefits of a product. It is not an invitation to pass new laws.

A strict, legal interpretation of the 1938 law would necessitate the conversion of ''active'' cosmetics to drugs. This would be a disaster of the highest magnitude that would immediately stifle innovation and creativity. Drug development is slow and costly, and requires proof of efficacy and safety. Cosmetics, on the other hand, do not require premarketing clearance and can be rapidly commercialized provided that the claims are not grossly misleading.

Over the years, and to its credit, the FDA has been flexible and permissive in the way in which it has viewed claims, some of which are unequivocally exaggerated. The trouble comes when some cosmetic manufacturers make frank drug claims for their products. In this case, the FDA sends out warning letters that require relabeling of the products without necessarily changing any of the ingredients. Competitors who are prudent and conscientious may be at a disadvantage if they make less aggressive claims, an issue highlighted by the ''antiaging'' claims made for certain α-hydroxy acids.

Cosmeceuticals enable cosmetic scientists to communicate with each other regarding the standards that must be met to justify performance claims, without resorting to hype.

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THE INTERNATIONAL SCENE

There are three main trading blocks, the United States, Europe, and Japan. Obviously globalization as an integrated free-trade network cannot work if each block classifies and regulates skin care products differently. Unfortunately, no international consensus currently exists, inevitably sparking disputes and trade practices that may place some producers at a grave disadvantage.

The situation is more complex and far more demanding in Europe. This is made obvious in the European Economic Cosmetic (EEC) Directive of 1993. The requirements for labeling cosmetics are formidable and daunting (4). The product information that must be made available to officials encompasses the following: qualitative and quantitative composition of the product: specifications of raw materials; methods of manufacture; safety assessments; and proof of effectiveness. In the United States, manufacturers are not required to demonstrate either safety or efficacy prior to marketing, as is the case for drugs. On top of all this, the EEC has prohibited testing on animals after January 1998 (which I judge to be completely unrealistic).

Japanese authorities have created their own laws in response to the problem that many skin care products are neither pure drugs nor pure cosmetics in the traditional sense, but mixtures of the two. The category we call cosmeceuticals they call quasidrugs (5). They allow cosmetics to include pharmacologically active ingredients, provided that the medicinal effects are mild and the products have been demonstrated to be safe. The legal wording leaves a lot of room for ambiguities and ad hoc interpretations that some perceive as a trade restraint.

Even a cursory look at these regulatory disparities shows the detrimental effects of not establishing uniform, international standards. The following examples illustrate the quandaries which now exist, a situation which is bound to get nasty without an international consensus.

In the United States, the following agents are regulated as drugs while in Europe (according to the European commission on cosmetics) they are sold as cosmetics.

1. Antiperspirants
2. Antidandruff shampoos
3. Sunscreens

This classification is detrimental to industry in the United States, especially in the case of sunscreens, which are more advanced and more effective in Europe because there is greater choice of ingredients. Paradoxes also abound in the United States. For example, retinol (vitamin A) can be sold as a cosmetic, but its oxidation product, retinoic acid, is regulated as a drug. Furthermore, claims

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allowed by the FDA for a recently approved retinoic acid product (Renova, Ortho Pharmaceuticals) are purely cosmetic and relate only to improved appearance. However, the product is still only available by prescription!

On the other hand, minoxidil, a drug that purports to grow hair and improve attractiveness, satisfies the basic definition of a cosmetic and is available without a prescription.

Sometimes, there are too many statutory exceptions and loopholes that are downright dangerous. For example, theophyllin is a powerful drug with a narrow therapeutic index, and is used in the treatment of asthma; blood levels should be monitored frequently. Yet, this same agent can be sold as an unregulated cosmetic when incorporated in topical formulations for the treatment of cellulite.

I recommend that all interested parties read the scholarly treatise prepared by Vermier and Gilchrest, respectively (6). They argue that cosmeceuticals already exist and are in fact desirable as intermediates between cosmetics and drugs and that they should continue to be regarded as cosmetics. The current legal definitions are archaic and unworkable. They move toward the European position and recommend that it is in the interest of manufacturers to prove the efficacy of active cosmetics.

COSMECEUTICALS: A DIVERSITY OF OPINIONS

The writings on this subject are fascinating and cover a remarkable range of divergent, confusing, and conflicting opinions from all over the globe (7). Recent papers strongly express the feelings and beliefs of major players in this field.

Dweck's paper provides the British perspective. He begins as follows: What on earth is a cosmeceutical (8)? Is it an attempt to convince the consumer that their skin care product is really a topical medicine without a proper license, or is it a genuine category that attempts to provide a mild product that has been more stringently tested than a normal skin care product? He recommends reading an official medicine leaflet as a guide to deciding what comprises a medicinal product. He concludes that future discussions will be marked by debate. The British have clearly reached no conclusions.

Wittern takes up the issue from a European perspective. He is decidedly not enamored of the term cosmeceutical (9). He considers ''that the existing legal regulations are precise and clearly distinguish between cosmetic and pharmaceutical efficacy. They do not allow for the introduction of a new class of products such as cosmeceuticals.'' He recounts that A. M. Kligman introduced the term but didn't bother to define it. ''Obviously, he didn't know what he was starting.'' I plead guilty to not knowing what controversial storms would follow the concept. The piece de resistance of the cosmeceutical imbroglio is the article by Urbach (10), who states:

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> At the moment, there is hardly a topic in the cosmetic industry as controversial as cosmeceuticals . . . Cosmeceuticals meet consumer demands for high efficacy. From a consumer and regulatory point of view, having a separate cosmeceutical class in neither helpful, scientifically suitable or juridically necessary. The cosmeceutical concept is superfluous. The most sensible and useful service we can give the consumer legislator and manufacturer is to advise against the further use of this term (11)!

Steinberg presents the American perspective (11). He endorses the term and thinks its introduction has made it necessary to reconsider the statutory definition of a drug and a cosmetic and to seek international agreements on the kinds of regulatory actions that might be enacted.

The situation is quite different in Japan, as described by Takamatsu (5). It turns out that the Japanese government recognized early on the problems that were coming to the fore as a result of the cosmetic industry's ability to create ''performance'' products that did more than beautify.

It is abundantly clear that, for the sake of fair trade, a rapidly expanding international marketplace will have to come to grips with the problems presented herein.

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Definition

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New insights about the function of the skin, as well as the development of new products for skin care, make it necessary to question or redefine the definitions of cosmetics and drugs. Moreover, in the United States, Europe, and Japan, different definitions of cosmetics are used. The definition of a drug is more or less equivocal on these countries. According to the Food, Drug, and Cosmetic (FDC) Act, a drug is defined as an article intended for use in the diagnosis, mitigation, treatment, or prevention of disease or intended to affect the structure or any function of the body.

In the United States, according to the FDC act of 1938, a cosmetic is defined as an article intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting structure or function (1). It is noteworthy that in this definition the cosmetic is not allowed to have any activity (i.e., without affecting structure or function). In Europe, the definition of a cosmetic was reevaluated and described by the council directive 93/35/EEC of June 14th, 1993 (2). The cosmetics directive contains 15 articles. The definition of a cosmetic is described in article 1 and is as follows:

> A ''cosmetic product'' shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body epider-

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> mis, hair system, nails, lips and external genital organs or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition.

The other 15 articles describe the following topics: overall safety requirements, controlled substances, potential ban of animal testing, inventory of ingredients, labeling, harmonization, product information requirement, procedure for adaptation, list of permitted ingredients, safeguard clause, and implementation.

According to the pharmaceutical affairs law, the Japanese definition of a cosmetic is as follows:

> The term cosmetic means any article intended to be used by means of rubbing, sprinkling or by similar application to the human body for cleansing, beautifying, promoting attractiveness and altering appearance of the human body, and for keeping the skin and hair healthy, provided that the action of the article on the human body is mild.

The Japanese definition is only slightly different from the definition of a cosmetic within Europe. Both definitions allow a cosmetic to have mild activity and possess pharmaceutical activity. This is in sharp contrast to the definition of a cosmetic in the United States.

Moreover, in article 7a of the European cosmetics directive, which describes the product information requirement, it is stated that a proof of effect should be included (2). In the United States, however, a product would be regarded as a drug if a proof of effect was mentioned.

Extensive research on the physiological activity of the skin has provided evidence that even small changes in the environment can modify the activity of skin tissue (3,4). Application of inert creams (5), humidity, UV light (4), water (6), etc., all influence the activity of the skin and therefore possess pharmaceutical activity that may affect structure or function of the skin. Thus even water or the humidity of the air could be defined as a drug, according to the FDC act! As mentioned by Gilchrest, the Food and Drug Administration asked her to define water as a drug, when water was applied on the skin under experimental conditions (7).

Registration of a product as a drug requires many elaborate and costly procedures; therefore, the manufacturer of a product with pharmaceutical activity would prefer to have the product registered as a cosmetic. This might mean that the pharmaceutical activity of the product is not mentioned and/or investigated, and, as a result of these confusing and old-fashioned regulatory rules, important information is not given to the public.

The introduction of the term ''cosmeceutical'' enables us to classify more precisely a product with an activity that is *intended* to treat or prevent a (mild) skin (abnormality). In order to avoid introducing new definition criteria, we suggest that cosmeceuticals are only regarded as a subclass within the domain of a

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Table 1 Cosmeceuticals as a Subclass of Cosmetics (Europe and Japan) and as a Subclass of Drugs (U.S.)

	Cosmetic	Cosmeceutical	Drug
Pharmaceutical activity	+	+	+
Intended effect in skin disease	−	(+)	+
Intended effect in mild skin disorder	−	+	(+)
Side effects	−	(±)	+

cosmetic or drug. In Europe and Japan, cosmeceuticals can be regarded as a subclass of cosmetics; however, in the United States cosmeceuticals can only be regarded as a subclass of drugs. Cosmeceuticals could be characterized as follows: (1) The product has pharmaceutical activity and can be used on normal or near-normal skin. (2) The product should have a defined benefit for minor skin disorders (cosmetic indication). (3) As the skin disorder is mild the product should have a very low-risk profile (see Table 1). The definition of minor skin disorders or mild skin abnormalities is difficult and can be regarded as cosmetic indications. Even socioeconomic factors may have an impact on whether a skin disorder is regarded as a disease or as a cosmetic indication (8,9). Nevertheless, in most western countries there is no written consensus that skin abnormalities that are treated by over-the-counter drugs may be regarded as mild skin disorders or may be termed cosmetic indications (9,10).

The procedure for registration of a cosmeceutical should not be as cumbersome as for drugs. The intended activity of the cosmeceutical for treatment of a minor skin disorder should be demonstrated by clinical studies within the framework of good clinical practice. Moreover, it should be shown that safety requirements are optimal and that no side effects can be expected (11). The safety evaluation is mandatory for cosmetics in Europe, according to articles 2, 12, and 13.

In the United States, this would mean that a subclass of drugs (cosmeceuticals) are registered in a similar manner as over-the-counter products (12). It would be beneficial if these countries could agree on the definitions of cosmetics and drugs and, in so doing, define cosmeceuticals as a subclass of cosmetics. This would prevent the current situation in which certain products are registered as drugs in the United States (sunscreens) and as cosmetics or cosmeceuticals in Europe and Japan.

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3
Photoaging

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INTRODUCTION

Aging and photoaging of the skin are now well-accepted concepts, but formerly it was equivalent to heresy to propose that these were anything but natural events. Aging skin was to be accepted as an inevitable, irreversible, and trivial consequence of getting old.

It became obvious early on that skin damage was an inevitable sequela of the medical use of x-rays; only in the past two to three decades has the extremely damaging nature of ultraviolet radiation (UVR) become increasingly clear to both scientists and the general population, and attempts to circumvent and reverse such damage have become extremely popular. These observations have coincided with several pertinent phenomena: (1) the incredible growth of scientific knowledge in recent years; (2) people in western populations living longer and spending increased leisure time exposed to sun in outdoor activities; and (3) the rampant cosmetic claims for products that will ''turn back the clock'' to youth overnight.

In the midst of this chaos, there exist two opposing hemispheres. One is the northern hemisphere, where life is rigid, cold scientific proof is difficult, and only the hardiest survive in the frozen tundras of pharmaceutical bureaucracy and governmental regulation. The southern hemisphere is friendly and warm and things that make you ''feel'' better are considered good, rather than inherently evil because they are not ''natural'' and may prevent us from looking our age.

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War has inevitably existed between these two spheres ever since south's cosmetics were defined as bad and the north's pharmaceuticals were defined as good. Advocacy of the term cosmeceutical, as an attempt to compromise and bridge the gap between cosmetic and pharmaceutical, greatly enlivened the debate.

In fact, the debate has forced us to reevaluate what we truly believe, even made us iconoclasts, willing to listen to new ideas. It has taken place during an era of unprecedented discovery about the structure and functioning of the skin, and the discussion has begun to rise above the former shrill hysteria and is now on a higher plane of logic and scientific facts.

Photoaging is the ideal skin condition to focus the debate. On one hand, appearance of the facial skin makes this condition so obvious to the subject and observers, which in turn makes the use of cosmetic products so appealing. On the other hand, only a pharmaceutical product can truly and meaningfully effect change in the substantial pathology of the condition. The clear demonstration of the clinical efficacy of tretinoin, a pharmaceutically active retinoid topically applied for a cosmetic condition, speaks to the utility of the term cosmeceutical.

BACKGROUND

The term cosmeceutical implies cosmetic utility with an activity of at least a mild pharmacological or pharmaceutical nature. Cosmetic effect should be at least a partial result of structural or functional change, which can be reproducibly demonstrated by some reliable, accurate, and validated methodology—be it clinical or instrumental. Topical products presently predominate in the discussion, yet nontoxic, systemic substances such as vitamins and naturally occurring substances should also be considered in the definition.

Distinction between intrinsic aging of the skin and photoaging has been repeatedly emphasized, but in the context of this discussion it has little relevance; skin that is visible and cosmetically deteriorated is invariably sun exposed and usually highly sun damaged. In the vast majority of individuals, photoaging overshadows intrinsic changes, especially in the skin of the face, neck, and dorsal forearms (1).

The terms photoaging and photodamage have frequently been used interchangeably, although we have previously preferred to define photoaging as a process and photodamage as a description of the clinical or histological condition at any point in time. Photoaging begins at a very early time point, even in infancy, as a result of repetitive, chronic exposure of the skin to ultraviolet radiation. Clinical changes recognizable as photodamage may appear in early childhood, especially where exposure is high. A study of teenagers' skin in Australia demonstrated that 70% of Australians have detectable sun damage by the age of 14

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years (2). As the process of photoaging continues, additional clinical signs of wrinkling, texture, and pigmentary change become progressively noticeable. Cessation of exposure to UVR or treatment allows partial reversal of the clinical and histological change. Both from the clinical and chronological standpoints, the process is a continuum with change possible in either direction.

THE COSMECEUTICAL PERSPECTIVE

The concept of photoaging is scientifically and psychologically complex and inclusive of a broad perspective (Table 1). A subject's self-assessment of appearance, the marketing claim made for the product, and the physical attributes of the cosmetic product are frequently strong forces in the equation, yet to the practitioner, investigator, and regulator, a rigorous endpoint of cosmetic improvement or demonstration of pharmacological activity may be most central. Evaluation for cosmeceutical effect must account for the following specific and distinct needs.

The customer's perspective is more related to the individual's perceptions of their skin appearance than to a meticulously quantitated numerical assessment of its condition. These perceptions are more global than specific, and mandate an evaluation that is weighted toward overall appearance but adequately accounts for specific concerns of dryness, texture, wrinkling, skin color, and pigmentary unevenness. The consumer asks, ''How old do I look?'' first and ''How can I get rid of these wrinkles and liver spots?'' second, although much scientific investigation seeks to preferentially quantitate specific parameters.

Cosmetic or therapeutic effects produced by the product are important to the consumer, but so are physical aspects of the product itself. A product that is not cosmetically elegant or that is drying or irritating to the skin will be less acceptable to the consumer in spite of alleged pharmaceutical properties. These aspects and physical attributes of cosmetics can be well quantitated by both consumer panel testing of the product as well as by specific instrumentation.

Table 1 The Cosmeceutical Perspective

Subject's self-perceptions
Customer cosmetic expectations
Product physical attributes
Regulatory aspects
Marketing claims
Degree of pharmacological activity

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Cosmeceutical properties of a therapeutic intervention are central to the discussion and may require both well-conducted studies of consumer satisfaction as well as adequate documentation and substantiation of cosmetic or therapeutic claims (e.g., improvement in overall appearance, percent reduction of wrinkles, improvement in roughness and pigmentary unevenness). Adequate methodology exists to evaluate rigorously these cosmeceutical aspects.

Pharmaceutical testing of pharmacological effect is, by definition, the most stringent, requiring not only adequate trial design and execution but substantially more documentation of statistically significant changes that are also clinically and consumer relevant. This is the area where proper application of biometrics to photoaging is most helpful and important (3).

THE CLINICAL CONDITION OF PHOTOAGING

Cosmetic Deficits

The subject or patient complains that they look ''old'' to themselves and others (Table 2). They note that their skin is rough, dry, wrinkled, and that their face and hands, in particular, have numbers of variously colored flat spots. Tanning no longer produces an even darkening of the skin and, especially on the legs, numerous white spots have appeared. Occasionally they are aware of a less resilient quality of their skin, which in some areas tends to sag and not bounce back when it is stretched. Raised unsightly growths of cosmetic or medical concern to the patient may have appeared.

Clinical Presentations

Photoaging is most frequently progressive, yet modified by both environmental exposure and genetics (Table 3). The clinical presentation of photodamage is therefore highly polymorphic but with many characteristic signs and symptoms (4).

Table 2 Photoaging Signs and Symptoms

Overall appearance older than chronological age
Wrinkles, fine and coarse
Diverse pigmentary alterations
Rough texture
Dryness
Sallow complexion
Various neoplasms, benign and malignant

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Table 3 Functional Abnormalities of Photoaging

Uneven tanning
Skin easily distends
Slow return to normal contour
Thinned skin easily traumatized
Sensory decrease
Decrease in immune competence

The most casual observation of the face or neck of an individual with photodamaged skin, even by the untrained observer, consciously and subconsciously gives an overall impression of a person older than their chronological age. Visually, wrinkles both fine and coarse are frequently the hallmark of sun-damaged skin in many individuals, although genetic differences may, in some, favor pigmentary alterations or thinning of the skin as the most prominent presenting sign.

An overall sallow, or yellowish hue, is common and presumably due to the complex interplay of light absorption and reflection in photodamaged skin that is characterized by uneven thickness of the stratum corneum and abnormalities of melanization. Additionally, circulatory alterations of endogenous origin or as a consequence of photodamage produce variable contributions of heme pigment to overall skin color.

Pigmentary alteration of photodamaged skin is very common but highly variable. Discrete alterations consistent with actinic lentigos, especially prominent on the face and hands, may alternate with mottled hyperpigmentation consisting of patchy and alternating lighter and darker macules due to diffuse abnormalities of melanogenesis and melanosome distribution in keratinocytes. Diffuse pigmentary change may also present as melasma on the face due to either epidermal melanin abnormalities or dermal macrophages containing melanin or heme pigment.

Hypomelanotic macules are vitiligenous and are frequently observed most prominently on the lower extremities.

Dryness and surface roughness, best perceived by tactile rather than visual means, are among the most common complaints related to aged and photoaged skin, but are not specific for either. Scaling due to dryness or perturbation in epidermal turnover is also common, but not specific to photodamage.

Multiple neoplastic lesions as a consequence of photodamage are common. Benign seborrheic keratoses are mostly cosmetic growths that appear in sun-exposed body areas.

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Functional Alterations

Photodamage-related structural alterations in epidermis and dermis are mirrored by functional abnormalities that may be of either cosmetic or medical consequence. The skin no longer tans evenly and areas of hypopigmentation may sunburn after minimal UVR exposure. The skin is easily distended and does not quickly return to its original contour. Grossly observed thinning and histological stratum corneum irregularity, epidermal thinning, and abnormal collagen and elastin result in skin that is easily traumatized with abrasions, cuts, and tears. Blood vessels can be easily seen through the skin and, because of epidermal thinning and decreased dermal integrity, the skin bruises and bleeds more easily than normal.

Sensory decrease, not usually clinically obvious, has been partially documented in increasing age, though not specifically in photoaging. Utilizing skin compliance and a two-point discrimination testing on the pad of the index finger, increasing age was correlated with decreased tactile sensitivity and said to be likely related to change in the nervous system (tactile discrimination), rather than change in skin itself (skin compliance) (5). An actual increase in intraepidermal nerve fibers, correlated with severity of photodamage, was observed in a recent study of the ultrastructure of photodamaged skin and was theorized to be indicative of a neural involvement in the pathophysiology and/or repair of photodamaged skin (6). The known effects of capsaicin on skin sensory function as well as the observations of synthesis of nerve growth factor (NGF) and other biologically active factors by keratinocytes should stimulate further research in this interesting area.

A decrease in immune competence of aged skin has been repeatedly demonstrated and UVR is known to be highly immunosuppressive. This complicated interaction continues to be an extremely important area of research (7).

COSMECEUTICAL PRODUCT TESTING

Product Attributes

The consumer can personally judge certain charateristics of the product and, although there is a wide range of acceptable characteristics, certain general principles apply. Since the products will be applied at least to the face, and probably to other areas of the body, it must be cosmetically acceptable and preferably refined or elegant. Exceptions are those individuals who will use a greasy ointment or malodored product thinking it must be therapeutic if it has those undesirable characteristics.

Product qualities and potential acceptance can be tested more rigorously by using a panel of trained individuals who note the various properties of the

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Table 4 Product Testing

Product attributes by dermatosensory panel
Claim substantiation
Instrumentation
Pharmaceutical testing

formulation (Table 4). Even untrained individuals may be able to distinguish overall acceptability but use of trained panelists allows much greater refinement of the overall and individual aspects of the product. Commercial testing is available (entitled the DermatoSensory Profile), which describes and grades or compares products for their characteristics of appearance and feel on the skin. Testing includes evaluation of the rate of absorption of the product into the skin, including spreadability and stickiness, immediate afterfeel, including shininess, greasy or oily feeling, drag (the sensation of resistance to motion over the skin), and residue (the sensation or perception of something remaining on the skin). Perception of residue after set periods of time such as 5, 15, and 30 min is called delayed afterfeel. Various descriptions of the product itself, aside from its characteristics on the skin such as color, odor, thickness, substantivity, consistency, grittiness, or smoothness can also be described. Many of these product characteristics are important in consumer acceptance of the cosmeceutical as well as in their perception of benefit.

Cosmeceutical Testing

No matter if cosmetic or pharmaceutical endpoints are sought, adequate trial design is critical for accurate, precise, consistent, reproducible, and valid observations in photoaging. The optimum trial for pharmaceutical, and to some extent cosmeceutical, purposes is double-blind, placebo (vehicle)-controlled, multicenter, and frequently, for the chronic process of photoaging, of at least several months' duration. Study of a relatively large, genetically homogeneous population of a narrow range of similar skin type (usually skin types I–III) is often required to observe statistically significant differences. Cosmetic testing may be of much shorter duration in fewer subjects but should optimally follow the same basic logic. Study of the parameter that is most important to the product is essential; a facial moisturizer designed for older females living in the north should not be artificially tested in male and female college students in the south. Similarly, establishment of change of transepidermal water loss (TEWL) is irrelevant if the product is claiming to affect wrinkles.

Overall severity rating for study entry and follow-up of global appearance has been accomplished with a photographically derived rating scale, with 0 =

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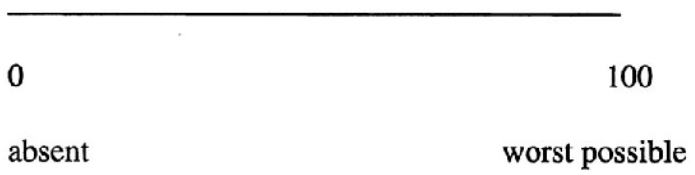

Figure 1

none, 1 to 3 = mild, 4 to 6 = moderate, and 7 to 9 = severe photodamage (8). These evaluations may be performed by the investigator, by the subject, or by third persons comparing photographs of before and after in a randomized and blinded manner.

This and similar rating scales may also be applied at baseline and follow-up to specific parameters such as wrinkles, surface roughness, mottled pigmentation, overall color, skin dryness, and texture.

A 100-mm visual analogue scale has been successfully utilized to rate the overall appearance of the skin and specific parameters of fine wrinkles, discrete pigmentation, shallowness, and texture. The scale may be continuous from 0 to 100 (Fig. 1), rating the condition as absent to severe, or balanced (Fig. 2) with a score of zero designating no change from baseline, improvement recorded to the right side of zero (to a maximum of +50 mm), and worsening recorded to the left side of zero (to a minimum of −50 mm).

A clinical panel evaluation technique has been described that potentially allows precise, consistent, and completely unbiased evaluations of clinical state (9). Very high-quality photographic slides are obtained, prepared in matched carousels, and placed on two random-access projectors in a rear-screen projection booth. Side-by-side comparison of each patient's time-randomized baseline and end-of-treatment photographic slides by trained, but uninvolved, evaluators is thus accomplished in a completely blinded and randomized manner. Both global response and specific parameters of overall appearance, fine wrinkles, and discrete pigmentation can be judged and graded on a 13-point balanced categorical

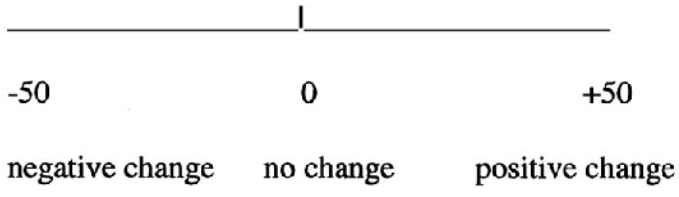

Figure 2

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scale with zero representing no difference between the two photographs. A score of +1 to +6 is assigned when the end-of-treatment photograph is perceived to be better and a score of −1 to −6 when the baseline photograph is perceived to be better.

Trials of isotretinoin have successfully utilized the clinical panel and demonstrated the therapeutic effect compared to vehicle (10,11).

Claim Substantiation

If the above-described pharmaceutically oriented trials demonstrate significant effect, claim substantiation may be relatively easy and clear-cut.

However, distinction may be made among the various aspects of a claim for a product. There are, in each country, very different cultural and legal perspectives dictating what can be stated about a product designated for photoaging. These claims are generally related to the broad perception of whether the cosmetic or skin-care aspects, the potential cosmeceutical action, or a proven pharmaceutical effect is being claimed and advertised. In this regard, everything from consumer statements, consumer panel testing, instrumentation results, clinical testing results, to absence or presence of statements regarding alteration of structure or function of the skin must be considered in evaluating, regulating, and advertising a product.

Use of Instrumentation

With no other methodology is there the same potential for accurate, precise, reproducible, consistent data to used appropriately or to be nefariously manipulated to purport therapeutic effect as with the now-available and sophisticated instrumentation (12). Evaluation of the obtained instrumental data must take place in a clinically appropriate context for them to be truthful and meaningful (Table 5).

Table 5 Instrumentation

Optical profilometry
Fluorescent photography
Ultrasound
Transepidermal water loss
Skin hydration by capacitance/conductance
Laser Doppler
Colorimetry

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Optical Profilometry

Profilometry is one of the most useful techniques in photodamage evaluation. A skin replica obtained with silflo dental impression material is oriented and side-illuminated to produce shadows of various widths and depths that are captured by high-resolution video interfaced to a computer containing image-analysis software (13). The width and depth of the ''peaks'' and ''valleys'' of the skin surface may correspond to roughness, wrinkling, or other surface contours or markings and can be differentiated by the analysis software. The technique is reproducible and blinded, can be utilized even in large clinical trials, and has correlation with the clinical condition. Evaluation of the cheek and crows feet area of the face consistently demonstrate approximately a 10% improvement in wrinkles after treatment with tretinoin (14). It can also be useful in evaluation of skin roughness that may change after tretinoin or alpha hydroxy acid treatments.

Fluorescent and Polarized Photography

Another highly informative technique utilizes photographs obtained with illumination of UVR of 365-nm wavelength to highlight differences in epidermal melanin content and distribution. The photographs of photoaged skin may be highly dramatic in accentuating mottled and diffuse pigmentary alterations and may also be quantitative, utilizing visual counting of macules and evaluation of diffuse change compared to a 20-point gray scale (15). Polarized photography allows selective enhancement of the appearance of wrinkles, pigmentary change, or erythema (16).

High-Resolution Facial Photography

A very sophisticated research tool, high-resolution facial photography allows direct visualization and measurement of individual wrinkles and appears to be potentially highly sensitive and precise for determination of this parametner (17). Present systems are proprietary, however, and have not been commercially available.

Ultrasound

High-frequency B-scan ultrasound consistently demonstrates an echo-poor band in the upper dermis corresponding to the location of dermal elastosis of photoaging (18). Refinement of ultrasound techniques may soon allow more precise definition of epidermal and dermal thickness and changes resulting from therapy.

Transepidermal Water Loss

Although a nonspecific technique, measurement of transepidermal water loss (TEWL) is frequently utilized. TEWL may be increased whenever barrier func-

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tion of the epidermis is altered. The underlying changes of stratum corneum may be a result of acute or chronic insult and correlation of TEWL with a specific condition or its therapy is frequently not a simple matter. Aged skin has been variously demonstrated to have changes in TEWL (19). Nonspecific interventions, such as use of moisturizers, may quickly effect substantial changes in TEWL and more specific hydrating measures, such as the inclusion of humectants, hyaluronic acid, lactic or glycolic acid, and lipids, which can be absorbed into the intercellular space, may also affect TEWL.

Skin Hydration

Measured by capacitance or conductance, skin hydration may (similar to TEWL) demonstrate acute, chronic, narrow, or wide oscillations in a variety of skin conditions and experimental circumstances. Hydration status is not specific to photodamage but may be improved as a result of some treatments for photodamage.

Cutaneous Blood Flow

Blood flow through the skin can be measured by laser Doppler instrumentation and may reflect local flow as with increased capillary growth or as a result of local inflammation or overall vascular response to environmental factors such as exercise. As with many other techniques mentioned herein, interpretation of results is critical to reliable conclusions about pharmacological effect of a product.

The Dansyl Chloride Technique

This relatively simple method allows estimation of epidermal turnover by staining stratum corneum with dansyl chloride (or other stains) and subsequently evaluating the time to elimination of the stain from the skin by visual or instrumental observation. The time to elimination of the stain (in days) reflects the rate at which the desquamating stratum corneum has been replaced by underlying proliferative epidermis. Agents purporting skin ''rejuvenation'' are frequently evaluated with modifications of this technique.

Skin Color by Colorimetry

With the use of appropriate filter, this technique is more specific, sensitive, and reproducible than visual observation of skin color. It offers an instrumental approach to enable separation of skin color effects related to melanin or heme pigments and is especially useful in precisely quantitating erythema.

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Miscellaneous Instrumentation

Mechanical properties of the skin measured by twistometry, indentometry, levarometry, and ballistometry reflect some of the most important aspects of the mechanical functioning of the skin such as elasticity, ability to reconform after deformation, strength to resist torsional and abrasive trauma, etc. Although many techniques exist to evaluate these characteristics, in the absence of notable products that improve these functional aspects, they are not yet of widespread pharmaceutical testing utility.

PRODUCTS POTENTIALLY CLASSED AS COSMECEUTICALS

It is clear from the above-described diversity of product testing that many extremely sensitive and reproducible clinical and instrumental techniques are widely available to study products for their potential cosmeceutical effect. The issue of classification of any product thus becomes partly definitional and partly perceptual. As beauty is in the eye of the beholder, so the consumer, investigator, or regulator may view any objective fact in disparate fashion. There is not a single or universally defined and accepted endpoint with any of the above-elaborated techniques that determines if a product is cosmetic, cosmeceutical, or pharmaceutical. Most of the below products described are accepted as at least cosmetic in effect, many as pharmacologically active, and most as cosmeceutical as defined above (Table 6).

Moisturizers

As pointed out in a previous chapter, it is now clear that even simple occlusion of the skin, with a ''moisturizer'' such as petrolatum, has definite effects on skin

Table 6 Potential Cosmeceuticals

Moisturizer
Retinoids
Estrogens
Various vitamins and minerals
Alpha-hydroxy acids
Beta-hydroxy acids
Hydroquinones
Hyaluronic acid
Natural cartilage polysaccharides

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structure and probably on function and thus it is appropriate to begin the discussion of potential cosmeceuticals with this seemingly simple product (see Chap. 1). In fact, however, the matter is far from simple, as modern moisturizers have achieved a sophistication and multiplicity of potential effects in parallel with our increasing knowledge of stratum corneum barrier function and kinetics of skin hydration and transepidermal water loss.

There is no universally accepted clinical or biomechanical definition of dry skin, although photoaged skin is frequently described as dry. Dry skin may be clinically rough, scaly, less flexible, and dry to the touch. Instrumental measurements may reveal changes in skin surface topography such as irregularity of stratum corneum and abnormal desquamation by profilometric evaluation or sticky tape application. An increase in TEWL indicative of impaired barrier function of the stratum corneum may be due to stratum corneum disruption and loss of intercellular lipids. A decrease in moisture of the viable epidermis can be determined by capacitance (Corneometer) or conductance (Skicon®-100)techniques.

Although older or photodamaged skin is frequently perceived as ''dry,'' there is substantial evidence that TEWL is actually decreased in chronologically aged skin, possibly due to increase in stratum corneum barrier function or decreased moisture content of the viable epidermis (20).

Moisturizers and emollients may exert their positive effects in several ways. Simple occlusion effects may acutely allow retention of more water in the skin and acutely lead to lowered TEWL. Healing of damaged stratum corneum and replacement of intercellular lipids may reestablish normal barrier function and allow normalization of TEWL. Strictly cosmetic effects of change in perception of dryness and skin smoothness also may be noted.

Retinoids

Retinoids, with pleotropic biological effects including modulation of epidermal cell differentiation and sebocyte dedifferentiation, have been extensively studied and have, in a way, become the prototypic cosmeceutical by which others are judged in treatment of photoaging. Without an immediately perceived and clinically obvious pharmacological effect, after many weeks of application they produce a number of definite, observable, and quantifiable improvements in photodamaged skin; these changes are best described clinically as cosmetic in appearance but with definite, modest, chronic pharmacological effect on epidermis and dermis.

Tretinoin has been well studied in multiple, large, open, and blinded clinical trials over the past 20 years and is unquestionably active in reversing some of the clinical, histological, and instrumentally determined manifestations of photodamage. This molecule can be considered as a prototypic cosmeceutical with clear pharmacological action producing a definite cosmetic effect. The various

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clinical and histological grading systems consistently demonstrate improvement (21–23). Excellent, good, or fair responses were obtained in 5%, 21%, and 42% of 76 tretinoin emollient cream–treated patients and in 0%, 11%, and 32% of 72 vehicle-treated subjects—results that were clinically obvious, cosmetically meaningful, and statistically significant ($p < 0.001$) (24). The mean percent change from baseline in this study was 16.5%, a cosmetically noticeable change indicative of a modest long-term pharmacological effect. The individual parameters of fine wrinkling, roughness, and mottled pigmentation are consistently improved and can be observed and quantitated by the subject. The investigator or a third party can confirm this utility with any and all of the clinical grading systems, visual analogue scoring, clinical panel assessment, fluorescent and polarized photography, optical profilometry, and histological assessments described above.

Some months of treatment are necessary to reach meaningful clinical effect and the treatment, while generally adequately tolerated, does frequently produce some undesired dryness and erythema especially at the onset. Roughness, fine wrinkles, and mottled pigmentation respond but meaningful improvements in skin function, such as elasticity have not been consistently described. A small study of six women aged 68 to 79 years who applied 0.025% tretinoin cream to the nonsun-exposed skin of the inner thigh for 9 months demonstrated alteration of the involutional structural changes in intrinsically aged sun-protected skin (25). Thus, the reparative abilities of retinoids may not be limited only to photodamage.

Isotretinoin has also been adequately documented in double-blind, vehicle-controlled trials to improve the same parameters of photoaging as tretinoin and appears to be well tolerated.

Adapalene has been proven effective in treatment of acne (26). It most likely will be studied and proven efficacious in reversing some of the stigmata of photoaging.

Tazarotene, another recently studied retinoid, has been shown to be effective in psoriasis and acne will likely be effective in photoaging (27).

Retinol, the prototypic retinoid, is the alcohol of retinoic acid and has been shown to be somewhat active in animal models of photodamage. It has recently been incorporated into two products marketed as AFIRM and as Healthy Skin Anti-Wrinkle Cream with claims relative to photoaging in humans stating ''. . . a retinol product . . . clinically proven effective at reducing the appearance of fine lines, wrinkles, and mottled hyperpigmentation, while improving skin texture and tone'' (28). Data to substantiate this cosmetic claim (not the same as pharmaceutical efficacy substantiation) are not included in the advertisements.

Hormones and Vitamins

As many hormone and vitamin deficiencies adversely affect the skin and as many of the skin functions and structures have been shown to be affected by hormonal

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or vitamin treatment, it is surprising that there has not recently been more quality research in the area of hormonal and vitamin effects or attention focused on the therapeutic use of androgen, thyroid, or growth hormones topically applied in photoaging.

Androgens

Androgens play a major role in skin physiology and are especially important in regulation of hair growth and sebum secretion. Less is known about their anabolic capabilities in skin but one would conclude that they should produce beneficial effects on aging and photoaging skin although conclusive proof is presently lacking.

Estrogens

A modest literature does exist, however, supporting the utility of estrogens in reversing at least some of the sequelae of skin aging. An open study of 98 postmenopausal women found a skin difference of 7 to 15% thickening of the skin and a 35% increase in sebum in those women who had been using estradiol gel or hormone replacement therapy (HRT) compared to the women who had not been receiving HRT (29). The mean duration of HRT was 58 months.

A pilot comparative study of eight perimenopausal women treated with 0.3% estriol, and 10 perimenopausal women treated with 0.01% estradiol to the face for 6 months duration showed improvement in symptoms of skin aging in both groups, with no changes in vaginal epithelium or in serum estradiol, follicle stimulating hormone, or prolactin (30). Clinically, improvement of elasticity, firmness, skin moisture, vascularization, and wrinkling were noted. Instrumental determination of skin moisture (Corneometer CM 420) showed increases from 55.9 ($\pm$ 30.5 S.D.) to 80.0 ($\pm$ 28.4 S.D.) in the estradiol group and comparable results in the estriol group. Optical profilometry (Hommeltester T 2000) results in five patients (estradiol group) and seven patients (estriol group) showed statistically significant changes in wrinkling in both groups with mean RZ-D measurements of 38.6 μm pretreatment and 24.8 μm at end of treatment in the estradiol group, and means of 40.7 and 28.2 μm in the estriol group.

A randomized, double-blind study of 54 women aged 52 to 70 years with moderate-to-severe facial cutaneous aging compared treatment with either 1 g Premarin cream (0.625 conjugated estrogens per gram) or placebo vehicle cream applied to the face for 24 weeks (31). A statistically significant difference in skin thickness measured by B-scan ultrasonic echography was demonstrated at week 24 in the Premarin-treated group. Skin thickness of epidermis plus dermis increased from a mean of 1.56 $\pm$ 0.20 mm at baseline to 1.68 $\pm$ 0.19 mm at end of treatment with Premarin ($p = 0.013$). At weeks 12 and 24, Premarin cream was significantly ($p = 0.010$ and $p = 0.012$) more effective in improving fine wrinkles as measured by mechanical profilimetry. Clinically, significant improve-

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ments in roughness, laxity, and mottled hyperpigmentation were noted by the investigator, but no differences from baseline or between the two groups were noted in subjects' self-evaluations of overall facial appearance or wrinkling of the crow's feet area. Although pre- and posthormone determinations were not obtained in this study, a significant difference from baseline in the vaginal maturation index was noted in the Premarin-treated group, indicating probable systemic effect.

A 6-month study compared the effects of 0.01% estradiol and 0.3% estriol topically applied to the facial skin of 59 preclimacteric women with skin aging symptoms (32). Both groups demonstrated significant decreases of wrinkle depth measured by optical profilimetry as well as clinical improvement in elasticity and firmness of the skin. Significant increases of type III collagen labeling by immunohistochemistry and increased collagen fibers were noted. No evidence of systemic hormonal effect was noted except for an increase in prolactin levels.

Vitamins

Vitamin D

Many vitamin D analogues have demonstrated effects on epidermal cells and fibroblasts and they have achieved quick acceptance in treatment of psoriasis. As some of their properties resemble those of retinoids, modulation of epidermal differentiation is possible and should be investigated in photoaging.

Vitamin C

As with claims for retinol in cosmetic products, the claims made for topical vitamin C are still more cosmetic than documented pharmaceutical.

Vitamin E

Vitamin E is an antioxidant in many systems and has been proposed, studied, and promoted for a large number of diverse systemic and skin conditions (33). On a theoretical basis, the concept of utility of the antioxidant effect of vitamin E is appealing, but, although the literature is voluminous, it is not completely convincing of a pharmaceutical effect in most conditions including skin disease, photoprotection, or photoaging. A 4-week study of 5% RRR alpha-tocopherol oil-in-water cream applied to the crows feet area demonstrated, by optical profilometry, decreased skin roughness, length of facial lines, and depth of wrinkles compared to placebo (34). A 10-day study by the same investigators claimed

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enhanced skin smoothness with topical vitamin E. Larger, longer trials are needed to substantiate a true and long-lasting effect.

Miscellaneous Agents

Alpha-Hydroxy Acids

A now substantial literature demonstrates a definite cosmeceutical effect of alpha-hydroxy acids (AHA). In a pilot study, a 25% increase in skin thickness was noted, comprised of both epidermal and dermal contributions. Increased acid mucopolysaccharides, improvement in elastic fiber quality, and increase in collagen density were also noted (35). A 22-week double-blind study confirmed utility of both 8% glycolic and 8% lactic acid in treatment of photodamaged skin in overall appearance and in specific parameters of mottled pigmentation, sallowness, and roughness (36).

The beta-hydroxy acid, salicylic acid, has been studied for its effects on photodamage in a large number of women during a home-use trial versus a proprietary glycolic acid cream and was observed to be superior on global improvement of appearance (37).

Hydroquinones

These agents, as weak depigmenting agents, may occasionally be of some utility in treatment of the epidermal pigmentary irregularities associated with photoaging. Higher concentrations, better delivery systems, and combination with other active products may enhance their utility in treatment of pigmentary abnormalities related to photoaging.

Alpha-Interferon

An interferon-containing cream was demonstrated to increase cutaneous CD1a+ cells and HLA− DR+ cells in aging skin and in skin treated with PUVA implying that this increase in epidermal Langerhans cells may benefit photoaging-reduced immunosurveillance (38). Substantiation of clinical effect requires additional study.

Minerals

The legends surrounding Cleopatra, the ancient Queen of Eygypt, are numerous. Frequently referenced in advertising of cosmetics, she is said to have claimed the rights to the Dead Sea's mineral ingredients and, most naturally, these ingredients have claimed numerous cosmetic properties (39). Cosmetics derived from this source are varied in their composition. Some contain a high concentration of divalent cations, magnesium, and calcium and a lower concentration of mono-

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valent cations sodium and potassium as well as miscellaneous other cations and anions. Zinc and selenium have been frequently studied in dermatological conditions, most often inconclusively for true pharmacological effect, but these and other minerals unquestionably play major roles in normal physiology of the mammalian organism. Most notably, their vital roles as cofactors in enzymatic processes means that they cannot be completely dismissed in spite of sometimes extravagant marketing claims. Much confirmatory work is needed to fully substantiate cosmeceutically oriented claims of skin penetration, restoration of moisture because of hydroscopic characteristics, and, importantly, actual participation as cofactors in enzymatic regulation activities in the metabolism of healthy or photoaged skin.

Hyaluronic Acid

Hyaluronic acid plays a key role in both epidermis and especially dermis. Its water-holding properties are well established and, more recently, its involvement in control of cell growth, membrane receptor function, and adhesion have been demonstrated. A progressive reduction in electron-dense granules of hyaluronic acid has been observed with increasing age (40). Numerous observations support the concept that application of exogenous HA may be beneficial in various types of tissue remodeling, repair, and healing (41). Furthermore, application of topical tretinoin to photoaged skin has been demonstrated to increase both epidermal and dermal hyaluronic acid theoretically increasing water-holding capacity and possibly facilitating other intercellular transports (42).

Hyaluronic acid stimulation by electrorydesis has been demonstrated. Retinoids have long been known to stimulate HA in epidermis and dermis, but recently a cosmeceutical device that produces a specific, pulsed electromagnetic field (electrorydesis) has been studied in three patients and demonstrated ability to stimulate a significant increase in electron-dense granules corresponding to hyaluronic acid in collagen, elastic fibers, and soluble matrix (43). Clinically, swelling of skin and decrease in prominence of wrinkles was noted, apparently related to increased hydration of the dermis related to increased GAGS.

Natural Cartilage Polysaccharides

Vivida, an oral formulation containing 500 mg/day of purified natural polysaccharides from cartilage of marine fish was compared with a placebo in a study of 30 women aged 40 to 50 years with sun-damaged skin (44). No changes were noted in the placebo group, but in the Vivida group, after 90 days, the epidermal thickness increased from 0.11 to 0.29 mm, dermal thickness from 0.74 to 1.39, skin elasticity index from 44 to 73%, and the erythemal index decreased from 0.301 to 0.205.

Imedeen, a different commercial preparation of natural cartilage polysaccharides containing 380 mg of active substance was compared to Vivida 500 mg/

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day in a second study of 30 women aged 40 to 60 years (45). After 90 days, statistically significant differences ($p < 0.001$) in favor of Vivida were demonstrated, with mean epidermal thickness increased from 0.14 to 0.26 mm, dermal thickness from 0.90 to 1.51 mm, and elasticity index from 47 to 71%.

Confirmation of these results in larger, controlled multicenter studies, as well as explanation of how an oral preparation of polysaccharides would survive the digestive system and reach the systemic circulation and, therefore, the skin, is needed.

The substances and products described above are at least partially clinically substantiated as having a cosmeceutical action in photoaging and in many cases have a theoretical basis for action. A host of additional putative agents has been described with new additions monthly. A recent review discusses potentially skin-active naturally occurring botanicals including familiar substances such as chamomile, ginseng, hops, etc. (46). Substantiation of effect in photoaging is not available, but in view of the long-standing folklore surrounding some, an open mind and meticulous investigation are prudent.

Minoxidil

Topically applied minoxidil provides another example of modest pharmacological action with cosmeceutical impact and is modestly effective in treatment of androgenetic alopecia by conversion of some telogen follicles to anagen possibly via K-channel mechanisms. It would be interesting to study this class of molecule in photoaging to see if the ''resting'' aged epidermis and dermis could be similarly stimulated to a more active state.

Last, although it is not the specific purvue of cosmeceuticals, multiple interventions from conventional surgery to laser techniques have been detailed to affect photoaging. One new device has recently been described which may foretell the future treatment of many diseases and conditions of the skin. A depth-targeted gene delivery and expression in the skin utilizing pulsed electric fields has been discussed in the context of skin aging (47). It has become increasingly clear that manipulation of gene action by various techniques, be they indirect such as hormonal, receptor-mediated up- or downregulation of genetic control, or direct intervention by substitution of new gene material into the cell nucleus, are feasible and productive in regulation of various skin dysfunctions. A new and important frontier is being explored that will have profound effects on the future treatment of human disease.

SUMMARY AND CONCLUSIONS

In the face of raging clinical, regulatory, and philosophical debates over whether photoaging is a condition, a disease, or a strictly cosmetic view, science has

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been progressing. The cosmetic and pharmaceutical efficacy of tretinoin and other molecules has been unequivocally established by cosmetic, clinical study, and instrumentation methods. That the inherent structure and function of the skin can be modified to produce essentially and primarily cosmetic change is indisputable and the term cosmeceutical is the ideal designation for many of the above products advocated for the care and treatment of aged and photodamaged skin. Whatever the cultural, political, scientific, and regulatory polemics, the cosmeceutical in treatment of photoaging remains a fact.

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4
Hydroxyacids

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INTRODUCTION

Hydroxyacids are organic carboxylic acids classified into the α- and β-types (AHA and BHA) according to their molecular structure. Both AHAs and BHAs are used worldwide and most probably for centuries as active dermatological drugs and cosmetic ingredients. Their acceptance by physicians, cosmetologists, and consumers contrasts with the few independent, well-controlled studies demonstrating long-term effects. In addition, little is known about the relevance of distinguishing AHAs and BHAs as far as the biological consequences of their application onto the skin is concerned.

Health care and cosmetic regulations differ among countries, although skin biology is the same throughout the world. In general, physicians consider that the current legal definitions of drugs and cosmetics are archaic and unworkable in some countries. It is evident that any environmental threat and topical product may exhibit some biological effect on the skin. Hence cosmetics should be viewed as skin physiology modifiers. Should they all be classified as real bioactive agents? This is a matter of definition because bioactivity differs by several degrees of magnitude among product categories. There is a huge difference between decorative, supplement, and real active compounds in cosmetology (1).

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Nowadays, there is much controversy about the concept of cosmeceuticals. What is in a name? The term was coined by Kligman to describe products that fall between the two groups: cosmetics and pharmaceuticals. This concept received immediate acceptance by many dermatologists. However, some corporate leaders contend that cosmeceuticals are neither scientifically sensible nor juridically necessary. In fact, with the exception of Japan, national regulatory agencies have not formally recognized the class of cosmeceuticals, or ''quasidrugs.'' The lack of clarification might in time have negative effects. Some products are at risk of being banned, although they could be valuable in cosmetology; the reverse might also be true, and some products could be used in cosmetology without adequate evaluation of their potential biological effects. One example is the widespread use of AHAs. Despite the obvious antixerotic and caustic effects of AHAs at given concentrations, there is little information available about their general toxicity and secondary biological effects. In contrast, the toxic effect of the BHA salicylic acid is well known when the percutaneous absorption is high.

CHEMICAL STRUCTURE AND NATURAL SOURCES OF AHAS

AHAs range from simple aliphatic compounds to complex molecules. Many of these substances can be derived from natural sources and are often referred to as fruit acids. However, a number of synthetic sources provides access to new structural analogues. The AHAs used in dermatology and cosmetology are usually produced by chemical synthesis. They are characterized into chemical groups based on the number of incorporated carboxylic groups (Table 1).

According to their configuration, AHAs may be present under different stereoisomeric structures called enantiomers termed ''l'' and ''d'' or ''R'' and

Table 1 Hydroxyacid Classification

α-Hydroxyacids	
monocarboxylic acids:	glycolic acid
	lactic acid
	mandelic acid
dicarboxylic acids:	malic acid
	tartaric acid
tricarboxylic acid:	citric acid
β-Hydroxyacids	
salicylic acid (ortho hydroxybenzoic acid)	
2-hydroxy-5-octanoyl benzoic acid	
tropic acid	

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''S.'' Some of the common AHAs occur naturally in an enantiomerically enriched form and both enantiomers may be available.

Glycolic acid (2-hyroxyethanoic acid) is a constituent of sugar cane juice. Lactic acid (2-hydroxypropanoic acid) was first isolated in 1780. The l-lactic acid is produced by the microorganism *Lactobacillus* and is responsible for the taste and odor of sour milk. The other enantiomer, d-lactic acid (also called sarcolactic acid) is formed during anaerobic muscular contraction and is also found in apples, ergot, foxglove, opium, and tomatoes. Mandelic acid (2-hydroxy-2-phenylethanoic acid) can be obtained from hydrolysis of an extract of bitter almonds. Malic acid (2-hydroxy-1,4-butanedioic acid) was first isolated from unripened apples in 1785. Tartaric acid (2,3-hydroxy-1,4-butanedioic acid) was first isolated in 1769. It is widely distributed in plants, particularly in grapes and lees of wine. Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) was first isolated from lemon juice in 1784. It is also found in pineapples and other citrus fruits.

BIOLOGICAL ACTIVITIES OF HYDROXYACIDS

Many aspects concerning the mechanisms of action of hydroxyacids still remain unknown. During the past few years numerous cosmetics containing hydroxyacids have appeared on the market with unfounded claims of performance. Hasty conclusions have been drawn from uncontrolled studies. Erroneous information and incorrect statements flourished behind promotional objectives obscuring the facts.

At least one facet of the hydroxyacid biological activities may be ascribed to the native acid strength of the compounds. Such physicochemical characteristics are measured by the proton dissociation in solution and are expressed as the pKa. A hydroxyacid has a stronger acid strength when its pKa number is lower. A decrease of 1 unit in pKa represents a tenfold increase in the strength. If the acid strength influences some of the biological effects of hydroxyacids, it does not, however, correlate with the potency of all their biological actions.

The pH of the formulations varies with both the nature of the hydroxyacid and its concentration. In order to avoid irritation as much as possible, it is desirable to formulate a cosmetic preparation with a pH close to the normal pH range of the skin. This may be achieved by partial neutralization and by the addition of an effective buffer. However, data suggest that neutral pH AHA products induce little effect on skin.

In order to prevent misunderstandings and misstatements about the effects of hydroxyacids, one should also consider biological actions related to their chemical structure regardless of their acidity. Such a hypothesis is not yet supported by scientific data. The exquisite enantioselectivity exhibited by many biological systems suggests that enantiopurity is an important parameter in any phar-

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macological effect, including pharmacokinetics, metabolic rate, and toxicity. Thus the components of a racemate can differentially interact with biomolecules of the skin. Whether such concern is of importance for some effects elicited by AHAs is not settled.

Both AHAs and BHAs exert indisputable direct effects on the stratum corneum, at least when it is affected by xerosis, ichthyosis, and analogous conditions. Comedonal hyperkeratosis in acne-prone subjects might also be improved by the same compounds. In the field of tumors, benign keratoses and viral warts may also be affected by high-concentration formulations. The efficacy is largely related to the pH-related chemical burn. Such caustic effect is also induced in order to realize AHA skin peeling. The effect of hydroxyacids, if any, on heliodermatosis appears more complex, involving multifaceted mechanisms boosting physiological aspects of aging skin.

Most of the aforementioned effects are in part hydroxyacid dose-dependent. In the present review, we arbitrarily define the category low concentration when there is less than 4% of active compound in the formulation. Medium concentration is applied in the range 4 to 12%, and high concentration for dosages higher than that.

EFFECTS ON CORNEOCYTE COHESION AND STRATUM CORNEUM FUNCTIONS

During the formation and maturation of the stratum corneum the intercorneocyte linkages corresponding to desmosomes become modified into so-called corneosomes. Their numbers normally decrease toward the surface of the skin, most notably during the stratum compactum to stratum disjunctum transition (2). In xerotic and ichthyotic conditions, ordered desquamation is impaired because desmosomes persist up to the outer stratum corneum leading to the unruly accumulation of corneocytes and to skin scaling and flaking (3).

Salicylic acid is the reference BHA used since the early days of dermatology to improve xerotic conditions. Although this compound at low and medium concentrations seems to have little or no effect on the normal stratum corneum, there is growing evidence that complete desmosomal degradation is helped in various xerotic and ichthyotic disorders (4–6). It appears, therefore, that applying the term keratolytic to such a compound is a misnomer, while desmolytic agent is more appropriate and explicit.

Quite recently, a lipophilic derivative of salicylic acid was tested on normal human skin (6,7). It corresponds to the 2-hydroxy 5-octanoyl benzoic acid, also called β-lipohydroxyacid (β-LHA). One of its main targets is clearly the corneosomes, which appear to be weakened following altered chemical bonds in the junctional complexes. Subtle differences in desmolytic activity of salicylic acid

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and β-LHA have been described and ascribed to the respective hydrophilic and lipophilic natures of these compounds (6).

Various AHAs, particularly lactic acid and glycolic acids in the medium range of concentrations, have profound effects on corneocyte cohesion (8–11). The usefulness of such formulations in xerotic and allied conditions is beyond doubt (12–17). The mechanisms of action of AHAs at that level are poorly documented. A desirable pH for inducing desquamation with AHA application lies between 2.8 and 4.8. The cutaneous surface pH changes cannot be taken lightly because they can persist several hours following applications and can affect a number of stratum corneum layers according to the product concentration. A discrete superficial desmolytic effect occurs in response to low dosages (6). However, in other circumstances, when an appropriate amount of a given AHA is topically applied, within a couple of days the stratum corneum abruptly becomes detached at its lower-most levels, and desquamates as large flakes or sheets (8,9,17). In such instances, no disaggregation of corneocytes at upper levels of the stratum corneum is apparent (17). Speculation has been made on the interaction between AHAs and various enzymatic processes involved in the maturation and disaggregation of the stratum corneum (17).

In addition to the therapeutic effect of the various hydroxyacids improving hyperkeratotic disorders, the same products yield cosmetic benefits by increasing plasticization and flexibility of the stratum corneum (18) without impairing the barrier function (7,11,19). This barrier function was even reported to be improved by some AHAs leading to increased resistance to SLS-induced skin irritation (20). The latter beneficial effect was not equal for all hydroxyacids, being more marked for AHAs characterized by antioxidant properties (20,21). A similar protection was not evidenced after applications of salicylic acid (22).

CAUSTIC EFFECTS

When applied to the skin in high concentration, AHAs cause necrosis and detachment of keratinocytes leading to epidermolysis (12,23,24). Such injury is a chemical peeling depending primarily upon the disruption of the skin pH. The farther away from the physiological pH, the greater the caustic effect, the greater the risk of side effects, but the more likely the patient is to receive the benefits of the peeling agents. A tolerable sense of burning itch is often experienced by patients.

The indications of such treatment encompass the destruction of slightly elevated seborrheic and actinic keratoses (12,25). The full-strength preparation must be applied carefully and exactly to the keratosis in an office procedure. After a few minutes, the entire lesion can be curetted off. Common warts can also be eradicated by hydroxyacids in a home-administered treatment with

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applications made daily for several days. To shorten the treatment period, the outer portion of the hyperkeratosis can be removed with a scalpel in an office setting.

ACNE AND PSEUDOFOLLICULITIS TREATMENT

Salicylic acid is listed among active products to treat acne (26,27). However, clearcut evidence for a significant benefit at low concentration in well-controlled experimental and clinical trials is scanty. Similarly, medium concentrations of AHAs, such as glycolic acid, lactic acid, and mandelic acid, are employed twice daily to improve mild acne (12). Such a treatment awaits validation by independent controlled studies. In our experience, the lower AHA concentrations present in some cosmetic products have no effect whatsoever on acne and comedones.

Another modality of acne treatment has been proposed using high concentrations of glycolic acid in an office setting (12). The procedure has to be repeated weekly or so. Improvement has been reported to be precipitous while patients were also taking tetracyclines (12). Discomfort, mild diffuse erythema, and fine scaling are often experienced by patients. In addition, there is a risk for stronger irritation leading to a papular and perifollicular erythema that can persist for a few weeks.

Pseudofolliculitis is another related disorder that can be improved by topical AHA treatment (28).

BOOSTING PHYSIOLOGICAL ASPECTS OF SKIN

One fascinating aspect in the effects of hydroxyacids is the boosted physiology that has been claimed to occur in the epidermis and dermis (29–35). Accordingly, some of these compounds have been used to correct skin atrophy (36) and to induce a gradual reduction in signs of aging, including dispigmentations (37) and wrinkles of fine and moderate depth (12,33,38–40). However, only a few controlled clinical trials and experimental studies have been conducted so far to validate these observations, and currently fuel controversies.

After a few days of application of 12% glycolic acid at low pH, fine wrinkles of the face may vanish as a result of irritation and dermal edema (41). Besides the untoward immediate effect of stinging, such smoothing effect is rapidly alleviated upon stopping topical treatment. Furthermore, in long-term applications, there is some concern regarding the presence of a chronic low-grade inflammation producing reactive oxygen species damaging collagen and elastic fibers. However, signs of reversal of aging and photoaging have been reported during long-

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term therapy (12,33,38–40). Such findings were not confirmed in other studies, however, which indicated almost an absence of AHA effects on major skin aging parameters (42–44). In fact, new deposits of glycosaminoglycans in the dermis represent a result of inflammation that has been mistakenly interpreted as a correction of aging. A comparative controlled study has shown that tretinoin is more active than medium concentrations of glycolic acid in the improvement of the facial skin tensile properties (42). It should be noted that the combination of tretinoin and AHAs may be beneficial as therapy for photoaged skin (39,45).

In contrast with salicylic acid, low concentrations of β-LHA elicit a dermoepidermal stimulation (7,44,46–48) that leads to increased keratinocyte proliferation and epidermal thickness. Such effect is more evident in older skin and remains within the physiological range of normal skin. The difference between this and other AHAs and BHAs is that angiogenesis is moderately increased by β-LHA. An increased number of Factor XIIIa-positive dermal dendrocytes has been seen after topical applications of AHAs and β-LHA (34,47). Adverse reactions are mostly represented by stinging sensations without any other clinical and histological signs of irritation.

CONCLUSIONS

AHAs and BHAs enjoy tremendous interest in dermatology and cosmetology. They also attract media attention and consumer curiosity. Claims and proven effects are sometimes contradictory. Much remains to be learned and speculation must be turned to fact. Improved regimens capitalizing on the various beneficial effects of hydroxyacids should be explored.

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5
Sebum

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SEBACEOUS GLANDS

Anatomy

Sebaceous glands are multilobular holocrine glands generally associated with hair follicles (1). The basal sebocytes sit on a basal membrane at the outer limits of the lobes, and as cells move from the basal layer toward the lumen of the gland they synthesize lipids, which accumulate as intracellular lipid droplets. As they synthesize lipid, the cells become larger, and the nucleus and other internal organelles are degraded. Ultimately, the entire mass of the cell is converted into a viscous liquid-phase lipid mixture. In most pilosebaceous units, sebum passes from the sebaceous gland into the hair follicle via the short sebaceous duct and outward onto the skin surface through the follicle. Generally, the hair follicle is large compared to the associated sebaceous gland; however, large sebaceous glands are associated with vellous hairs. These units are called sebaceous follicles and predominate on the forehead and cheeks.

Distribution

Pilosebaceous units are found over the entire surface of the skin except for the palmar and plantar regions (2). The density of follicles is greatest on the head,

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neck, and shoulders. In adults, the density of follicles on the scalp and face is in the range of 310 to 900 per square centimeter (1,3,4). On the torso and limbs the density of follicles is generally less than 100 per square centimeter (3). Large sebaceous glands are present in the submucosal connective tissue of the lip and buccal mucosa (5,6). Sebaceous glands in the oral mucosa often appear as slightly raised yellow spots, called Fordyce spots. Specialized sebaceous glands are also present on the edge of the eyelid (7) and the areolae of the nipples (3).

Sebum Secretion

Methods for Measurement

Early attempts to measure sebum secretion rates involved removal of lipid from the skin surface, followed by protection of a defined area of skin for a standard time (8,9). At the end of the timed interval, lipids were collected by extraction and analyzed either gravimetrically or chromatographically. These extraction-based methods tended to remove sebum from the follicles as well as some of the epidermal lipid from the stratum corneum. Therefore, methods based on direct extraction invariably overestimated the amount of lipid on the skin surface.

More recent investigations of sebum secretion have been based on adsorption of sebum as it is secreted. The adsorbents used for this purpose have included cigarette paper (10,11), bentonite gel (12), and Sebutape (Cuderm Corporation, Dallas, TX) (13,14). With all three methods, the most frequent site of measurement has been the forehead, and the skin surface is depleted of sebum at the outset of measurement.

With the cigarette paper method, the paper is delipidized by extraction with ethyl ether. After thorough drying, the paper is held in contact with the skin surface by means of a gauze strip. After a defined, standardized collection time, the paper is removed, and adsorbed lipids are extracted into ethyl ether and analyzed. Total lipid can be determined by evaporating the solution onto a tared aluminum planchet or by thin-layer chromatography in conjunction with photodensitometry (15). The latter analytical method gives composition in addition to total amount. Although the cigarette paper method has been useful, it may overestimate sebum secretion because the paper tends to deplete sebum from the follicular reservoir in addition to that which would have been secreted in the absence of an adsorbent.

The complications introduced by the follicular reservoir were most effectively addressed by the bentonite method (12), where bentonite gel is applied to the forehead 14 h before the start of the measurement period; the bentonite coating is replaced after 6 h. This pretreatment completely depletes the follicular reservoir of excess sebum. At the beginning of the measurement period, two

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small dacron disks are imbedded in freshly applied bentonite near the center of the depleted region. After 3 h, the disks are removed, and the lipids are extracted into ethyl ether and analyzed by quantitative thin-layer chromatography. This method yields the sustainable sebum secretion rate, which should reflect the rate at which sebum is synthesized. Although this method has been used in several studies of great importance (cited later in this chapter), it has not been widely used. This is at least in part because the suitability of bentonite for this application varies from one batch to another.

Currently the most widely used method for studying sebum secretion is based on a porous polymeric tape called Sebutape, which is coated with a weak adhesive sufficient to hold it in contact with the skin. As sebum is secreted from the orifice of a follicle, it is adsorbed into the pores in the polymer and the appearance of the tape changes from opaque to transparent. Densitometric and computer-assisted image analysis methodology can yield information on the sebum secretion rate per unit area of skin or per follicle, as well as follicle density.

A more extensive review of the above methods—as well as several variant methods based on the decrease in light scattering of a rough surface when it becomes coated with lipid—has recently been published (16).

Hormonal Control

Sebaceous glands are stimulated by androgenic hormones produced by the testes, ovaries, and adrenal glands (3,17,18). Testosterone and androstenediol are produced by the testes. The ovaries also produce some testosterone, androstenediol, and dehydroepiandrosterone; however, the significance of these steroidal hormones in regulation of female sebaceous gland activity is uncertain. Dehydroepiandrosterone and dehydroepiandrosterone sulfate produced by the adrenal glands are the major circulating androgens in women and are also significant in men. In the sebocytes, the androgenic hormone binds to a cytosolic receptor, which then translocates to the nucleus and modulates gene expression (19–21).

Variation with Age and Gender

Sebaceous gland activity is high in utero, and this is responsible for production of the vernix caseosa, a coating of sebaceous lipid and exfoliated stratum corneum material that coats the newborn (22). By 1 year after birth, the sebum secretion rate is extremely low and remains so until the onset of puberty (23). At that time, the increased concentrations of androgenic hormones cause a rapid increase in sebum secretion rates. Although there is great individual variation in sebum secretion rates, on average sebum secretion rates begin to decline in the late teen years (24). This decline continues for the remainder of life. Although there is

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considerable overlap, the average sebum secretion rate at any given age is greater for men than for women (24).

SEBUM COMPOSITION

Human

Lipid Class Composition

Human sebum from isolated sebaceous glands consists mainly of squalene, wax esters, and triglycerides with small proportions of cholesterol and cholesterol esters (25). As this viscous liquid flows outward through the follicle, lipases of both microbial and epithelial origin hydrolyze some of the triglycerides (26). Thus, sebum collected from the skin surface has a reduced proportion of triglycerides compared to sebum from the lumen of the gland, and free fatty acids are now present. The extent of triglyceride hydrolysis varies widely. Representative compositions of sebum expressed from isolated glands and from the skin surface are summarized in Figure 1. The large error bars associated with the triglyceride and fatty acid fractions from the skin surface lipid reflect the variability in triglyc-

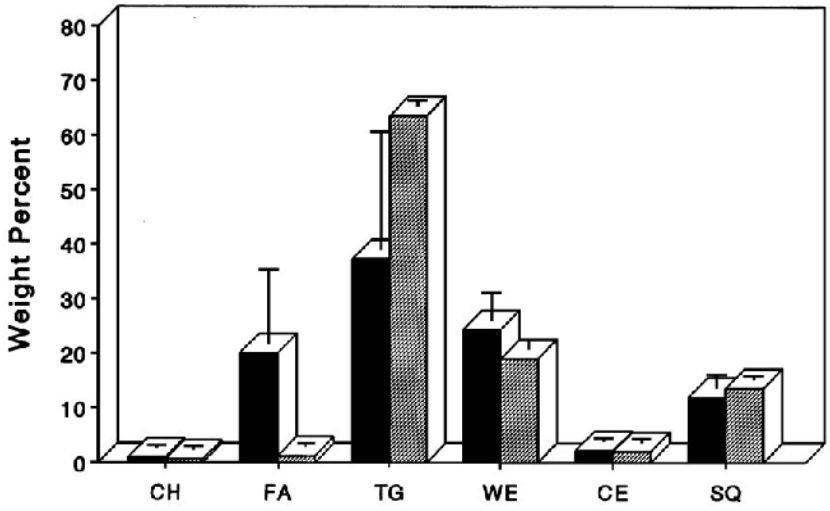

Figure 1 Composition of neutral, nonpolar lipids from the skin surface (solid bars) and isolated sebaceous glands (shaded bars). Error bars indicate 1 standard deviation. CH, cholesterol; FA, fatty acid; TG, triglyceride; WE, wax ester; CE, cholesterol ester; SQ, squalene. (Based on results from Refs. 59 and 60.)

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eride hydrolysis. Representative structures of the major sebaceous lipids are illustrated in Figure 2. Squalene is normally an intermediate in the synthesis of cholesterol (27); however, in differentiating sebocytes, the enzymes beyond this point in the biosynthetic pathway are not expressed. The small proportions of cholesterol and cholesterol esters present in sebum are derived from the original basal sebocyte membranes. It is also noteworthy that the wax ester fraction consists of fatty acids ester-linked to primary fatty alcohols.

Fatty Chains

Unsaturated Species The proportions of saturated and unsaturated fatty acids vary markedly among the several ester lipid classes in human sebum (22,25). The wax ester fraction contains about 60% monounsaturated and 40% saturated fatty acids (22), whereas in the cholesterol ester fraction 65% of the fatty acids are saturated and 30% are monounsaturated (22). Small proportions of dienoic acids are present in sebum (22,28). Both linoleic acid (C18:2 Δ9, 12) and the Δ5, 8 isomer of linoleic acid have been identified (28). The Δ9, 12 isomer is derived from the diet (29); whereas, the Δ5, 8 isomer is synthesized in the gland. Interestingly, the proportion of the Δ5, 8 isomer relative to Δ9, 12 is increased in acne patients (28). In the triglyceride fraction, the saturated and mo-

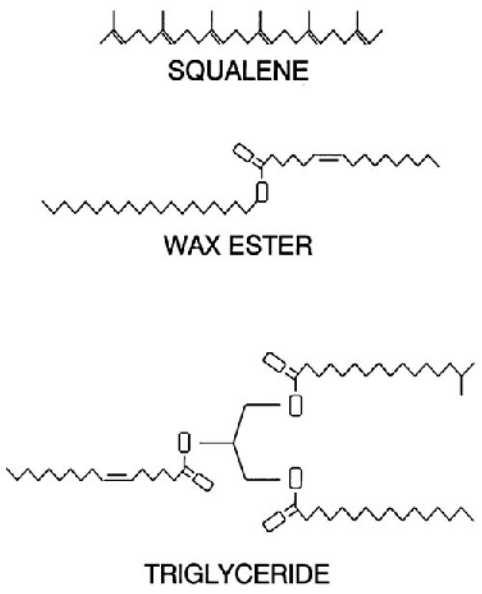

Figure 2 Representative structures of the major lipid classes of human sebum.

nounsaturated fatty acids comprise 70 and 30%, respectively (30). Most of the monounsaturated fatty acids are derived from C16:1 Δ6, or to a much lesser extent 18:1 Δ9, by extension or removal of 2-carbon units, and a small percentage of the monounsaturated fatty chains have iso or anteiso methyl branches (22,25). The chain lengths of the monounsaturates are almost entirely within the range of 14 through 18 carbons with C16:1 Δ6 (called sapienic acid) being the most abundant. Sapienic acid is shown in Figure 3.

Saturated Species The saturated fatty acids are almost entirely in the range of 12 through 18 carbons in length with palmitic acid (C16:0) being the most abundant (22,25). Generally, the straight-chain species predominate, but the proportions of methyl branched species can be highly variable (31). The methyl branched species include iso and anteiso branches (Fig. 3). There are also a wide variety of other mono and multi methyl branched saturated chains (32), but for a given individual the pattern of methyl branching appears to be constant (31).

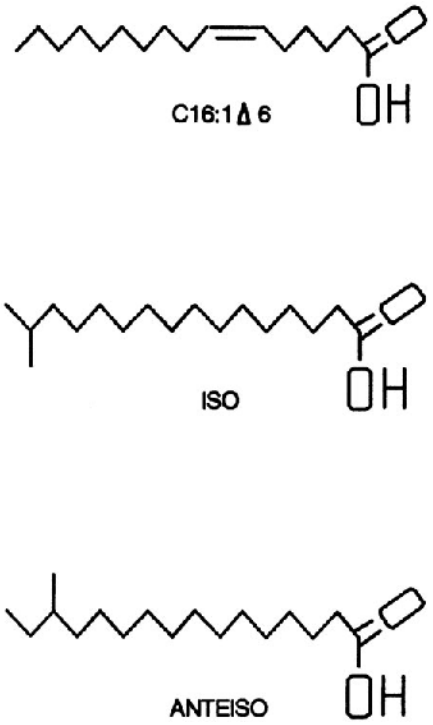

Figure 3 Fatty acids found in human sebum.

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Also, identical twins appear to have identical sebaceous fatty acid compositions, including the pattern of methyl branching, while nonidentical twins, although generally having similar branching patterns, sometimes differ as much as nontwin groups (33). All of this supports the contention that sebum composition is largely under genetic control.

Other Species

All terrestrial mammals produce sebum, and in all cases the lipid mixture is a viscous liquid consisting of several types of nonpolar lipids (34,35). The lipid class composition is species-specific. Among the most widely distributed sebaceous lipids are sterols and sterol esters. In addition to the wax monoesters found in humans, the sebum from many species contains type I wax diesters in which a normal fatty acid is ester-linked to the hydroxyl group of an α-hydroxyacid, which in turn is ester-linked to a fatty alcohol or type II wax diester in which two fatty acids are esterified to 1,2-diols. Sebum from the cow also contains triesters that contain 1,2-diols with an α-hydroxyacid esterified to one of the hydroxyls and a normal fatty acid ester-linked to the other (36). A second normal fatty acid is ester-linked to the α-hydroxyl group.

Members of closely related species tend to have similar sebum compositions. For example, sebum specimens from the members of the genus *Equus* all contain cholesterol, cholesterol esters, type II wax diesters, and giant ring ω-lactones (37,38). The lactones constitute from about 50 to 70% of the total sebum mass and are formed by cyclization of 30- through 36-carbon ω-hydroxyacids. In general, the degree of unsaturation and methyl branching of the giant ring lactones from the different species of the equidae are in accord with the taxonomic relationships among these species (38). *Equus caballus*, the domestic horse, produces lactones that predominantly contain one double bond and a methyl branch on the penultimate carbon. The lactones of the donkey, *Equus asinus*, are made from 30-, 32-, and 34-carbon straight-chained ω-hydroxyacids. The lactones of the mule, *Equus caballus/Equus asinus*, are monounsaturated and 50% of the chains have the methyl branch while the other 50% are straight (38). Again, this observation indicates genetic control of sebum composition.

SEBUM IN HEALTH

In hairy mammals, sebum serves two clear functions. First, it is a water repellent on the fur, which is clearly advantageous for aquatic mammals and for mammals living in moist environments. Second, 7-dehydrocholesterol secreted from the sebaceous glands onto the skin surface is photochemically converted to previtamin D, which is then converted to vitamin D in a temperature-dependent, nonen-

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zymatic reaction (39). When the animal licks its fur during grooming, the vitamin D is recovered by means of a salivary vitamin D binding protein (40). In humans, a function for sebum is less well established, and it is possible that sebum production is a functionless vestige of our ancestors. One clue in this regard comes from the species known to produce squalene as a component of their sebum. In addition to human sebum, squalene is found in the sebum of the otter, beaver, kinkajou, and mole, *Scalopus aquaticus* (41,42). The otter and beaver are aquatic; the kinkajoo lives in the canopies of tropical rain forests; and *Scalopus aquaticus* lives in moist–wet soil. Could it be that our ancestors spent a great deal of their time in water along coasts or rivers and benefited from the waterproofing afforded by a coating of squalene?

Sebum no doubt contributes a degree of lubrication to the skin surface, and it has sometimes been suggested that dry skin results from insufficient sebum production. However, two lines of evidence argue against this. First, as has been pointed out, prepubertal children produce almost no sebum but most do not suffer dry skin or other skin problems (43). Second, in one study in which the sebum secretion rate was measured and subjects were surveyed about the condition of their skin, no correlation could be found between the occurrence of xerosis and sebum production (44).

Sebum definitely does not contribute to the permeability barrier function of the skin. In fact, if human sebum is applied to neonatal rodent skin, barrier function is decreased (45).

One possible function of sebum is a contribution to the antimicrobial defense of the skin. It has long been known that fatty acids produced by sebaceous triglyceride hydrolysis have antibacterial properties (46), and it has more recently been demonstrated that sebaceous lipids can interfere with the adherence of yeast to the stratum corneum (47). In addition to a decline in function of the immune system, the decline in sebum secretion with age could contribute to the increased incidence of bacterial and fungal infections of the skin in the elderly (48). The fact that prepubertal children do not have a high incidence of skin infections may be attributed to their healthy immune systems. So sebum is clearly not essential for the avoidance of skin infections, but it may be helpful in this regard in some individuals.

SEBUM IN DISEASE: ACNE

There is a clear, positive correlation between the occurrence and severity of acne and the sebum secretion rate (49). In one study comparing age- and gender-matched subjects with moderate, mild, or no acne, the subjects with moderate acne had the highest sebum secretion rates, while those with mild acne had sebum secretion rates intermediate between those measured for subjects without acne

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and those with moderate acne (49). The average sebum secretion rate for all subjects with acne was three times that for the subjects without acne, and in no case did a subject with acne have a sebum secretion rate that was not greater than the sebum secretion rate of the matched control.

The development of an inflammatory acne lesion is a multistep process (3). The initiating event is the formation of a keratinous plug, or comedo, that blocks the pore of the follicle. Bacteria within the follicle then grow and it becomes distended. The follicular epithelium becomes thin, and an inflammatory response is induced as bacterial products diffuse into the surrounding tissue.

It has been suggested that the development of acne may result from essential fatty acid deficiency localized to the follicular epithelium (50). In experimental systemic essential fatty acid deficiency, the skin becomes scaly and more permeable (29,51). If sebaceous fatty acids were to penetrate into the follicular epithelial cells and compete with linoleic acid from the circulation for incorporation into lipids, a localized essential fatty acid deficiency could be produced. The resulting scaling could lead to comedo formation, and the defective barrier function would facilitate exchange of materials between the follicle and surrounding tissue. This would include an influx of water and nutrients into the follicle to support bacterial growth as well as the eflux of inflammatory mediators.

Reduction of the sebum secretion rate is therapeutic for acne. This can be achieved by oral administration of retinoids (52,53), estrogen (54,55), or antiandrogens (55,56). Estrogen is thought to act by reducing production of testosterone, and antiandrogens act by blocking the androgenic receptors on sebocytes, thereby preventing binding of androgens.

Orally administered 13-cis-retinoic acid is an effective treatment for moderate-to-severe acne vulgaris (52,53). Although the retinoids probably act through specific receptors (57), details of their mechanisms of action remain uncertain (58). Chapter 9 deals with retinoids, so further discussion will not be included here.

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6
Hair Growth Enhancers

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INTRODUCTION

Hair has played a significant role in the lives of people throughout history, not only impacting their outward appearance but also on their inner self. Both men and women regard an abundance of hair as ideal, providing them with positive self-image attributes such as beauty, strength, virility, youthfulness, and confidence. Conversely, the lack of hair is associated with negative attributes and historically people have gone to great extremes to conceal their baldness. In early times, Egyptians used ''artificial braids'' to hide their baldness and outlandish hair-growth concoctions (e.g., snake oil) were prolific. In more recent history, minoxidil topical solution (Rogaine® 2%, Pharmacia & Upjohn) became the first clinically proved, safe, and effective hair-growth stimulant after it was discovered that its active ingredient (minoxidil) caused hypertrichosis when taken orally for hypertension. The 2% concentration of minoxidil topical solution became available in 1986 for men and in 1991 for women; a higher, more effective concentration (5%) is now available as either a prescription or nonprescription product in over 20 countries including the United States. The favorable findings with minoxidil topical solution have led to a flurry of activity to find new pharmacological agents to treat androgenetic alopecia. One such agent, finasteride (Propecia® tablets, 1 mg, Merck), was recently introduced into several countries including the United States as a prescription product for males only. Other agents are as-

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suredly yet to come, as research endeavors remain intense. Since 1995 nearly 70 patent applications have been submitted for androgenetic alopecia, immunomodulatory related hair diseases, and antichemotherapeutic alopecia agents (1).

HAIR GROWTH BIOLOGY

Hair grows from primary follicles. Actively growing hair follicles penetrate the entire epidermis and dermis. There are approximately 5 million total body hair follicles, of which 100,000 to 150,000 are scalp follicles. In adults, 90% of the hair follicles are in the growing (anagen) stage and the remainder are in the resting (telogen) stage. Follicular density decreases with age (1135/cm^2 at birth to 485/cm^2 at $\sim$30 years to 435/cm^2 at 80 years). Scalp hair grows at a rate of 0.37 to 0.44 mm/day and normal scalp hair loss or shedding in adults ranges from 50 to 100 hairs per day (2).

The growth of hair in humans is controlled by complicated mechanisms that can differ among various body locations. Morphologically, there are three types of hair: vellus, terminal, and intermediate. Vellus hairs are short, fine ($<$0.3 mm in diameter), soft, usually nonpigmented, and unmedullated. Terminal hairs are large ($>$0.3 mm in diameter), darkly pigmented, and medullated. Ninety percent of the hairs on the chest, trunk, shoulders, legs, and arms of men are terminal hairs, whereas only 45% of hairs in the same regions on women are terminal (3). Intermediate hairs occur on the scalp, and they demonstrate a morphology between those of terminal and vellus hairs. Intermediate hairs are medullated and contain a moderate amount of pigment (i.e., less than that found in terminal hairs) (4).

There are four types of hair follicles: terminal, vellus, miniaturized, and senescent. Terminal follicles bear terminal hairs at some time during the life of an individual, whereas vellus follicles do not bear terminal hairs at any time during an individual's life. Miniaturized follicles are those terminal follicles that have lost their ability to produce terminal hairs and instead produce vellus hairs. Senescent follicles are any of the three types of follicles that no longer produce hairs and have lost histological evidence of the ability to produce hairs.

The character of human hair is constantly changing from the prenatal period to old age; and under given physiological conditions, the same hair follicle can successively form different types of hair. Despite differences among individuals, follicle development for all types of hair is virtually the same.

Hair undergoes repeated cycles of active growth and rest. The relative duration of each cycle varies with the age of the individual and the region of the body where the hair grows. The length of the cycle is often modified by a variety of physiological and pathological factors. The cyclic phase of the hair follicle is

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identified by an active growth period known as *anagen*, an intermediate period known as *catagen*, and a resting stage known as *telogen*.

In the anagen phase, which lasts from 2 to 8 years (2), the follicle reaches its maximum length, and there is a proliferation of the matrix cells. Anagen hair generally has a thick shaft, and in given segments its medulla is clearly visible. The proximal-most part of the bulb in anagen hair is deeply pigmented. The bulb gradually tapers and becomes lighter in color at and beyond the keratogenous zone of the follicle. Catagen hair, in its involutional form, differs from telogen (clubbed) hair in two ways: (1) its keratinized (proximal) part is darker than that of clubbed hair; and (2) its inner and outer root sheaths are better preserved (5). Unlike the anagen phase, the catagen phase is short, lasting from 2 to 4 weeks (2). Telogen hair, or clubbed hair, is easily recognized because it generally contains a thin shaft, which is transparent near the root and devoid of a medulla and keratogenous zone. The telogen phase also is much shorter than the anagen phase, lasting from 2 to 4 months (2). The normal anagen/telogen ratio is 9:1.

ANDROGENETIC ALOPECIA

Androgenetic alopecia is the most common type of hair loss in humans. Its prevalence in any population has not been accurately studied, but it occurs much more often in Caucasians than in other races (6). Androgenetic alopecia affects approximately 50% of men over 40 years of age and may also affect just as many women (7). It occurs in both men and women as a result of genetic and hormonal factors.

Morphology and Control

Androgenetic alopecia appears to be autosomal dominant with gene expression apparently determined by hair follicle location (7). Expression of androgenetic alopecia can vary considerably from one person to another. In androgenetic alopecia, genetically predisposed hair follicles become progressively miniaturized over time. In men, the thick, pigmented terminal hairs in the affected area of the scalp eventually are replaced by the fine, unpigmented vellus hairs. Eventually, the affected scalp may become completely devoid of any hair. Women, however, rarely become completely bald but usually experience thinning characterized by an intermixing of the normal terminal hairs with finer vellus hairs (7). In both men and women, the hair growth cycle is altered, with fewer hairs in the anagen stage and more hairs in the telogen stage for longer periods of time (7).

Although scalp hair growth is not androgen-dependent, androgens are necessary for the full expression of androgenetic alopecia whereby they diminish

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the size of the hair follicle and diameter of the hair fiber, as well as shift hairs from the growing to resting state (6). The main androgen circulating in the plasma of men is testosterone, whereas the most important androgen in women is androstenedione. These androgens are metabolized by the enzyme 5α-reductase, reducing testosterone to dihydrotestosterone (DHT) and androstenedione to testosterone (and some DHT). In balding scalps, DHT production and the level of 5α-reductase activity are increased relative to nonbalding scalps (7). Another important enzyme, aromatase, has recently gained some attention (8,9). Aromatase is specifically located in the outer root sheath of hair follicles. It converts androgens (e.g., testosterone) to estrogens (e.g., estradiol), and there may be a two- to fivefold increase in the amount of aromatase in the scalp of women relative to men (8,9). This finding may explain the different clinical presentation of androgenetic alopecia in men and women.

Clinical Presentation

The clinical presentation of androgenetic alopecia is different for men and women. It may occur as early as 17 years of age in normal males and by 25 to 30 years of age in endocrinologically normal females (6). There is no evidence, however, to suggest that there is an age at which the onset of the balding process is no longer initiated or a threshold age at which the progression of baldness ceases to continue (7,10). Invariably, both men and women see increased shedding of hair, which prompts them to seek out medical advice.

In men, androgenetic alopecia is usually progressive, typically receding from the normal hairline in an M-shaped pattern with an enlarging balding vertex (6). Several classification systems have been used to characterize the balding state of men, the most popular being the Hamilton scale as modified by Norwood (Fig. 1) (11) and the Savin scale (Fig. 2). Women often do not present with a distinct pattern, but rather have diffuse hair loss or thinning of the temporal and parietal areas with retention of the frontal hairline in most cases. Ludwig (12) has described this pattern commonly seen in women (Fig. 3) and this classification is widely used and accepted. Women may present with the typical male patterning of hair loss, with this occurring more frequently in postmenopausal women than premenopausal women (13). In both sexes, concomitant loss of hair in the temple–sideburn areas and nape of neck can be observed as well as occasional increases in body hair.

Psychological Factors of Hair Loss

Hair loss can profoundly affect people and the clinical significance of androgenetic alopecia should not be trivialized as just a cosmetic nuisance. The World Health Organization (14) in fact classified androgenetic alopecia as a disease

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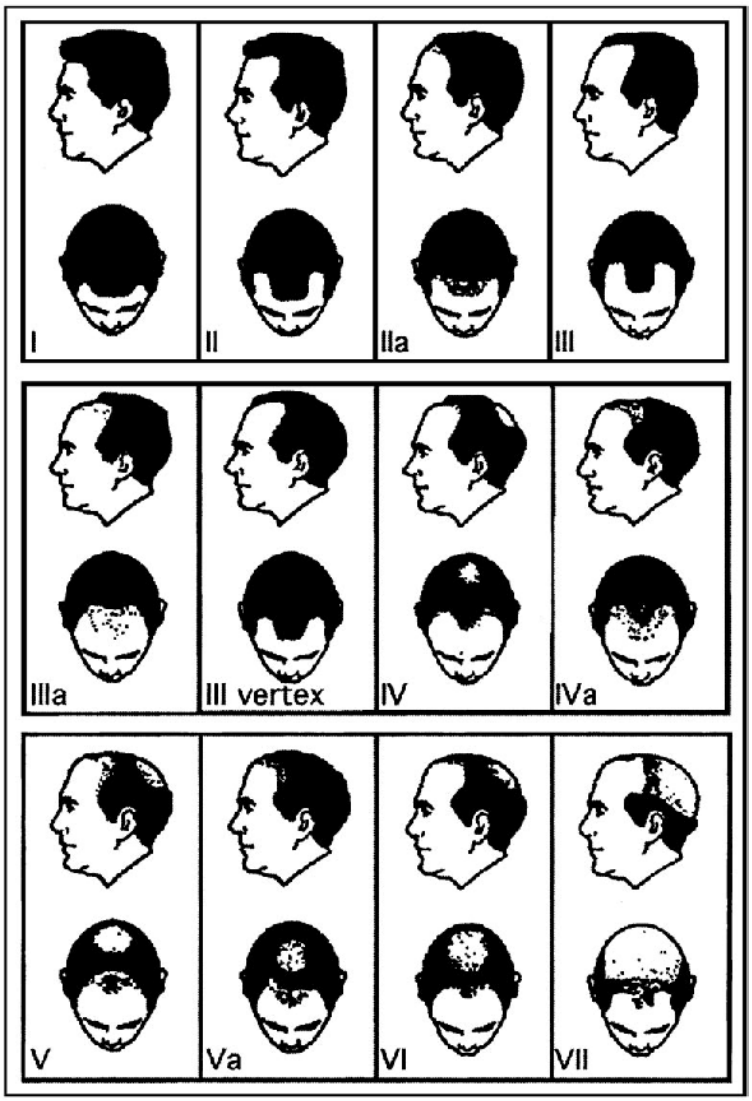

Figure 1 Hamilton baldness scale as modified by Norwood.

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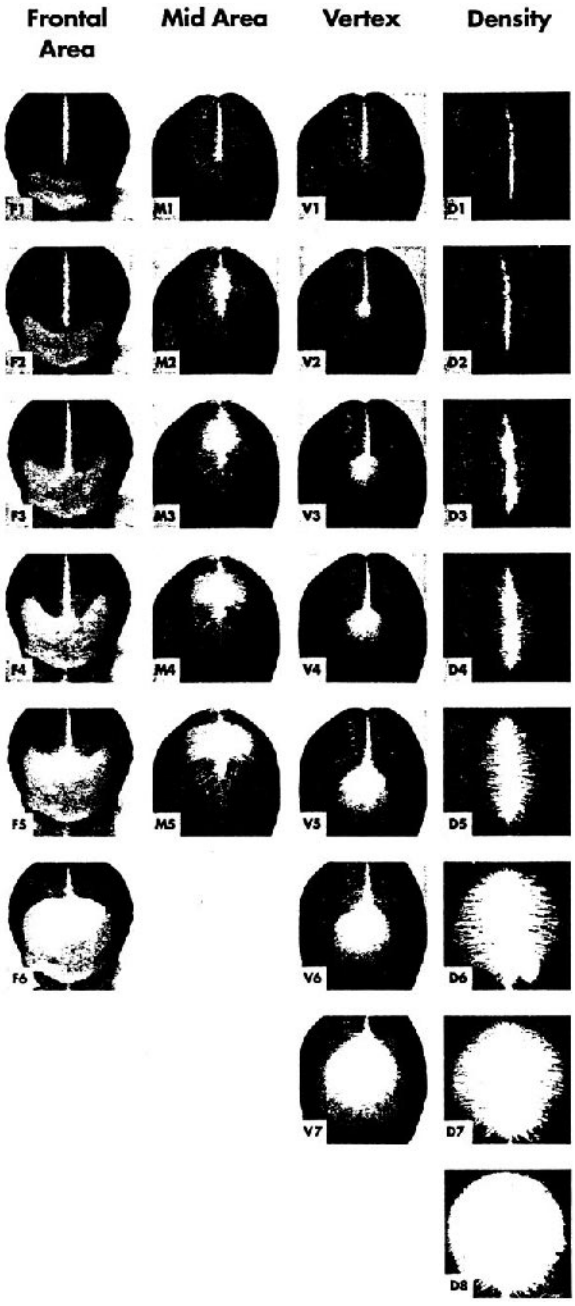

Figure 2 Savin baldness scale.

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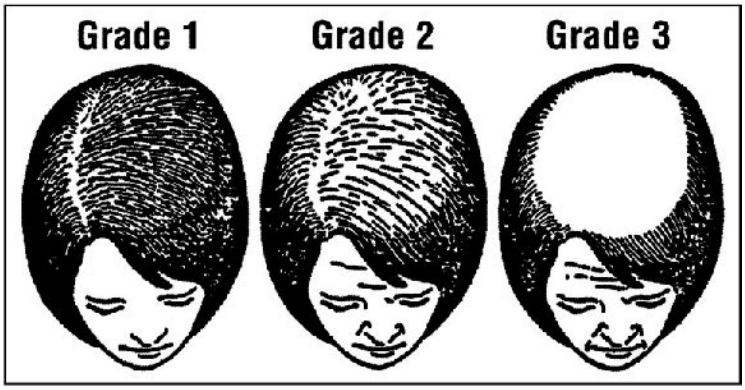

Figure 3 Ludwig baldness scale. (Reproduced with permission from Ref. 12.)

(L64). Although androgenetic alopecia is seen as a normal variant of aging by most individuals, it can have both psychological and pathological consequences and these effects are taken seriously by both the patient and physician (15,16). Despite the age of onset and the ongoing progression of the baldness per se, the main area of concern in individuals is a psychological one. The majority of men and women (90% or more) want to reverse or halt their hair loss, feel frustrated or helpless about the condition, and are self-conscious about their looks.

Psychopathological symptoms in patients with androgenetic alopecia have been well documented. Maffei et al. (17) showed that the prevalence of personality disorders in subjects with androgenetic alopecia proved to be significantly higher than the prevalence of such diagnoses in the general population. Cash (18) reported that, in men with androgenetic alopecia, the effects of balding resulted in considerable preoccupation, moderate stress or distress, and copious coping efforts. In another study by Cash et al. (19), women were nearly twice as likely as men to be ''very upset'' or ''extremely upset'' about their hair loss condition. Relative to a control group, women with androgenetic alopecia had a more negative body image as well as more social anxiety, poorer self-esteem, less of a sense of control over their lives, and less life satisfaction.

Treatment of Androgenetic Alopecia

Nonspecific Biological Response Modulators

Minoxidil Topical Solution How minoxidil topical solution affects hair growth has not been fully characterized, but it can be categorized as a nonspecific biological response modulator, with a direct effect on the hair follicle. This results

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in increased size of the hair follicle with treatment, increased proliferation of dermal papilla cells, and opening of potassium channels (20).

As mentioned previously, minoxidil topical solution was first available as a 2% product for use in both men and women with androgenetic alopecia, but now a higher, more effective concentration (5%), is available in over 20 countries. The 5% product is approved for use in both men and women in most of these countries, although in Australia, Denmark, and the United States it is indicated for men only. Further research is warranted to fully delineate this apparent gender-specific situation.

Efficacy and safety of the 5% product have been clearly established in men. The results of a controlled clinical trial involving 393 men with androgenetic alopecia showed statistically significant differences favoring the 5% product over the 2% product with regard to change in nonvellus hair counts; the placebo response was generally minimal (21). The nonvellus hair count data also support that the response to treatment occurred faster with the 5% product, with effects being realized as early as 2 months. The use of minoxidil topical solution (be it at the 2% or 5% concentration) is established as a safe treatment for androgenetic alopecia with systemic medical events rarely reported in over 10 years of consumer use. Local dermatological symptoms (e.g., drying, itching, erythema) are known to occur and these tend to be more frequent with the 5% product.

Topical minoxidil solution also has the ability to stabilize hair loss, that is retardation of the existing hair loss process or, simply, maintenance of an existing head of hair. Numerous anecdotal reports by patients and clinicians as well as clinical trial data support this clinical phenomenon. Price and Menefee (22) report the results of a placebo-controlled clinical trial in 32 men with androgenetic alopecia where both 2% and 5% minoxidil topical solution were found to promote hair regrowth and retard the hair loss process over a 96-week period. Comparatively, the 5% product provided a greater benefit than the 2% product, and both were better than placebo.

Minoxidil topical solution has also been used in combination with topical retinoids. The results of a pharmacokinetic study showed that absorption of 2% minoxidil topical solution was increased when used concomitantly with 0.05% tretinoin cream (23). In a small ($n = 36$), pilot study of 0.025% tretinoin and 0.5% minoxidil topical solution, over 50% of the patients had moderate-to-good hair regrowth (24). However, in a larger ($n = 136$), controlled trial that evaluated concomitant use of 0.05% retinoic acid with 2% and 5% minoxidil topical solution, results were equivocal (unpublished data, Pharmacia & Upjohn). Nevertheless, as reported by Comacho (25), clinicians use minoxidil topical solution (2% or 3%) in combination with tretinoin (0.01%) with acceptable results.

Other Biological Responses Modulators Several other biological response modifiers (i.e., diazoxide, viprostol, tretinoin, cyclosporin A) have been tested as treatments for androgenetic alopecia (25). None of these products has

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proved more effective than minoxidil topical solution, and in some cases the risks of the treatment outweighed any possible benefits. Thus, none of these products have been commercialized.

5α-Reductase Inhibitors

Finasteride Finasteride is a specific inhibitor of steroid type II 5α-reductase, an intracellular enzyme that converts testosterone to DHT. By inhibiting type II 5α-reductase, this conversion is blocked, resulting in significant decreases in serum and tissue DHT concentrations. Merck & Co. developed finasteride as an oral treatment for androgenetic alopecia after men taking finasteride (5 mg/day) for prostate enlargement noticed regrowth of their hair. Three controlled clinical trials were performed in men (18 to 41 years), with mild-to-moderate degrees of androgenetic alopecia. In these studies, 1879 men ingested either a 1-mg finasteride tablet or placebo tablet once daily for 12 months; after 12 months, finasteride-treated patients were switched to placebo and placebo-treated patients were switched to finasteride and they were followed for an additional 12 months (26,27). Clinical improvement was seen as early as 3 months in finasteride-treated patients and hair regrowth continued throughout the trial. Finasteride also had a stabilizing effect on hair loss, which was maintained through the second year of treatment. Hair counts in placebo-treated patients decreased during the study. Finasteride was generally well tolerated in these studies. Some men, however, experienced decreased libido, difficulty in achieving an erection, and decreased semen volume ($<$2% of patients in each case). These side effects resolved in 58% of the men who continued treatment and completely abated upon discontinuation of the drug (28).

Finasteride is indicated for use in men only. Women of childbearing age cannot take finasteride because it may cause hypospadia (a developmental abnormality of the penis) in the male offspring if taken during pregnancy.

Other 5α-Reductase Inhibitors Several other 5α-reductase inhibitors are currently being developed as treatments for hair loss (1), but no further information regarding their effectiveness is available at this time.

Androgen Receptor Inhibitors

RU58841 RU58841 (Roussel Uclaf) is a nonsteroidal topical antiandrogen without significant systemic effects apparently due to its metabolism in the skin. This appears to be a promising new hair growth agent that promotes hair growth on the scalp as well as retards hair growth of both beard and body hair follicles (29–32). RU58841 has produced highly efficacious results in stumptailed macaques (31,32).

The synergistic effects of RU58841 and minoxidil topical solution (2% and 5%) have also been reported in stumptailed macaques. The effect of combined

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treatment was most remarkable early on, but by 1 year no significant benefit of combined treatment was apparent relative to either treatment alone (33).

Other Androgen Receptor Inhibitors Other androgen receptor inhibitors are receiving a lot of attention as evidenced by development of several agents of this type (1). No further information regarding their effectiveness in treating hair loss is available at this time.

Unproved Treatments

With the increased interest in hair growth promotion, numerous products and remedies are marketed to consumers purporting their beneficial effects. The efficacy (and safety) of these products has not been established in large, controlled clinical trials as was done for minoxidil topical solution and finasteride.

ALOPECIA AREATA

Etiology and Clinical Presentation

Alopecia areata is a nonscarring, reversible hair disorder that can cause hair loss anywhere on the body. The occurrence of alopecia areata is reported as $<0.1\%$ of the Caucasian population and between 0.9% and 4% of dermatological patients (34). It appears to affect males and females equally. Although alopecia areata can occur at any age, its onset is before the age of 20 years in about one-half of the cases (35). The course of alopecia areata is extremely variable, with hair loss occurring rapidly, slowly, or intermittently. In some cases, spontaneous hair regrowth occurs immediately, whereas in other cases, it may take days or even years for normal regrowth to occur. Recurrence and severity of the disease are unpredictable, and it can have a life-long presence for many patients.

The etiology of alopecia areata is unknown but many theories have been postulated: (1) a chronic inflammatory disease with an autoimmune basis; (2) genetic predisposition; (3) a result of psychological factors (e.g., stress, anxiety) or exposure to chemicals or infectious agents; and (4) directly related to destruction of follicular or epidermal keratinocytes and melanocytes (35,36). It very well may be that one or more of these plays a role in the disease process.

The clinical appearance of alopecia areata varies from small patches of hair loss to total loss of scalp hair (alopecia totalis) to loss of all body hair (alopecia universalis). It is not usually associated with any symptoms, although some individuals may experience pruritus or paresthesias before or coincident with the loss of hair. A variety of other diseases may also occur in association with alopecia areata, including allergic rhinitis, asthma, atopic dermatitis, diseases of the thyroid gland, vitiligo, systemic lupus erythematosus, discoid lupus erythematosus, rheumatoid arthritis, pernicious anemia, scleroderma, ulcerative colitis, myasthe-

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nia gravis, and lichen planus. Alopecia areata also may occur in association with Down's syndrome and Turner's syndrome, as well as in diabetics and patients with human immunodeficiency virus (35). The diagnosis of alopecia areata can be challenging given the multitude of concomitant diseases and conditions; however, it can be absolutely confirmed by biopsy of hair follicles in the area of hair loss.

Treatment

In some cases of alopecia areata, no therapeutic intervention is necessary because of the patient's spontaneous regrowth of hair. In many cases, however, long-term therapeutic intervention is necessary in order for patients to see cosmetically acceptable hair regrowth. There are many treatment options available for alopecia areata but no one treatment stands foremost because of the variable nature of the disease and its unpredictable course (Table 1). The benefits and risks of each treatment must be carefully evaluated on a case-by-case basis.

DIFFUSE ALOPECIA

Etiology and Clinical Presentation

Patients afflicted with diffuse alopecia typically complain of hair loss all over the scalp not just in the areas usually seen in androgenetic alopecia. However, the differential diagnosis of diffuse alopecia versus androgenetic alopecia, particularly in females, can be difficult because of the similar presentation, and biopsy and histological assessment may be required to confirm the diagnosis. The course of the diffuse alopecia can be continuous or episodic. Diffuse alopecia may present as telogen or anagen effluvium and can be caused by drug and chemical exposure, thyroid disorders, nutritional influences, and psychological stress.

Telogen effluvium is characterized by abrupt, diffuse hair loss. Common causes are childbirth, febrile illnesses, surgery, psychological stress, crash diets, and drug therapy (38). The excessive shedding usually begins 3 to 4 months after the inciting event (39). Anagen effluvium is characterized by widespread or circumscribed loss of anagen hairs from growing follicles. Alopecia due to anagen effluvium is quite obvious because 90% of the hair follicles are in anagen (growing) phase. In contrast with telogen effluvium, loss of anagen hair begins within days to a few weeks after the inciting event. Common causes of anagen effluvium are radiation, toxic drugs, environmental and occupational exposure to hazardous chemicals, and loose anagen syndrome (39). Drug- and chemical-induced hair loss is usually confined to the scalp and is most often diffuse, but it can be patterned or localized. It can also manifest itself as a telogen or anagen effluvium. Hypothyroidism is directly correlated with diffuse alopecia, whereas

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Table 1 Treatment Options for Alopecia Areata

Immunomodulating agents	Topical sensitizers
Intralesional steroids (triamcinolone acetonide, triamcinolone hexacetonide)	Dinitrochlorobenzene
Topical steroids (fluocinolone acetonide cream, halcinonide cream, betamethasone dipropionate cream)	Squaric acid dibutyl ester
Intramuscular steroids (triamcinolone acetonide	Diphencyprone
Systemic steroids (cortisone acetate, prednisone, prednisolone)	Combined therapies
Cyclosporine (oral or topical)	Topical, intralesional, and/or systemic steroids
Thymopentin (intravenous or intradermal)	Minoxidil topical solution with betamethasone dipropionate cream, anthralin cream, diphencyprone, or oral prednisone
Nitrogen mustard (topical)	Topical diphencyprone and oral inosiplex
Azathioprine (oral)	Oral prednisone with topical fluocinolone acetonide cream and/or intralesional triamcinolone acetonide
Phototherapy with ultraviolet B light	Oral prednisolone with oral cyclosporine
Psoralen (topical or oral) plus ultraviolet A light	Other agents
Biological response modifier	Oral zinc
Minoxidil (topical or oral)	Cosmetic management
Irritants	Liquid nitrogen
Anthralin (topical)	Turbans
	Scarves
	Hairpieces
	Custom prostheses

Source: Refs. 35 and 37.

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alopecia due to hyperthyroidism is less clearly established (40). Examples of nutritional influences that can cause diffuse alopecia include caloric deprivation (crash diets), protein-calorie malnutrition, and deficiency in zinc, iron, essential fatty acid, and biotin levels (40). Finally, with regard to psychological stress, it is often difficult to determine its role in hair loss because hair loss itself can be very stressful, thus making it nearly impossible to ascertain which is the precipitating event, stress or hair loss (40).

Treatment

Diffuse alopecia is usually temporary and resolves without scarring. Once the cause is identified and eliminated, the prognosis for hair regrowth is usually good (40). In some cases (e.g., exposure to multiple chemotherapeutic regimens or ionizing radiation), hair regrowth may be incomplete and may be different in terms of color, shape, or texture (39).

In one of the most common causes of diffuse alopecia, chemotherapy, minoxidil topical solution may have utility in reducing the duration of the induced hair loss. In two studies, one controlled (41) and one uncontrolled (42), minoxidil topical solution was not effective in preventing hair loss associated with chemotherapy. In a placebo-controlled clinical trial by Duvic et al. (43) in which 22 women had undergone surgery and adjuvant chemotherapy for breast cancer, 2% minoxidil topical solution did not prevent hair loss, but it did decrease the duration of chemotherapy-induced hair loss relative to the placebo group.

FUTURE

The future for hair growth research and potential forms of treatment is very bright. The cross-disciplinary efforts of academia, the pharmaceutical industry, and clinicians have led to new understanding of hair growth regulation, both biochemically and genetically. Sawaya and Price (8) have recently shown that there are differences in the amounts of steroid-metabolizing enzymes in the hair follicles of males and females with androgenetic alopecia. The isolation of two forms (type I and type II) of the enzyme 5α-reductase requires further study to elucidate their specific roles in regulation of hair follicle growth/regression. The recent finding that the enzyme aromatase is specifically located in the outer root sheath of hair follicles refocuses our efforts to study the entire hair follicle, not just the dermal papilla cells (8). Based on the numerous patent applications since 1995 (1), it is clear that industry is highly involved in developing hair growth enhancers. And finally, Ahmad and colleagues' (44) discovery of the gene for hair loss in alopecia universalis provides momentous progress at the molecular level. These exciting findings highlight the great strides that have been made in hair

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growth research and provide impetus to researchers in their quest for new and/or refined therapies for hair loss (45,46).

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7
Moisturizers

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INTRODUCTION

Dry and chapped skin is a very common problem both in healthy individuals and in patients with skin diseases. Dry skin might be connected to some inherited disorders relating to the structure and function of the epidermis (e.g., ichthyosis, atopic dermatitis) and may also be secondary to other diseases (e.g., diabetes or renal failure). Moreover, the condition can occur in response to an environment with low humidity and/or low temperature. Exposure to solvents, cutting fluids, surfactants, acids, and alkalis may also produce dryness.

Several features give the impression of dry skin (1–4). The visible and tactile characteristics mentioned below are judged both by the dermatologist and the affected person, while the sensory characteristics are perceived solely by the affected person:

1. Visible characteristics—redness, lackluster surface, dry, white patches, flaky appearance, cracks, and even fissures
2. Tactile characteristics—rough and uneven
3. Sensory characteristics—dry, uncomfortable, painful, itchy, stinging, and tingling sensation

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The term ‘‘dry skin’’ is not generally accepted. Some relate it to the lack of water in the stratum corneum (SC), whereas others consider dry skin to belong to a group of disorders with a rough skin surface (5,6). It has not been conclusively shown that the water content of the stratum corneum is reduced in all dry skin conditions. For example, reduced water content has not been detected in the pruritic and dry-looking skin of patients with chronic renal failure (7) or in the clinically dry-looking skin of the elderly (8). There is also a discrepancy between the subjective self-assessment and the clinical assessment of the presence of dry skin (3,4). However, in other studies, a decreased water content of the SC has been found in elderly patients with xerosis (9,10) and in studies of winter xerotic skin the water content of the stratum corneum correlated inversely with clinical scores of dryness (1,2). Furthermore, the dry-looking skin of patients with atopic dermatitis and psoriasis is less hydrated and less capable of binding water than normal skin (8,11–15). In vitro studies have also confirmed that pathological stratum corneum from atopic and psoriatic patients is less capable of binding water than normal stratum corneum (14,16).

Products used for treatment or prevention of dry skin are called emollients or moisturizers. They are able to break the dry skin cycle and maintain the smoothness of the skin. The term ‘‘emollient’’ implies (from the Latin derivation) a material designed to soften the skin (i.e., a material that ‘‘smooths’’ the surface to the touch and makes it look smoother to the eye). The term ‘‘moisturizer’’ is often used synonymously with emollient, but moisturizers usually contain humectants, which hydrate the stratum corneum. In the present chapter, the term moisturizer will be used, but may also apply to creams without humectants.

Application of moisturizers to the skin induces tactile and visual changes of the skin surface. The ratio between oil and water is important, as well as the type of oil and the amount and type of other ingredients (emulsifiers, humectants, etc). The combination of substances influences the initial feel of the product, its spreading behavior on the skin, whether and how fast it is absorbed, and how the skin feels after its use. Water in the applied products has an immediate hydrating effect, due to penetration into the skin from their water phase (17). Other ingredients can also be absorbed into the skin, be metabolized, or disappear from the skin surface by evaporation or contact with other materials (18–21).

Recent studies indicate that moisturizers may have greater impact on the skin than is generally believed. Moisturizers affect the structure and barrier function not only of diseased skin, but also of skin that looks normal. The term ‘‘cosmeceuticals,’’ as proposed by Kligman, may be relevant to describe moisturizers that contain no recognized medicaments, but nonetheless have medicinal value (22).

The present chapter will give an overview of the structure and function of dry skin relating to the use of moisturizers.

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MOISTURIZERS IN RELATION TO SKIN STRUCTURE AND WATER CONTENT

Roughness and scaling are visible features of clinically dry skin in patients with atopic dermatitis (3). Closer examination of these areas by scanning electron microscopy shows that the surface morphology is changed from a regular pattern to a coarser one, with broad, irregularly running furrows and loss of minor furrows (3). Likewise, in xerosis, increasing derangement of minor furrows and later also of major furrows can be observed (23). A more coarse and irregular skin surface pattern with larger squares is also found in recessive X-linked ichthyosis (24).

Moisturizers are expected to increase skin hydration and to modify the physical and chemical nature of the surface to one that is smooth, soft, and pliable. Smoothing of the surface can be observed immediately after application of a moisturizer as a result of the filling of spaces between partially desquamated skin flakes (25,26). The surface friction is also changed after application of moisturizers (27). Besides mixing with material already present on the surface, topically applied substances may enter into the skin and affect its surface structure and water content.

Using instrumental evaluation of the skin topography the influence of moisturizers on the skin structure has been addressed (25,28–33). The roughness parameters and the distance between furrows/peaks can describe changes in the hydration status (28–35). Dry skin tends to have a larger number of high peaks and a larger distance between the peaks than normal skin (33,34). Hydration of normal skin has been reported both to decrease (28,29,35) and to increase (30) the roughness parameters. Cook found the distance between the peaks to be smaller after hydration (35). A single application of moisturizers has been found to decrease the roughness parameters and reduce the distance between the furrows during the first 2 h (31). No change in the roughness but a decrease in the distance between the peaks was found after a 21-day treatment period in a study by Cook (34).

Water in the SC is associated with the hydrophilic parts of the intercellular lipids and with the keratin fibers in the corneocytes (14,36). The fibrous elements in the corneocytes have hydrophilic properties and also contain a water-soluble fraction that enhances their water-holding capacity (37–39). In the hydrated SC, three types of water with different molecular mobilities can be found. At a water content below 10%, the primary water is tightly bound, presumably to the polar sites of the proteins (14,40,41). When the degree of hydration exceeds 10%, the secondary water is hydrogen bonded around the protein-bound water, and above 40 to 50% the water resembles the bulk liquid (14,40,41). It is the secondary water that contributes to the plasticity of the SC (14,39). The amount of tightly

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bound water, which does not seem to have any plasticizing effect, is almost the same in different types of pathological skin, whereas the amount of secondary water is much smaller in SC from psoriatic patients and from elderly persons with xerosis than in normal SC (14). For instance, in normal SC from glabrous skin the content is 38.2 mg/100 mg dry tissue, as compared with 31.7 mg in senile xerosis and 27.2 mg in psoriatic scales per 100 mg dry tissue (14). Prolonged exposure to water induces a pronounced swelling of the SC in the thickness dimension (42), with swollen corneocytes, and in the intercellular lamellar regions rough structures, water pools, and occasionally vesiclelike structures can be seen by means of freeze–fracture electron microscope (43). Proinflammatory substances are also released from the SC, which incites an inflammatory reaction (44) and increases blood flow in subclinically irritated skin (45).

Possible Roles for Humectants

Moisturizers often contain low-molecular-weight substances with water-attracting properties, called humectants. These substances are supposed to penetrate into the skin and increase the degree of hydration of the SC. In some vehicle-controlled clinical studies on dry and irritated skin, the improvements have been amplified by the content of humectants in the moisturizer (37,46–51).

A special blend of humectants can also be found naturally in the SC; it is called natural moisturizing factor (NMF) (52). NMF can make up about 15 to 20% of the total weight of the corneum and substances belonging to this group are amino acids, pyrollidone carboxylic acid (PCA), lactates, and urea (Table 1) (52,38). NMF is formed from the protein filaggrin and this formation is regulated by the moisture content in the SC (97). Extraction of NMF from the skin reduces the ability of the SC to bind water (38,39,53,54). Pyrrolidone carboxylic acid

Table 1 Composition of Natural Moisturizing Factor (NMF)

	(%)
Amino acids	40.0
Pyrrolidone carboxylic acid	12.0
Lactate	12.0
Urea	7.0
Na, Ca, K, Mg, phosphate, chloride	18.5
NH3, uric acid, glucosamine, creatinine	1.5
Rest unidentified	

Source: Ref. 52.

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(PCA) occurs primarily in the SC in the form of its sodium salt at levels reaching about 3 to 4% (53).

A deficiency of NMF is linked to dry skin conditions. In ichthyosis vulgaris (55) and psoriasis (56), there is a virtual absence of NMF. The amino acid composition of SC samples from old people are altered in xerotic skin (10,57). There is a decrease in the amount of water-soluble amino acids in relation to the severity of xerosis, a finding that has been suggested to reflect decreased profilaggrin production (10). A reduced content of amino acids has also been observed in experimentally induced scaly skin (58). Furthermore, the SC in patients with severe ichthyosis vulgaris with a low surface hydration state has a lower amino acid content than normal SC (10). The content of urea both in the normal and affected SC of patients with atopic dermatitis is also substantially reduced (59). In addition, a significant relationship has been found between the moisture-binding ability and the PCA content of samples of SC (53).

The water-binding capacity at various humidities differs between humectants (Table 2). For example, the sodium salts of lactic acid and PCA appears to be higher than that of glycerin and sorbitol (60,61). Urea also has strong osmotic activity (62,63). As may be anticipated, the water-holding capacity of normal SC and of scales from psoriatic and ichthyotic patients is substantially increased after treatment with urea and glycerin preparations (13,29,46,64). Likewise, PCA attracts water and increases the degree of hydration of solvent-

Table 2 Moisture-Binding Ability of Humectants at Various Humidities

Humectant	31%	50%	52%	58–60%	76%	81%
Butylene glycol						38[e]
Glycerin	13[c] 11[b]	25[a]	26[b]	35–38[c,f]	67[b]	
Na-PCA	20[c] 17[b]	44[a]	45[b]	61–63[c,f]	210[b]	
Na-lactate	19[b]	56[a]	40[b]	66[f]	104[b]	
Panthenol	3[d]		11[d]		33[d]	
PCA	<1[c]			<1[c]		
Propylene glycol				32[f]		
Sorbitol		1[a]		10[f]		

Description of test conditions can be found in the original articles.
[a] Ref. 60.
[b] Ref. 61.
[c] Ref. 53.
[d] Ref. 67.
[e] Ref. 68.
[f] Ref. 69.

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damaged guinea pig footpad corneum (37). However, which of these substances most efficiently increases the skin hydration is not known. Besides differences in water-binding capacity, their penetration characteristics are important for the effect. The amount of urea (65,66) and glycerin (28) absorbed into normal SC can be followed using a simple tape-stripping technique.

Although water is known to play an important role in maintaining skin suppleness and plasticity (70), the humectants in themselves may also affect its physical properties. For example, α-hydroxy acids and NMF increase skin elasticity (71–76) and stimulate the keratinocyte ceramide synthesis (77). Studies also indicate that if NMF is removed, water alone cannot restore elasticity (76).

Furthermore, humectants might influence the crystalline arrangement of the bilayer lipids (78). In dry skin, the proportion of lipids in the solid state may be increased, and putative moisturizers may then help to maintain the lipids in a liquid crystalline state at low relative humidity (78,79). Glycerin has been shown to interact with model lipids to maintain the liquid crystalline state even at low relative humidity (79,78). It has also been proposed that glycerin may aid the digestion of the superficial desmosomes in subjects with dry skin and thereby ameliorate dry flaky skin (80).

Possible Roles for Lipids

The lipid composition of the epidermis changes dramatically during epidermal differentiation (81,82). There is a marked decrease in phospholipids and an increase in fatty acids and ceramides (81,82). In the final stages of this differentiation, keratinocytes discharge lipid-containing granules—lamellar bodies—into the extracellular spaces in the upper granular layer, where they form intercellular membrane bilayers (Fig. 1) (36,82–84). This lamellar material greatly expands the intercellular compartment and constitutes about 5 to 10% of the total weight of human SC (85,86). The composition of these lipids is unusual and is important for the water-holding capacity of the SC (54,87–89). Exposure of the skin to solvents removes the structural lipids and produces a chapped and scaly appearance (54,88,90,91). Furthermore, lipid depletion enhances the susceptibility of water-soluble materials to be extracted by water (39,54,87). Unlike the lipids in all other biological membranes, those in the SC do not contain phospholipids, but are mainly composed of ceramides, sterols, and fatty acids (Table 3).

Application of lipids to the skin surface may increase skin hydration by several mechanisms. The most conventional one is occlusion, which implies a simple reduction of the loss of water from the outside of the skin. Common occlusive substances in moisturizers are lipids, for instance, petrolatum, beeswax, lanolin, and various oils. Although they reduce water loss (17,92), their effect may be diminished when combined with other ingredients in skin-care products (93,94). These lipids have long been considered to exert their effects on the skin

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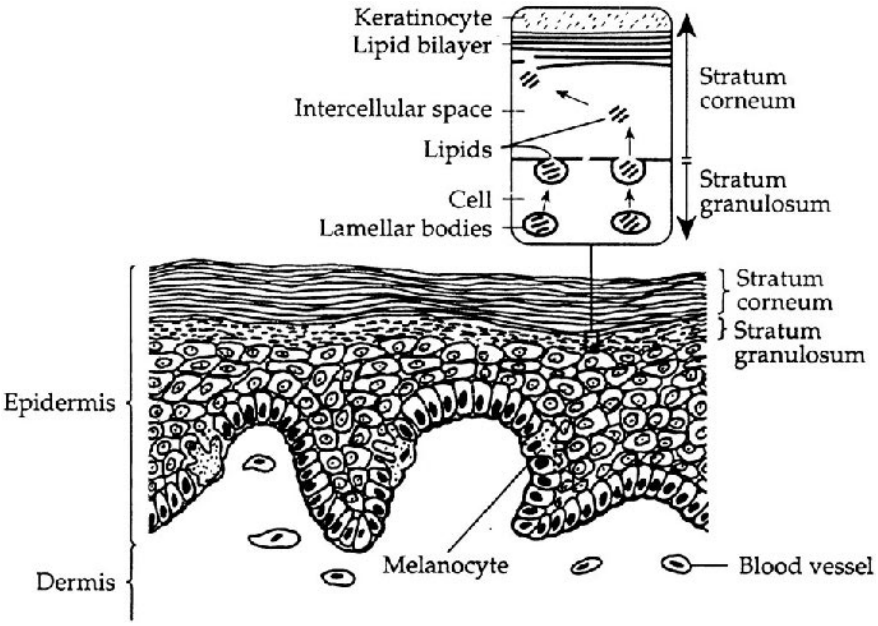

Figure 1 Structure of the epidermis and a schematic presentation of the formation of the intercellular lipid bilayer.

Table 3 Composition of Human SC Lipids

Lipid	Facial skin (Ref. 86)	Facial skin (Ref. 82)
Ceramides	19.9	39.1
Fatty acids	19.7	9.1
Triglycerides	13.5	0.0
Free sterols	17.3	26.9
Cholesteryl esters		10.0
Cholesteryl sulfate		1.9
Sterol/wax esters	6.2	
Squalene, *n*-alkanes	9.7	
Others	6.7	11.1

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solely by forming an inert, epicutaneous, occlusive membrane. However, topically applied lipids penetrate the skin (21,95–103). For example, the syndrome of EFA deficiency is readily reversed by topical treatment with linoleic acid or sunflower seed oil, which is rich in linoleic acid (101–103). Furthermore, application of structural lipids from SC increases skin hydration and reduces scaling (89,104).

A more speculative mechanism behind the beneficial effects of lipids are their possible anti-inflammatory action. Polyunsaturated fatty acids in oils have been suggested to be transformed enzymatically by the epidermis into ''putative'' anti-inflammatory products (105). Treatment of UVB-induced acute inflammation shows that dietary supplementation with fish oil (106,107) and purified ethyl ester of eicosapentaenoic acid (20:5, n-3) from fish oil (108) has some anti-inflammatory effects. Topical (96,98), as well as oral (109), treatment with fish oils rich in omega-3 fatty acid is claimed to be effective against psoriasis, although this has been questioned (110–112). In patients with atopic eczema, no difference between fish oil and maize oil was detected in a double-blind multicenter study (113).

Atopic dermatitis has been reported to benefit from oral treatment with evening primrose oil, a vegetable oil rich in gamma linolenic acid (GLA), a fatty acid of the omega-6 family (114,115), although this has not been confirmed in other studies with topical (116) or oral treatment with evening primrose oil (117,118) or another oil rich in GLA—borage oil (119). Moreover, the GLA-containing borage oil is claimed to have good effect against infantile seborrheic dermatitis (99,100). The biochemical mechanisms of the possible therapeutic effects remain unclear, but it has been suggested that the enzyme δ-6-desaturase, which converts linoleic acid into GLA, might play a role, since it has been suggested that this enzyme is less active in atopic eczema and seborrheic dermatitis (99,120).

MOISTURIZERS IN RELATION TO THE BARRIER FUNCTION

Dry, scaly skin is usually associated with impaired barrier function (8,58,103,121–123). Impairment in the barrier function might be due to cracks in the skin, resulting from a decreased softness and flexibility of the SC (39,70). The projected size of the flattened corneocytes is also considered to influence the barrier function, and in dry, scaly skin the projected size is reduced, indicating a shorter penetration pathway through the skin (1,58,124). Furthermore, the lipid content and organization of these intercellular barrier lipids have broad implications for the permeability barrier function (36,83–85,125,126). The lipid composition of the SC is highly variable among individuals, depending on a number of factors (Table 4). In dry skin and in skin exposed to organic solvents, the lipid

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Table 4 Factors Influencing the Lipid Composition of the Skin

Anatomical region (86)
Sex (136)
Age (136,137)
Season (137)
Exposure to surfactants (89,139)
Exposure to solvents (88,90,91,104)
Tape-induced scaly skin (58)
Atopic dermatitis (138,140–144)
Psoriasis (145)
Ichthyosis (146)
Essential fatty acid (EFA)-deficient states (103)

composition and normal bilayer structure are changed (58,88,90,91,104,127, 138,140–146). However, dryness of the SC may not necessarily increase skin permeability. For example, if the dryness is confined to the outermost SC layers and the major permeability barrier resides in the lower part of SC, no correlation between these parameters could be expected (89).

Improvement of the SC barrier function is central to the improvement of all dry skin conditions, in particular contact dermatitis and atopic dermatitis. Contact dermatitis is a major occupational skin disease and protective creams, also marketed as barrier creams or invisible gloves, have come to play an important role in protecting the skin from toxic substances. Protective creams are expected to be used on normal skin and form an impermeable film on the surface that can prevent noxious substances from entering into the skin. Such creams may also contain substances that trap or decompose the hazardous substance. Experimental studies also show that some creams can delay the contact with certain substances, whereas others enhance the penetration of the hazardous substance (128–133,147). Treatment can also reduce skin susceptibility to alkali, SLS, and DMSO, but increase absorption of hexyl nicotinate (134).

Considering the range of effects, the benefit of using protective creams in the prevention of contact dermatitis in industry or in wet working occupations is controversial (148). In a prospective study on metal workers, the beneficial effect from protective cream treatment was not confirmed, whereas an ordinary moisturizer decreased the prevalence of irritation (149). Moisturizers may also prevent contact dermatitis to a similar degree as barrier creams, but with the possible advantage of enhanced user acceptance (132,135).

In assessing the effects of moisturizers on skin barrier function (Table 5), studies evaluating the effects on diseased skin need to be distinguished from those

Table 5 Factors to Consider in Evaluating the Effects on Skin Barrier Function by Creams

Composition of the cream
Cream thickness; drying time
Test skin; animals or humans; normal or diseased
Single application versus repeated applications
Expected time course for effect
Biological endpoint
Challenging substance; application method; dosage

on normal skin (i.e., treatment or prevention). Furthermore, single or repeated treatment might be important for the outcome. One way to monitor changes in barrier function as a function of time is to measure TEWL (100,150–154). The level of TEWL has been suggested to serve as an indicator of the permeability of the skin to topically applied substances (155,156) and high basal values have also been found to predict increased skin susceptibility to chemical irritation stimuli (157–159).

Another method to assess the barrier function is to expose the living skin to substances with biological activity and to measure the response (Table 6) (132,133,160–165). However, long-term studies under real conditions are considered necessary to support the results from predictive testing (148,149).

Possible Roles for Humectants

In studies on dry skin, one might expect an improvement in the impaired skin barrier function in association with improvement of the clinical signs of dryness. TEWL has also been reduced in ichthyotic (46), atopic (163,166), and dry (167) skin by treatment with moisturizers containing humectants, such as urea or glycerin (46,166–169). In a placebo-controlled study, it has also been proven that urea

Table 6 Examples of Substances That Have Been Used to Test the Skin Barrier Function

Substance	Biological response	Refs.
Surfactants	Irritation	132, 133, 160, 158, 162, 163
Alkali resistance	Burning, itching, erythema	189, 161, 134
DMSO	Urticaria	190, 134
Nicotinates	Vasodilation	134, 165, 164, 185
Toluene	Irritation	133, 161

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promotes barrier recovery in SLS-induced dry skin (170). However, a moisturizer without humectant (171) and another with ammonium lactate as humectant (32) had no effect on TEWL, despite clinical improvement.

Despite the widespread use of moisturizers, scant attention has been paid to their influence on the permeability barrier of normal skin. It may be anticipated that the use of moisturizers on normal skin will increase the permeability, since increased hydration of normal skin is known to reduce its diffusional resistance (172–175). Hydration may create interfacial defects in the lipid bilayer caused by phase separation (43,176). In vitro experiments on SC also indicate that humectants increase TEWL (61,92) and certain humectants are known as keratolytics (see Ref. 177 for an overview). However, studies in healthy volunteers show no increase in TEWL by repeated application of moisturizers, although the treatment appeared to increase the skin hydration significantly (162,178–180).

The use of moisturizers with urea has been questioned, with reference to the risk of reducing the chemical barrier function of the skin to toxic substances (62). Some single-application studies also show that urea may act as a penetration enhancer (164,181–185). However, not all studies support this belief (165,186,187) and repeated applications (10–20 days) of urea moisturizers on normal skin actually reduce TEWL (162,167,169).

In vivo TEWL measurements have also been combined with challenge of the skin with a vasodilator (nicotinates) and with an irritant [sodium lauryl sulfate (SLS)] to further elucidate changes in barrier function due to treatment with moisturizers (148,162,163,165,169,188). Single exposure to sodium lactate, sodium-PCA, and sorbitol show these to reduce the penetration of benzyl nicotinate (165). Furthermore, an increased resistance to SLS-induced irritation has been found after long-term treatment with urea (132,162,163,169), glycerin (132), and α-hydroxyacids (188). However, absence of effects has also been found for a moisturizer with glycerin (162) and, likewise, *increased* skin susceptibility to irritation has been shown after treatment with a moisturizer without any humectant (148).

B. Possible Roles for Lipids

A disturbance of the epidermal barrier function induces a rapid response of the keratinocytes to restore cutaneous homeostasis. The mRNA coding for proinflammatory cytokines, adhesion molecules, and growth factors is upregulated (191). Likewise, there is an increase in DNA synthesis, leading to epidermal hyperplasia, and in lipid synthesis (91,152,153,192–194). The synthetic activity includes unsaponifiable lipids (91,152,194), fatty acids (152), and sphingolipids (151). Sterols and fatty acids are synthesized immediately after barrier disruption, whereas the increase in sphingolipid synthesis is somewhat delayed (151). Over time, the content of lipids in the SC is restored to the normal level in parallel with the return of barrier function (91,151–153,193,194).

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Topically applied lipids may also penetrate the skin and affect its barrier properties (90,99,100,103,126,154,195). For instance, sunflower oil, rich in linoleic acid, has been found to reduce abnormally high rates of TEWL in sodium-laurate-irritated rat skin (103) and borage-oil normalizes TEWL in infantile seborrheic dermatitis (99). Petrolatum has also been found to be absorbed into delipidized skin and to accelerate barrier recovery to water (154). In contrast to these findings, an inverse relationship was found between recovery of normal TEWL and the amount of sunflower seed oil in emulsions used for treatment of sodium lauryl sulfate (SLS)-induced irritation in humans (195). Moreover, applications of ceramides, linoleic acid, and a variety of other fatty acids alone delay barrier recovery in acetone-treated murine skin; likewise, two-component mixtures of fatty acid plus ceramide, cholesterol plus fatty acid, or cholesterol plus ceramide delay barrier recovery (90). The only treatments that allowed normal barrier recovery were applications of complete mixtures of ceramide, fatty acid and cholesterol, or pure cholesterol (90). Commercially available moisturizers have also been found to reduce elevated TEWL values in acetone-treated mice skin compared to untreated areas at various times during a 24-h test period (196). Furthermore, not only lipids but also emulsifiers can reduce TEWL in surfactant-irritated human skin (197). In normal forearm skin, a moisturizer without humectants has been found to increase skin susceptibility to SLS, without prior increase in TEWL (148).

DISCUSSION AND CONCLUSION

A lack of water may be too simple an explanation for all types of problems covered by the term dry skin, such as redness, scaling, roughness, itching, and a feeling of discomfort. Rather than just aiming at a general increase in the water content, the abnormal epidermis should probably be treated according to the underlying pathogenesis. The possibilities to correct or prevent abnormalities in the skin by different treatments may also help to explain the differences in preference for different moisturizers among individuals. This opens up new possibilities for further improvement in the treatment of different dry skin disorders.

The interesting findings that moisturizers also can affect barrier homeostasis clearly indicate that ingredients are not as inert to the skin as previously considered. A number of different mechanisms behind the barrier-improving effects from moisturizers have been suggested. It is obvious that a reduction in TEWL may be due to a simple deposition of lipid material to the surface, and not to any deeper effects in the skin. Another explanation is increased skin hydration, which increases SC elasticity and decreases the risks of cracks and fissures. Interference with the lipid layer around the corneocytes may also help to retain the moisture content in the corneocytes and prevent cracking of the SC (54,87–

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89,104,154). Moreover, it is possible that the applied moisturizer decreases the proliferative activity of epidermis, which increases the size of the corneocytes. With a larger corneocyte area, the tortuous lipid pathway gives a longer distance for penetration, which reduces the permeability (58,124,198). Reduction in mitotic activity and cell proliferation has been found by treatment with lipids and urea (199–201).

Topically applied lipids may also penetrate deeper into the skin and interfere with endogenous lipid synthesis, which may promote, delay, or have no obvious influence on the normal barrier recovery in damaged skin (90,126). Furthermore, other substances in creams may influence the composition of the SC lipids (e.g., lactic acid has been found to stimulate the production of ceramides by keratinocytes in vitro) (77). Other mechanisms, such as anti-inflammatory actions, are also conceivable explanations to the beneficial actions of moisturizers on the skin.

Whether changes in TEWL are predictive also for the permeability to substances other than water is likely to be dependent on the mechanism for the change in TEWL. For example, TEWL may be reduced by absorption of certain substances from the moisturizer, but this may facilitate absorption of other exogenous substances into the skin.

In conclusion, we can foresee that the increased understanding of the interactions between topically applied substances and the epidermal biochemistry will improve the formulation of future skin care products (202). Furthermore, noninvasive bioengineering techniques will allow us to monitor treatment effects more closely and in the future we can also expect new devices that can diagnose specific skin abnormalities noninvasively.

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8
Botanical Extracts

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The existence of the word ''cosmeceuticals'' is very much linked to the U.S. FDA definition of drugs and cosmetics in the 1938 FD&C Act. One can only speculate as to why 60 years of scientific knowledge and research have been ignored by the FDA in not revising the definition! The European Commission has been wiser and its 1976 definition of cosmetics was modified in 1993 to acknowledge the fact that everything put on the skin or hair may have a physiological effect (1). It puts the responsibility on the industry to ascertain product safety and efficacy (claims justification) (2).

Natural extracts, whether from animal, botanical, or mineral origin, have been used as ''active ingredients'' of drugs or cosmetics for as long as human history can go. Oils, butter, honey, beeswax, lead, and lemon juice were common ingredients of the beauty recipes from ancient Egypt. Many botanical extracts are used today in traditional medicine and large pharmaceutical companies are rediscovering them.

The major differences between the drug and the cosmetic approach rely on the intent (i.e., ''cure or prevention of a disease'' vs. ''beautifying'') as well as how the extract is considered. In the cosmetic industry, the botanical extract *is* the active ingredient. It may contain hundreds of chemical structures and it has a proven activity. In the drug industry, you need to know the chemical structure of the active ingredient *within* the extract, very often to synthesize it, to purify it, sometimes to discover that isolation and purification leads to a loss in the biologi-

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cal activity, or to realize that, despite all the skills of organic chemists, nature is *not* easy to reproduce.

ORIGIN OF BOTANICAL EXTRACTS

Botanical extracts have been used for centuries and are present in today's products either for their own properties or as substitute of animal materials that may have to be removed from products because pressure of animal rights associations or diseases like bovine spongiform encephalopathy (BSE). There are plant powders for hair coloring (Henne), scrubs (apricot kernel, corn), or masks (oat flour); plant extracts (''as is'' or purified); and biotechnology extracts obtained through fermentation, cloning, soilless culture (aquaculture, artificial media, etc.), which are developed from microorganisms, plant organs, total plants, or through the use of specific enzymes (3).

EXTRACTION PROCESS

Active ingredients ae not present in equal amounts in the plant or the organism. Most of the time, a higher concentration can be found in certain parts. Therefore, it is usually only one part of the plant that is used: fruit, bark, root, bud, flower, leaves, etc.

Depending on the future use of the extract, various extraction processes can be used. As mentioned, it is industry's responsibility to ensure the absence of toxic substances that could lead to unwanted side effects. The drug approval process allows side effects to be present provided the benefits outweight the disadvantages, while the cosmetics consumer has the choice of using a product that may have side effects or using another that has none, the product with side effects would not be acceptable.

Total Extracts

Total extracts are most common in the cosmetics industry, rarely, if ever, used in drugs. They are generally known from traditional usage, which has a long history. Their activity is often empirical and their active ingredients are not always identified, but their benefits are, very often, without possible doubt. Their mode of preparation can be found in traditional pharmacopeas (China, India, Africa, Europe, America), or from observing shamans or traditional practitioners. Very often, plants are blended in order to better control or synergize their effects, but sometimes also to preserve the secret of the active ingredient.

Modern techniques include: (a) pressing—for plants rich in water (e.g., juice, fresh plants, fruits, vegetables, cactus) or oil; (b) percolation, with one

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solvent or a mixture of solvents (water, glycols, ethanol) at room temperature or at elevated temperature (this process is the same as the one used to obtain coffee); and (c) maceration, with the same type of solvents (this process is the same as the one used to obtain tea).

These processes allow for better controls: stability, preservation, manufacturing reproducibility.

The content of the extract is very much a function of the type of solvent, the temperature, the plant:solvent ratio, the time of contact, the part of the plant used and its species. Sometimes it is also dependent of the plant culture conditions and the season of harvest.

In the drug industry, especially, the extract must be concentrated and the active material isolated by selective precipitation, chromatography, electrophoresis, etc.

Solvents have to be carefully chosen, not only for their extraction properties, but also for their compatibility with the final formulation and their harmlessness.

Selective Extracts

Special extraction processes or the use of specific solvents will lead to the obtention of a specific class of molecules.

The fragrance industry has for centuries obtained essential oils or floral water by water vapor extraction or ''enfleurage''—a process by which the plant flowers are put in contact with solid fats and terpens and sesquiterpens migrate into the oil phase.

The use of vegetable oils as solvent allows for the extraction of oil-soluble vitamins or lipids. More recently the use of supercritic CO_2 has been developed to extract aroms, essential oils, and oleoresins.

Purification

Extract purification to separate specific molecules from others are done following classic physicochemical processes—cryoprecipitation, column chromatography, electrophoresis, use of selective solvents and salts, etc.

Biotechnology Extracts

Biotechnology can be used to obtain, purify, or transform extracts. The use of enzymes as tools in this area is booming (4). One can find different enzymes to be used for very specific reactions in certain conditions. They could become an alternative to chemical reactions as they provide stereospecificity or eliminate the risk of solvent residues. Today, protein hydrolysates obtained by enzymatic

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reaction are free of the chlorine residues formed when acid hydrolysis is used. In addition, the use of exo-, endo-, or amino-acid-specific proteases allows for a better control of the end result.

Enzymes will allow for better yields by transforming or releasing specific molecules (use of pectinases, β-glucosidase, β-glucanase, lipases, transferases, esterases, etc.).

Amino acids, polyols, esters of fatty acids, polyol organic acids, more stable liposoluble vitamin esters with slow release properties, and new molecules (5) can be obtained.

Usage

Extracts or purified botanical molecules can be incorporated directly into solutions, emulsions, or vectors or can be used to form a vector (liposomes, phytosomes, phytospheres) (6). They can be topically applied, ingested, or injected, depending on the intended use and provided absence of toxicity has been shown.

Activity

Are botanical extracts really active? How does their activity compare to that of synthetic materials? Are all nautral ingredients safe?

Certainly one learns a lot on these questions by studying traditional uses. Centuries of human experience can prove safety. For example, lilium bulb oil extract use for sunburns has been reported since ancient Greece, while the water extract has been shown to be toxic. Natural ingredients have been shown to have a broad spectrum of activity, including hallucinogenic mushrooms and cardiotonic belladona. Scientific research conducted on plant extracts described in traditional pharmacopeas (7,8) has led to a broader range of potential applications.

Furthermore, research conducted during the last 10 years on skin biology allows us to better understand the biological mechanisms involved in dehydration, aging, etc. This, in turn, leads to the search for extracts with specific activities for targeted applications.

Antioxidants

Free radicals have been shown to play a major role in sun damage as well as in aging or in pollution (tobacco, stress). They act by degrading the skin structural fibers (collagen, elastin), cell membranes, DNA, or by creating inflammatory reactions (9). Free radical actions can be blocked by the following

Vegetable oils rich in tocopherols and tocotrienols. α-Tocopherol contributes directly to cell membrane structure by stabilizing it and allowing

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for proper functioning of membrane enzymes. Wheat germ oil and palm oil are particularly rich in tocopherols and α-, β-, γ-tocotrienols.

Carotenoids, such as β-carotene, found in plants or in part of plants exposed to the sun. Of particular interest is a unicellular microalgae, *Dunaliella*. Under normal conditions of light, temperature, or salt, these algae are green. However, under extreme conditions (high salinity, low pH, high sunlight, lack of nitrogen or phosphorus), they protect themselves by multiplying their β-carotene concentration by 10. The ponds become red, and the β-carotene concentration can reach 14% of their dry weight.

As first shown by Kligman (10), the action of retinoids and carotenoids (11) on sun damage has led to numerous works.

SOD is an enzyme that deactivates free radicals. Its concentration decreases with age. It has been possible to obtain *Bifidus* extracts that are rich in SOD (12).

Ascorbic acid, which can be found in *Rosa canina* (dog rose) fruits, actinidia (kiwi fruits), or *Malphigia punicifolia* (West Indian cherry) is an antioxidant that is also used for many of its other properties.

It is active in the synthesis of carnitin, a molecule intervening in the transfer of lipids inside the mitochondria. Ascorbic acid thus plays a role in improving cell resistance due to a better use of lipids. Ascorbic acid is an anti-inflammatory agent that degrades and eliminates histamine. It can be used in after-sun products; it protects against free radical damage, helps maintain the elasticity and the integrity of the extracellular matrix (ECM), and has immunostimulating activity.

Flavonoids, rich extracts from *Gingko*, *Fagopyrum* (buckwheat), *Eucalyptus sambucus* (European elder), or *Sophora japonica* are used for their antioxidant and anti-free-radical properties (13).

Rosmarinus (rosemary) extracts, rich in carnosic acid, are very potent antioxidants, used to protect food.

Syzygium aromaticum or *Germanium thumbergii* extracts can be used to protect collagenase activity and ECM from free radicals (14).

Lipids of the Epidermis and Barrier Function

Fish oils rich in polyunsaturated fatty acids (PUFA) of the n-3 type [e.g., EPA (eicosapentaenoic acid) or DHA (docosapentaenoic acid)] act directly on cell membranes by increasing their fluidity. They favor the exchanges between the inner and the outer compartment of the cells or between cells. In addition, they have anti-inflammatory activity (15).

Thanks to the use of microalgae cultures in photobioreactors, plant oils rich in PUFA (EPA and DHA) can be produced.

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Other plant oils rich in PUFA of the n-6 type [e.g., *Oenothera biennis* (evening primrose), *Borage officinalis* (borage), and *Ribes nigrum* (black currant)] are important in bringing essential fatty acids to the skin contributing to the maintenance or the restoration of epidermal lipids.

Oil and plant butters (rice, wheat, coffee, mango, sorgho, baobab, soya, corn, carob) are rich in essential fatty acids (EFA) (e.g., oleic and linoleic) or squalene (olive oil), which maintain skin suppleness and reduce water loss. They also contain a nonsaponifiable fraction rich in sterols. Some of these have exceptional healing properties that make them of particular value in sun or antiage products: *camelia* (tea), *argania, medicago* (alfalfa), *spinacia* (spinach), *Butyrospermomum* (shea butter), *Cucurbitaceae, Pongamia* (hongay or pongamia oil). β-sitosterol is well known for its inflammatory properties. The insaponifiable fraction is also a stimulant of collagen or elastin synthesis.

Phytosterols slow down the aging process by favoring fatty acid desaturation, which in turn maintains membrane fluidity and catalytic activity. γ-Orizanol (ferulic esters of cycloartanol, cycloartenol, and β-sitosterol) extracted from rice, topically applied, stimulates sebaceous gland activity, which slows down with age.

One can also find plant waxes (sugar cane, *Camauba, Ceroxylon, Jojoba*, rose) which are used to protect lips, hands, or face from dehydration.

Certain plants (yeast, wheat, apple, potatoes, rice bran, *Agaricus, Morus alba*, or white mulberry) are rich in ceramides and glycosylceramides. These may be used for their action on skin or hair to provide hydration or reconstitute epidermal barrier function.

Other plants are rich in oils containing very long-chain fatty acids (C22, 24, 26) like *Pentaclethra* or ewala oil used in Africa as a massage oil, or *Limnanthes alba* or shambrilla oil.

Fat Storage and Slimming

We are currently using botanical extracts with very specific actions that act at various levels of adipocyte metabolism.

Garcinia cambodgia decreases the transformation of sugars into fat.

Extracts of *Guarana*, tea, coffee, cocoa, which are rich in methylxanthines (caffeine, theobromin) are cAMP-phosphodiesterase inhibitors and thus accelerate lipid degradation.

Flavonoids, like quercetin or its derivatives, are also inhibitors of this enzyme and could lead to a 40% increase in cAMP.

Methylxanthins of the same plants will act on lipoprotein lipase (LPL), reducing the passage of fatty acids into the adipocyte.

Phytosterols from plant oils are being investigated for their potential action

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on fat storage or degradation, on adipocyte differentation or multiplication.

Antiage

Ascorbic acid is a key element in collagen synthesis (also in ''botanical collagen''). It stimulates the production of RNA coding for collagen and contributes to the synthesis of hydroxyproline and hydroxylysine (which is responsible for collagen three-dimensional structure).

Tests on cells have shown that PCO (procyanidol oligomers) from pine barks or grape pits were active in reinforcing and protecting the structure of the ECM. They improve microcirculation leading to a better irrigation of the tissues and thus to nutrition, hydration, hormone transport, etc.

Protection of elastic fibers (collagens, elastin) is promoted by extracts having free-radical scavenging properties, activating the synthesis of these proteins or inhibiting the enzymes responsible for their degradation: *streptomyces*, black currant, *Centella asiatica* (rich in asiatic acid), *Rudbeckia purpurea*, *Coleus*, *Areca*, . . .

Apigenin, extracted from *Chamomile* and its derivatives, and rutin from *Fagopyrum* have anti-inflammatory properties (by inhibiting histamine release), but they are also β-glucuronidase inhibitors. They protect mucopolysaccharides from degradation. Other extracts rich in polyphenols—tanins—also have antihyaluronidase activity (16–18).

Amino acids obtained by biotechnology through the action of microorganisms or enzymes on plant extracts are used for stimulation of systems that are active in aging as well as slimming (arginin, glutamin, HGH), hair growth (glutamic acid), or immunity (arginin) (19,20). Recent studies show the importance of amino acids in protecting the skin barrier function.

Tryptophan (from *Spirulina*, soy bean, pumpkin), vitamin B3 (from *Saccharomyces*), vitamin B6 (from avocado, banana, yeast, wheat germ), calcium, or magnesium all stimulate melatonin (MSH) synthesis. This hormone is very important to many biological processes and decreases rapidly with age. Melatonin is present in animals as well as plants. The highest concentration is found in *Festuca*, oats, corn, rice, and ginger (21).

Alpha- or beta-hydroxyacids that have been in vogue in recent years, not only in cosmetics but also in OTC drugs, are common in the botanical world. Whether from fruits (e.g., bilberry, apple, lemon, orange, kalanchoe), *Tamarindus*, *Hibiscus*, sugar cane (*saccharum officinalis*), *Accer saccharum* (sugar maple), *Salix, Betula* (sweet birch), or *Gaultheria* (Wintergreen) (22), their efficacy has been shown in smoothing, brightening, and sloughing skin. They contribute to the elimination of dead cells from the skin surface, hydration, as well as cell renewal. These acids are broadly used in facial, body, and even scalp care.

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Oligoelements and minerals like silicium can be found in *Equisetum* (Horsetail), *Oryza* (rice), or *Diatoma*. They contribute directly to the synthesis of collagen or proteoglycans and to the stabilization of ECM (23).

Selenium (*Astrogalus*) is said to play an important role in antiaging (immunity, inflammation, free radical scavenging), zinc (*Taraxacum*) in hair growth (action on testosterone) (24), and mother of pearl from shellfish in wound healing or tissue repair.

Saponins, a huge family of compounds, whether of a steroidal or triterpenic structure, are known for their detergent activity. They probably have other activities, which are yet to be established. Constant research shows that saponins, present in botanical extracts, have tremendous pharmacological and metabolic properties.

Ginseng and bupleurum—stimulate biosynthesis of proteins, RNA, cholesterol or lipogenesis.

Centella asiatica (asiaticosides)—stimulates synthesis of collagen and fibronectin.

Hedera, ficaria (hederagenin)—inhibits proteases.

Sterols from *sabal, serenoa as* well as $\Delta 7$ sterols are inhibitors of 5-α-reductase, an enzyme involved in androgenic alopecia, hyperseborrhea of the scalp or the skin, as well as acne.

Glycyrrhizin from *glycyrrhiza* and harpagosides from *harpagophytum* are broadly used for their anti-inflammatory properties.

Saponins have also been shown to increase stress resistance by increasing cortisol and prostaglandins, to protect membranes (*Eleutherococcus*), to increase metabolic efficacy (*Medicago*), to stimulate cells (*Ginseng, bupleurum*).

Extracts from *ganodema* are immunostimulating, immunoregulating, prolong all life in culture, and act on endocrine functions. They have been used in traditional Chinese medicine to slow down aging. This mushroom is rich in polysaccharides, triterpenes, and steroids.

Extracts from *arctophylos uva-ursi, coactis*, and *adenotricha* rich in arbutin and methylarbutin are used for their depigmenting effect. So are kojic acid, ascorbic acid and its derivatives, and SOD rich bifidus extracts. Rosmarinic acid from rosemary also has a tyrosinase-inhibiting activity.

CONCLUSION

Many other activities of botanical extracts have been shown and are used in cosmetics or drugs (OTC or traditional). The main difference between the two is really the intention of the manufacturer (i.e., cure or disease prevention rather

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than improvement of overall condition of the skin or hair), by maintaining or improving the natural processes.

Most cosmetic products today address both the rational and the emotional aspects that characterize their need in society, while they are often still considered as a ''dream in a bottle'' (Charles Revson).

Botanicals are playing an increasingly important role in the activity and safety of cosmetics; they allow for a renewal of the source of active ingredients in drugs.

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9
Topical Retinoids

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OVERVIEW

The retinoids are a diverse class of pharmacological compounds, consisting of vitamin A (retinol) and its naturally occurring and synthetic derivatives, which possess biological vitamin A activity (Tables 1 and 2). Vitamin A generically encompasses retinol (vitamin A alcohol), retinal (vitamin A aldehyde), and retinoic acid (vitamin A acid) (Fig. 1). In clinical use, retinoids have established their effectiveness in treating acneiform eruptions (e.g., isotretinoin), disorders of keratinization, such as psoriasis (e.g., acitretin), as well as some neoplastic processes (e.g., tretinoin for leukemia, isotretinoin for squamous cell carcinomas). Additional retinoids are currently being investigated, as are novel uses of retinoids already established in clinical practice. The main focus of retinoid usage in cosmeceuticals has been its role as the mythical ''fountain of youth'' (i.e., reversal of photoaging) (Table 3). Retinoids, like all drugs, have adverse effects, the most infamous one being teratogenicity. Over 2000 derivatives have been developed in the hope of finding retinoids with increased therapeutic efficacy coupled with diminished local and systemic toxicity. The recent focus of retinoids has been on topical delivery systems, as this route not only provides a safer adverse effect profile, but also delivers a higher dose to a targeted area (i.e., the skin).

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Table 1 Classification of Retinoids

Generation	Retinoid
First generation	Tretinoin (All-trans-retinoic acid)
	Isotretinoin (13-*cis*-retinoic acid)
Second generation	Etretinate (Ro 10-9359)
	Etretin (Ro 10-1670)
Third generation	Arotinoid (Ro 15-0778)
	Arotinoid ethylester (Ro 13-6298)
	Arotinoid methyl sulfone (Ro 14-9706)
	Adapalene (CD271)
Naturally occurring in humans	Retinol (vitamin A)
	Retinal (vitamin A- aldehyde)
	Retinoic Acid

This chapter provides a review of topical retinoids, focusing on the potential cosmeceutical applications of this class of drug. Oral retinoids with no significant cosmeceutical activity, such as acitretin, will not be covered. Note that the definition of drug versus cosmeceutical for this class is regulatory (man made) and not biological.

HISTORICAL BACKGROUND

The ancient Egyptians recognized the importance of vitamin A activity as early as 1500 BC, as evidenced by early writings in ''Eber's Papyrus'' describing the benefits of liver in treating night blindness (1). However, it was not until the early twentieth century that definitive knowledge of this substance was discovered. In

Table 2 The Roles of Naturally Existing Retinoids

Retinoid	Role
Retinol	Growth promotion
	Differentiation/maintenance of epithelia
	Reproduction
Retinal	Vision
Retinoic acid	Growth promotion
	Differentiation/maintenance of epithelia

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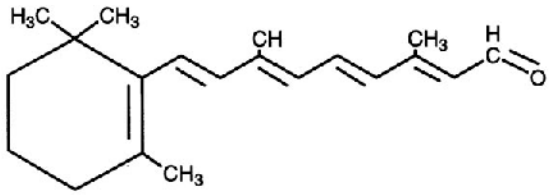
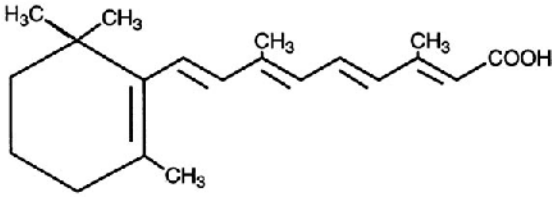
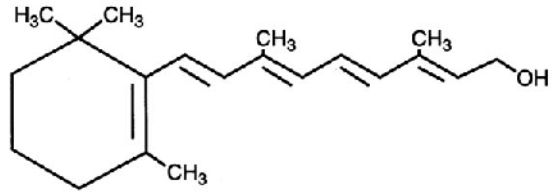


all- trans retinal



all- trans retinoic acid



all- trans retinol

Figure 1 Structure of retinoids.

1909, a fat-soluble extract from egg yolk was found to be essential for life (2). This substance, initially termed ''fat-soluble A'' (3) and later named ''vitamin A'' (4), was also found in butter fat and fish oils, demonstrating growth-promoting activity (5). Synthesis of vitamin A was achieved in the 1940s and from then on an upsurge of interest in the therapeutic uses of vitamin A became apparent.

Topical tretinoin was first used successfully by Stüttgen to treat disorders of epidermal keratinization in the 1960s (6). However, the irritation produced by the concentrations and formulations used in these studies inhibited widespread acceptance. Subsequently, Kligman proved the therapeutic efficacy of topical tretinoin in acne vulgaris (7), and went on to pioneer and popularize the use of retinoids in cosmetic dermatology by demonstrating its effects on photoaged skin (8).

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Table 3 Uses of Topical Retinoids

Retinoid	Proprietary name	Uses	
Tretinoin (All-trans-retinoic acid)	Retin-A Renova	Acne vulgaris	Primary indication
		Photoaging	
		Actinic keratoses	Secondary indication
		Lichen planus	
		Melasma	
		Postinflammatory hyperpigmentation	
Isotretinoin		Acne vulgaris	
Alitretinoin (9-*cis*-retinoic acid)	Panretin	Kaposi's sarcoma	
Retinol		Cosmetic ingredient	
Retinyl palmitate		Cosmetic ingredient	
Retinyl aldehyde		Cosmetic ingredient	
Adapalene		Acne vulgaris	
Tazarotene		Psoriasis	
		Acne vulgaris	
Motretinide		Acne vulgaris	

COSMECEUTICALS

The major forms of retinoids that may be of significant interest to the cosmeceutical industry are retinol, retinal, and possibly, retinoic acid. The main role of retinoids in cosmeceuticals are in extrinsic aging (photoaging). Currently, topical retinoic acid is FDA-approved for the treatment of acne, and in the adjunct treatment of fine skin wrinkling, skin roughness, and hyperpigmentation due to photoaging, as well as reducing the number of senile lentigines (liver spots) (9–11). At present, retinol is becoming an increasingly utilized ingredient in cosmetic preparations, such as moisturizers and hair products. One reason for this is that retinol is a nonprescription preparation. It has also been demonstrated to be less irritating topically than retinoic acid (12), which makes retinol a more favorable cosmetic ingredient than retinoic acid. It is therefore necessary to review the scientific basis for use of retinoids and their purported efficacy.

RETINOL

Vitamin A is a necessary dietary nutrient, required for growth and bone development, vision, reproduction, and the integrity of mucosal and epithelial surfaces.

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Vitamin A deficiency results in visual problems, such as xerophthalmia and nyctalopia (night blindness), hyperkeratosis of the skin, epithelial metaplasia of the mucous membranes, and decreased resistance to infections. Vitamin A is fat soluble, and occurs as various stereoisomers. Retinol (vitamin A1) is present in esterified form in dairy products, meat, liver, kidney, and oily saltwater fish.

For clinical purposes, vitamin A is available as retinol (vitamin A alcohol) or esters of retinol formed from edible fatty acids, primarily acetic and palmitica acid.

PENETRATION, ABSORPTION, AND CUTANEOUS METABOLISM OF TOPICAL RETINOIDS

Any active substance administered to the skin must penetrate the skin in sufficient amounts in order to have a pharmacological effect. This section presents evidence that the topical retinoids can be utilized effectively. Several methods have been utilized, including enzyme induction as a marker of effective penetration, radiolabeling, and HPLC.

Duell et al. (13) studied the penetration characteristics of all-trans-retinol (ROL), all-trans-retinoic acid (RA), all-trans-retinaldehyde (RAL), and retinyl palmitate (ROL palm) in human skin in vivo. An enzyme marker was utilized to demonstrate that penetration had occurred and to measure the potency of each retinoid. As the enzyme, cytochrome P-450-dependent RA 4-hydoxylase, is induced by retinoic acid, its induction can identify whether sufficient ROL, RAL, and ROL palm penetration and metabolism to RA occur. Therefore, this enzyme can qualitatively reflect penetration and potency in the epidermis.

Utilizing microsomal preparations from human skin biopsies, a significant induction in this enzyme was noted following topical application to human skin in vivo. After 48 h of occlusion, ROL (0.025% and greater) increased the enzyme activity significantly; however, lower concentrations did not cause significant induction. The increase in enzyme induction was nonlinear, with the higher doses only causing a small increase in activity.

RAL also caused a significant induction of enzyme activity after 48 h of occlusive application. Similarly to ROL, induction was seen at concentrations greater than 0.025%, but not lower. Enzyme activity increased in a dose-related manner, with similar peak activity to equivalent concentrations of ROL. At lower doses (0.01% and 0.025%), RAL was a greater inducer than ROL, but at higher concentrations (0.05%, 0.25%, 0.5%, 1%), ROL and RAL were equally effective inducers.

RA itself was a more potent inducer of the hydroxylase enzyme than ROL and RAL. Induction was seen in RA after 24 h of occlusion, compared to 48 h for ROL and RAL, and the degree of induction was much greater.

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ROL palm applied under occlusion also induced enzyme activity. 0.6% ROL palm significantly induced the enzyme, while lower concentrations, vehicle, or equivalent concentrations of palmitate alone did not. However, ROL palm was applied for 4 days, in contrast to the 24- and 48-h studies outlined above (Table 4).

The effect of occlusion on the ability of ROL, RA, and ROL palm was also assessed. While unoccluded RA significantly induced RA-hydroxylase, a significantly greater induction occurred under occlusion. A similar effect was seen for ROL palm. However, this occlusive effect was not seen with ROL: both occluded and unoccluded sites produced a similar significant increase in enzyme induction compared to vehicle. Enzyme activity induced by 0.25% ROL (either unoccluded or occluded) was similar to that induced by 0.025% RA (under occlusion).

Whether the induction of this or other enzyme markers in the skin reflects the ability of retinoids to produce a pharmacological effect is not clear. However, cosmetic-type preparations mandate sufficient retinoid concentrations to allow adequate penetration for a pharmacological effect. As a threshold level could be identified for enzyme induction in the above study, there may also be a threshold for a pharmacological effect. An insufficient concentration in the cosmetic, or inadequate application by the consumer, may render the formulation relatively ineffective.

Cellular Uptake of Retinol

In addition to sufficient delivery of the retinoid to the skin, the retinoid should be delivered in the correct form to allow cellular uptake and metabolism. Retinoids occur in human plasma bound to proteins: retinoic acid is bound to albumin and ROL to retinol-binding protein (RBP) (14). Therefore, the possibility that

Table 4 Assaying Retinoid Effects Utilizing Cutaneous Markers

Compound	Marker	Time to induction (under occlusion)	Minimum inducing concentration (%)	Does occlusion enhance induction?
ROL	cP450-OHase	48 h	0.025	No
RA	cP450-OHase	24 h	0.001	Yes
ROL palm	cP450-OHase	4 days	0.6	Yes
RAL	cP450-OHase	48 h	0.025	N/A

Source: Ref. 13.

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protein binding can determine the ability of a cell to take up retinoids has been considered. (The influence of protein binding on metabolism is discussed later.)

Hodam and Creek (15) studied the uptake of retinol, either free (in ethanol) or bound to RBP, in cultured human keratinocytes. Utilizing radiolabeled compounds, they demonstrated that the retinol uptake was much greater in the free than in the bound form. Free retinol added to the culture medium had a maximum uptake of 35% of the applied dose within 3 h of incubation, falling to 20% by 12 h. In contrast, RBP retinol had a peak uptake of 7.5% of the applied dose, detected at 24 h. Therefore, keratinocytes demonstrated a much slower uptake of RBP retinol compared to free retinol.

Cutaneous Metabolism

The metabolism of vitamin A and its derivatives in the skin is considered important to the understanding of their pharmacological effect. It has been hypothesized that the effects of ROL and RAL may result from their cutaneous metabolism to RA. While some investigators have shown that this metabolism may occur, pharmacological effects have also been seen in the absence of measurable RA. This section discusses the evidence that RA is an essential metabolite in the activity of ROL and RAL.

In vitro, metabolism of ROL, RAL, and RA was studied utilizing human skin and dermal fibroblasts (16). Radiolabeled ROL, RAL, and RA were applied either topically to the skin biopsies or to the culture media of the fibroblast suspensions and the metabolites were identified by HPLC after 24 h of incubation. The skin cultures demonstrated a gradient distribution of the retinoids within the skin: 75% of absorbed activity was in the epidermis, 20% in the dermis, and 2 to 6% in the culture medium for the three retinoids tested. Of the epidermal extracts, 60% of applied ROL remained unmetabolized. The main ROL metabolites in the epidermis were retinyl esters (18.5%), a finding that has also been demonstrated in keratinocyte cultures. RA (2%), RAL (1.6%), 13-*cis*-retinoic acid (1%), and polar compounds were also found. The dermis yielded similar metabolites, but a higher proportion of polar compounds.

RAL was also metabolized in the epidermis: 43% of the absorbed radioactivity was RAL, 9% retinyl esters, 14% ROL, and 0.8% RA. When RA itself was applied, 66% of the epidermal radioactivity was from RA, 17% from 13-*cis*-RA, and 10% from polar compounds. RA was not metabolized to ROL or RAL. Dermal fibroblasts also metabolized ROL, RAL, and RA in culture medium, but the significance of this in vivo is not yet clear. It is possible that these cells may contribute to the role of the dermis in the kinetics and dynamics of these substances.

These skin studies demonstrate the capacity for topical ROL, RAL, and RA to penetrate the skin in a gradient manner from the epidermis to the dermis.

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The activity in the epidermis was five times greater than in the dermis, suggesting an accumulation of compounds in that layer. While a proportion of the absorbed compound remains unchanged within the skin, significant metabolism was seen. ROL and RAL were metabolized to RA, which may play a role in the pharmacology of these substances.

Randolph and Simon (17) utilized ROL and RA bound to their endogenous binding proteins in their in vitro study: retinol was bound to retinol-binding protein and retinoic acid was bound to albumin, as has been found in human plasma (14,17a). Dermal fibroblasts, cultured either in collagen gel or on plastic dishes, were exposed to radiolabeled ROL or RA, and metabolites were detected by HPLC. In contrast to Bailey et al. (16), ROL was not metabolized by the dermal fibroblasts, although their findings for RA metabolism did concur. This may have been because of decreased availability of ROL to the dermal fibroblasts because of its protein binding. It was therefore suggested that the metabolism of ROL might only occur under pharmacological conditions. Supporting this explanation are the findings of Hodam and Creek (15) described previously, which demonstrate decreased uptake of RBP retinol compared to free ROL in cultured keratinocytes. However, the role of protein binding in uptake of ROL in dermal fibroblasts requires further elucidation.

As certain cell types preferentially metabolize different forms of retinoids, the cell content of a tissue may influence the availability of retinol and its metabolites to the surrounding tissue. The significance of this finding in cosmetic use is not yet clear. Hodam and Creek (15), in addition to determining the effect of protein binding on cellular uptake of retinoids, also considered whether protein binding affected the cellular metabolism of the retinoids once intracellular. In both cases, retinyl esters were the major metabolite and the percentage of ROL cell-associated radioactivity that was converted to retinyl esters was independent of the mode of delivery.

Several studies have therefore demonstrated a metabolic capacity for topical ROL and RAL. Retinyl esters appear to be the major metabolite, while the formation of RA from these substances constitutes a small proportion of the metabolites formed. However, this conversion may be sufficient for pharmacological activity. In vivo studies may better quantify both metabolism and dose-response relationships.

Pharmacological Effects of Retinol In Vitro and In Vivo

In vitro and in vivo studies of retinol and its derivatives have demonstrated several pharmacological effects on the skin. However, whether these effects are caused by RA as a derivative of ROL or RAL applied to the skin is not clear. The evidence is discussed below.

Kang et al. (12) found that epidermal changes could be demonstrated in vivo following topical application of ROL, without measurable retinoic acid lev-

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els. This suggests that ROL itself is active in the skin. Following 4 days of occlusive application of ROL, epidermal thickness increased significantly compared to vehicle control. This increase was dose dependent: a significant increase was seen with 0.05% ROL, and the maximum concentration used (1.6% ROL) caused an increase similar in magnitude to 0.025% RA applied over the same period. Further evidence of the pharmacological activity of ROL in the epidermis was an increase in the number of mitotic figures and in epidermal spongiosis (ranked on an ordinal scale).

Interestingly, the authors were not able to detect RA, or found only trace amounts, in the time points tested (0 to 96 h). Reverse-phase high-pressure liquid chromatography (HPLC) yielded ROL, 13-*cis*-ROL, and retinyl esters (RE) in the samples, which had been tape stripped to remove the stratum corneum prior to biopsy. These results differ from those presented above where RA was found in vitro utilizing human skin. Nevertheless, cellular retinoic acid binding protein (CRABP-II) mRNA was increased, indicating CRABP-II gene activation, which supports the idea of ROL conversion to RA. The same laboratory also demonstrated an increase in a retinoic-specific hydroxylase enzyme in vivo in a previous study (REFS). However, it is possible that ROL may indirectly mediate CRABP-II gene expression by an unidentified mechanism, other than conversion to RA.

Goffin et al. (18) compared a retinol cream to a vitamin E preparation on humans in vivo utilizing bioengineering methods. In this crossover study, subjects were exposed to environmental insults, such as ultraviolet (UV) irradiation and a topical surfactant (sodium lauryl sulfate), and assessed utilizing squamometry, corneosurfametry, and optical profilometry. The authors suggest that the retinol preparation may provide some beneficial effects against these insults and also reduce the trend in shallow wrinkling induced by the irradiation. However, these data are difficult to interpret because of the crossover study design, and also because the retinol preparation was a complex cosmetic formulation. Therefore, the effects seen cannot be attributed to the effect of retinol alone. Additionally, no vehicle control was utilized.

TRETINOIN THERAPY IN PHOTOAGING

Chronic exposure to sunlight causes a characteristic collection of signs presumed to be due to aging in the past, but are now recognized primarily as the consequences of solar and other environmental injury. This is termed photoaging or dermatoheliosis. The familiar stigmata of photoaged skin are rough, leathery skin with coarse wrinkles and yellow or mottled complexion. Histologically, the dermis exhibits changes known as solar elastosis; the collagenous connective tissue in the upper dermis is replaced by fragmented, disorganized elastic fibers (19).

Ultraviolet radiation stimulates collagenases (UV-responsive matrix metalloproteinases), thereby enhancing collagen degradation and resulting in this deficiency of dermal collagen (20). Irregular epidermal thickening is seen in photoaged skin, sometimes accompanied by irregularities in cell and nuclear size, shape, and staining reactions. Melanocytic hyperplasia is a frequent feature in chronically sun-exposed skin, seen diffusely as a background of increased pigmentation, or focally as ''senile lentigines'' (21). A telangiectatic network is often seen in photodamaged skin as the disorganized dermis fails to support vessel walls, allowing them to dilate passively (22).

Topical tretinoin (all-trans-retinoic acid), used for the past two decades as antiacne therapy, has also been found effective in the treatment of photoaging. Its role in photoaging was first described and subsequently popularized by Kligman (23). He observed that women treated with tretinoin described smoother skin with less wrinkles. This clinical observation prompted him to perform clinical trials comparing the effects of tretinoin on photoaging to an inert cream. In the first of these studies, 0.05% tretinoin in a cream base was applied twice daily for 3 months on dorsal forearms of elderly volunteers, and the results compared with similar application of an inert cream to the opposite forearms. Punch biopsy specimens, taken before and after treatment, were examined using light and electron microscopy. Skin bioengineering data were also obtained. In the second study, 0.05% tretinoin cream was applied to photodamaged facial skin and specimens obtained and analyzed in a similar fashion. A third, uncontrolled study consisted of long-term facial application of 0.05% or 0.1% tretinoin cream in over 400 healthy females. The studies demonstrated significant beneficial effects on photodamaged skin, including reversal of epidermal atropy, dysplasia, and atypia, eradication of microscopic actinic keratoses, uniform dispersion of melanin granules, new collagen formation in the papillary dermis, and angiogenesis (8). Kligman reinforced this work with animal studies using the photodamaged hairless mouse model (23).

These results were consolidated by Weiss et al. (24), who similarly demonstrated in a 4-month randomized, blinded, vehicle-controlled study that 0.1% tretinoin improved photodamaged skin, both histologically and ultrastructurally. Volunteers in the tretinoin-treated group showed significant reduction in lentigines, epidermal thickening, compaction of the stratum corneum with presence of glycosaminoglycan-like substance, increased mitoses in keratinocytes, and increased number 3 of anchoring fibrils at the dermoepidermal junction. Ellis and Weiss (9) then extended the tretinoin therapy in an open-label trial, utilizing the same subjects for up to 22 months, indicating that clinical improvement was sustained during long-term tretinoin therapy. They found that 71% of discrete lentigines had disappeared after this prolonged period. Further, the problems of dryness, erythema, and flaking of the skin associated with retinoid use had

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diminished or declined after the 22-month period, with maintenance of clinical benefit.

The findings in these earlier studies have now been reinforced by a solid background of formal clinical trials (25–27). [Tretinoin reverses photoaging by epidermal and dermal effects. The epidermal effects include epidermal thinning, reduction in corneocyte adhesion, decreased melanin production, and increased Langerhans cells. The dermal effects include increased collagen production, increased angiogenesis, and decreased collagenase and glycosaminoglycans (24).]

More recently, the emphasis on research in tretinoin has branched out, for instance, fine-tuning the optimum conditions for tretinoin therapy and new uses. In a recent double-blinded, vehicle-controlled comparison of 0.1% and 0.025% tretinoin creams in patients with photoaged skin, tretinoin 0.025% showed similar efficacy to 0.1%, while showing significantly less irritation.

Having more than proved its efficacy in the reversal of photoaging, the logical question is: Can retinoid therapy also improve intrinsically aged skin? The answer to this may be on the horizon. Varani et al. (28) completed an in vitro study utilizing cell culture techniques to investigate the effects of tretinoin on skin. Retinoic acid stimulated growth of keratinocytes and fibroblasts and stimulated extracellular matrix production by fibroblasts. Adult skin from sun-exposed and sun-protected sites responded equally well, whereas neonatal skin responded minimally. The implications are that retinoids may be able to repair intrinsically aged skin as well as photoaged skin, and that retinoids may modulate skin cell function in a manner that is age-related, not simply a response to photodamage.

TOXICITY

The adverse effects of retinoids are legion, and are mostly associated with hypervitaminosis A (acute or chronic). Fetal malformations, spontaneous abortions, hyperlipidemia (particularly elevated triglycerides), bone abnormalities, skin and mucosal dryness, retinoid dermatitis, pruritus, hair loss, pseudotumor cerebri, arthralgias, myalgias, and abnormal liver function tests (increased liver transaminases and alkaline phosphatase) are among the myriad potential adverse effects of retinoid therapy (29). Most of the above effects are reversible upon discontinuation of the retinoid, although some serious effects, such as fetal malformations and bone abnormalities, are not. We do not have sufficient case population data to be certain of cause and effect and no true double-blind studies exist. Recently, two classes of nuclear receptors, the RARs (retinoic acid receptors) and the RXRs (retinoid x receptors) have been identified, which are thought to play an important

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role in mediating retinoid-induced toxicity. The details of this mechanism are beyond the scope of this chapter and the reader is directed toward a recent review for elucidation (30).

Topical application has the benefit of a significantly better adverse effect profile. The most common sequelae are mucocutaneous effects, characterized by skin and mucosal dryness (xerosis, cheilitis, conjunctivitis), desquamation, erythema, and pruritus. These effects typically start after several days of therapy, peak within the first few weeks, then wane as tolerance develops (31). They are easily treatable—frequent application of emollients and other precautionary measures (such as avoidance of harsh soaps, astringents, abrasives, and excessive bathing) will ameliorate the situation. The mucocutaneous effects are dose dependent and reversible upon discontinuation of the retinoid.

Teratogenicity, well documented as the most serious side effect of oral retinoids (32), is logically the potential concern with topical retinoids. With oral retinoids, most aromatic retinoids cross the placenta; in utero exposure results in limb and craniofacial deformities, as well as cardiovascular and central nervous system abnormalities. Systemic absorption of topical retinoids, however, is thought to be negligible (33). A large retrospective study of birth defects in offspring born to mothers exposed to topical tretinoin (all-trans-retinoic acid) during pregnancy has demonstrated no significant risk (34). Animal studies by Willhite et al. (35) support these data, suggesting that the drug would not be expected to cross the placenta unless present at extremely high concentrations. Even in light of this evidence, many clinicians feel strongly about avoiding topical retinoids in pregnancy (36).

Reports of enhanced photocarcinogenicity in experimental mice exist (37), but no evidence exists of a comparable process with humans (38). Conversely, topical retinoids appear to have a protective effect against ultraviolet-induced premalignant and malignant lesions. However, skin treated with topical retinoids is more reactive to chemical and physical stresses (including ultraviolet light), because of the thinner horny layer and amplified vasculature. The concomitant use of sunscreens is therefore a necessary precaution.

THE FUTURE

Retinoids have revolutionized dermatological and cosmeceutical therapeutics for the past 2 decades. The successful trials of topical tretinoin have inspired the pursuit of other topical retinoids that could be effective in photoaging with fewer adverse effects. Undoubtedly, newer derivatives with safer adverse effect profiles will be forthcoming. Specifically, two new retinoids, adapalene and tazarotene,

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licensed for the treatment of acne and psoriasis, respectively, will almost certainly be investigated for photodamage.

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10
Depigmentation Agents

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INTRODUCTION

There are a variety of facial pigmentary disorders, as listed in Table 1. Among such diseases, malignant tumors should be diagnosed and treated properly because some of them are quick to develop, destructive, or fatal. Hyperpigmentation of the face of middle-aged women, is most common; however, it is benign, and, if diagnosed and treated early, it can be prevented in the future.

Melasma is commonly observed among middle-aged women (average age of 43) (1) and is rare in men. It is a diffuse or well-circumscribed noninflammatory brown hyperpigmentation that frequently occurs around the eyes, mouth, cheeks, and forehead.

Subjective symptoms such as itching or irritation are lacking (2). Melasma is present in middle age, but is rare in women over the age of 70. An experienced old Japanese dermatologist in Kyoto City often told melasma patients, ''You need not treat melasma. Just live until the age of 70 and then the melasma you suffer from will disappear.''

The main cause of melasma is considered to be an increase in progesterone (P4) in the serum at luteal phases. Sato (1) measured various hormones by tritium (3H) radioimmunoassay in two groups of age-matched middle-aged women (average age 43) with and without melasma on the seventh days of the ovarial and

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Table 1 Pigmentary Skin Disorders of the Face

I. Acquired
1. Melasma (chloasma)
2. Solar lentigo
3. Pigmented cosmetic dermatitis
4. Sun tanning
5. Tattoo
6. Ochronosis
7. Pigmentation due to atopic dermatitis
8. Phototoxic hyperpigmentation (Berloque dermatitis)
9. Posttraumatic hyperpigmentation
10. Others (lichen planus cum pigmentatione, pigmentsyphilis, etc.)

II. Hereditary
1. Nevus pigmentosus
2. Nevus spilus
3. Nevus of Ota
4. Ephelid

III. Skin Tumors
1. Melanoma
2. Basal cell carcinoma/epithelioma
3. Spitz nevus
4. Solar keratosis
5. Bowen's disease
6. Blue nevus
7. Others

luteal phases. Significant differences were only present in the increased levels of progesterone (P4) and 17OH progesterone in the plasma in the luteal phases of melasma patients as compared to the age-matched female controls without melasma (Fig. 1). Other hormones, such as estradiol, follicle stimulating hormone, luteinizing hormone, prolactin, androstendione, and cortisol (Fig. 2), showed no differences between groups during the ovarial and luteal phases. The increase in plasma progesterone may be attributed to the fact that melasma is exacerbated by pregnancy where plasma progesterone is increased or by contraceptive pills that occasionally contained progesterone; there is gradual decline of melasma after climacterium by 70 years of age.

Histopathology of melasma shows an increase in melanin pigments in the epidermal cells especially in the supranuclear region of the basal cells (Fig. 3). The number of epidermal melanocytes has not increased and, therefore, the hyperpigmentation of melasma is considered to be functional and reversible. Two links, however, are still missing: the connection to the increase in serum progesterone

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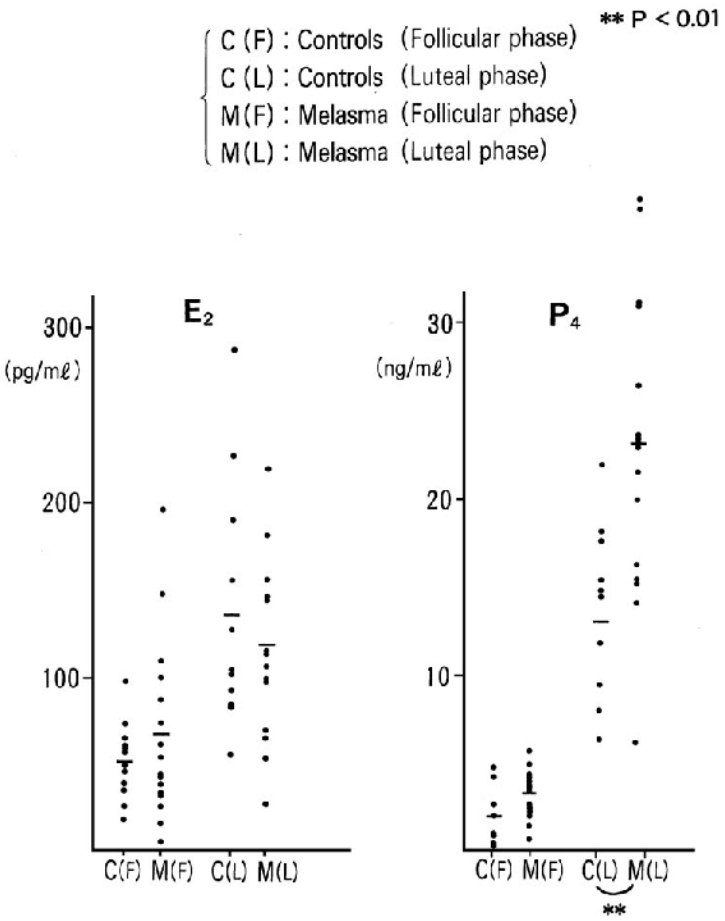

Figure 1 Serum progesterone (P4) and estradiol (E2) levels of melasma patients and matched controls in follicular and luteal phases.

since the intracellular receptor in the melanocytes is not known, and the photosensitivity of melasma patients has not been clarified.

When minimum erythema dosis (MED) was measured in melasma patients, 18 (24.7%) of the 73 melasma patients showed clear photohypersensitivity by lowered MED and minimum pigmentation dosis (MPD) to UVA and UVB. Further study showed that reactivity to UVA was normal but hypersensitivity to UVB was remarkable in all 15 patients. With such photohypersensitive melasma patients, MED was lowered to approximately one-third of normal persons in sum-

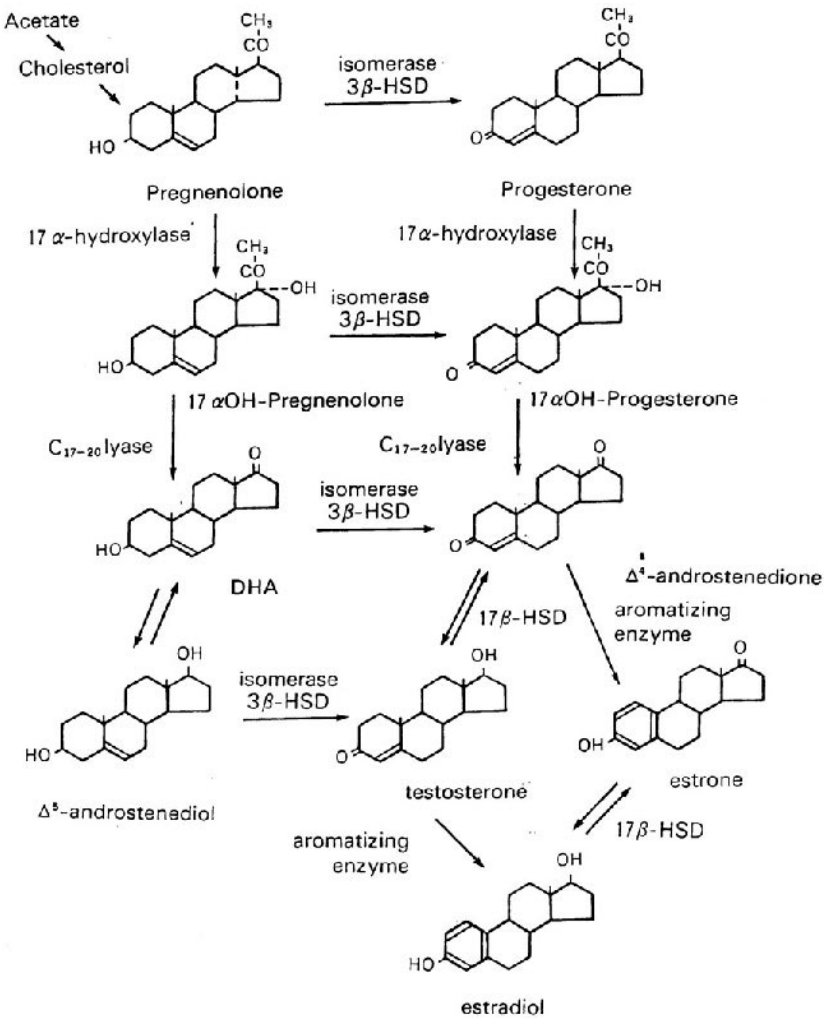

Figure 2 Biosynthesis of steroid hormones.

mer, and a palpable erythema was observed above 2 MEDs of UV-B which produced long-lasting hyperpigmentation for weeks. Therefore, 2 MEDs were almost equal to 1 minimum quaddel dosis (MQD) and to 1 MPD (Table 2; Fig. 4). All these patients did not have any medication when MED was measured, uro- and copro porphyrin levels were normal in urine, and the effect of common photoallergens such as musk ambrette or thiazides was denied. Plasma 17OH progester-

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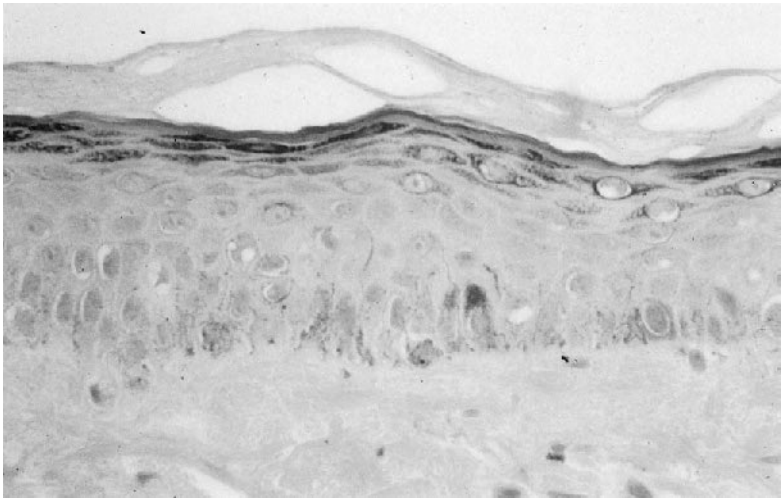

Figure 3 Histopathology of melasma shows increased melanin pigment at the basal layer and the lower part of prickle layer of the epidermis.

one levels were elevated only in one case, but nine other cases showed normal results when these photohypersensitive cases were again examined. Therefore, the mechanism of UVB photohypersensitivity in melasma should be investigated in the future.

Melasma has been regarded as an excellent target for newly developed depigmentation agents because many middle-aged melasma patients want to return their skin color to normal. Long-term therapy is necessary so that depigmentation occurs slowly, without provoking severe depigmentation (as with hydroquinone monobenzyl ether) or severe hyperpigmentation of ochronosis (as with hydroquinone at 2 ~ 4% concentrations under a tropical climate) (3). Historically, both disorders had been reported (4) and, therefore, both are disastrous pitfalls for those developing depigmentation agents.

First, unlike hydroquinone monobenzyl ether, the depigmentation agents under development should not kill melanocytes. Second, hydroquinone itself is not cytotoxic to melanocytes; however, it degenerates dermal elastic fibers under strong sunlight at high concentrations of 2–4%, which results in another disastrous strong brown hyperpigmentation called ochronosis (5). Therefore, the best depigmentation agent inhibit tyrosinase in melanocytes, and toxicity to epidermal cells, melanocytes, dermis, and other systemic organs is negligible. Also, depigmentation agents should not be strong sensitizers, oncogenic, or teratogenic. They should be stable chemically at least for more than a year.

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Table 2 MED and MPD with Melasma Patients (1983 ~ 1987)

Apparatus: NS-9[a]
Results: 1 MPD ≒ 1 MQD ≒ 2 MED (general rule)

1. MED

	Shortened	Normal	Total
Spring	9	13	22
Summer	18	55	73
Autumn	0	3	3
Winter	1	10	11

2. MPD

	Shortened	Normal	Total
Spring	10	12	22
Summer	23	49	72
Autumn	0	3	3
Winter	4	7	11

[a] Light sources: FL-20 BA-37, 20W × 2 (UV-A)
FL-20 E , 20W × 2 (UV-B)
Tube-skin distance: 10 cm
Automatic irradiation time: 10–90 s, at 10-s intervals
Performance
1. Normal individuals
MED: 70 ~ 90 s (Spring–Summer)
MQD, MPD: more than 90 s.
2. Photodermatitis patients
MED: shortened to 10–60 s
MQD, MPD: delayed erythema, etc., is detectable.
NS-9 is a modified version of the previous type NCA-6, added with an inverter to shorten the irradiation time for MED.

Hydroquinone cream changes color from white to brown after 3–4 months; therefore, it can be produced at pharmacies and hospitals on the condition that it is disposed of after the color changes. Therefore, it cannot be used in cosmetics or cosmeceuticals. Hydroquinone cream is an excellent preparation for the treatment of melasma with or without mild chemical peeling (6,7). However, the color change and the production of ochronosis have inhibited its usage in cosmetics and cosmeceuticals.

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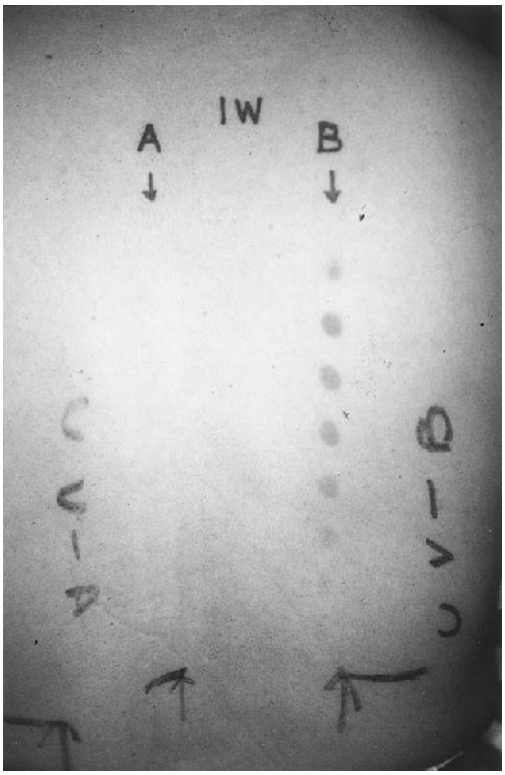

Figure 4 The results of MED and MPD (Table 2) showed that UVB hyperpigmentation was demonstrated at 1 week of UV irradiation. Note that no reaction occurred under UVA irradiation, even though the doses were the same.

SCREENING TESTS FOR DEPIGMENTATION AGENTS

A standard method for screening depigmentation agents is the isolated tyrosinase inhibition test. Mushroom tyrosinase has been commonly used, and the suppression of tyrosinase could be demonstrated when dose-dependent inhibition was demonstrated with hydroquinone as an effective control. Another kind of tyrosinase assay is noninhibitory or nonsuppressive-type reactions of melanogenesis. According to Mishima (8), melanogenesis can also be hindered by tyrosinase production inhibition, inhibition of tyrosinase transfer, and cytotoxic inhibition (Table 3). Cultured B-16 melanoma cells have been used in this field and are useful in demonstrating several new mechanisms of melanogenesis inhibition: glycosylation turned out to be another process of the production, along with matu-

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Table 3 Mechanism of Melanogenesis Inhibition

Mechanisms	Example
1. Suppression of tyrosinase	Kojic acid Hydroquinone Ascorbic acid Arbutin Ellagic acid
2. Other mechanisms	
a. Decrease in tyrosinase synthesis	Biomein®
b. Decrease in tyrosinase transfer	Glucosamine Tunicamycin
c. Cytotoxicity to melanocytes	Hydroquinone monobenzylether APTA[a]

[a] n-2,4-Acetoxyphenyl thioethyl acetamide.

ration of melanogenesis. Its inhibition also decreased the amount of melanin, and depigmentation agents were also found. Tyrosinase activities in ribosomes and the production of premelanosomes can also be targets for melanin production inhibition (8). There are two melanins, eumelanin (black ~ brown) and pheomelanin (yellow or red), and eumelanin production inhibition is usually considered with depigmentation agents.

Dose-dependent reactions are requested for depigmenting agents in in vitro tests, like tyrosinase inhibition or B-16 melanoma cell assay. This is needed because melanogenesis inhibition increases in parallel with the concentration of the depigmentation agents in the medium. When some chemical is added to the medium and the inhibition of melanogenesis disappears, it means that the added substance (Fig. 5) could successfully block the active site of metabolism, and thus the mechanism of this depigmentation agent becomes quite clear.

An example is shown in Figure 6, where we can see that a dose-dependent melanogenesis inhibition of kojic acid was completely blocked when cupric acetate was added to the medium. These results showed that the main mechanism of kojic acid was to chelate copper ions that are indispensable for tyrosinase so that a remarkable decrease of its activity was seen by the addition of cupric acetate.

Streptomyces fervens produces melanin when it is cultured in a liquid medium, and the melanin synthesis can be inhibited by the presence of depigmentation agents. An example that also shows the dose-dependent effect of kojic acid can be seen in Figure 7. The important fact is that streptomyces was alive in all the culture medium, even though black eumelanin was not produced or decreased in production after kojic acid was added in various concentrations: when streptomyces was transferred to another culture medium without kojic acid, it produced

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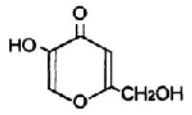

Molecular weight : 142.11

5-Hydroxy-2-(hydroxymethyl)-4-Pyrone

Procedure

glucose

← *Aspergillus albus*

crude KA — 30~32℃, aerobic conditions

↓ — Culture for 5 or 6 days

filtration

↓

charcoal treatment

↓

concentration

↓

recrystallization by water

↓

pure Kojic acid

[only one peak
with gas chromatography]

Figure 5 Kojic acid (KA).

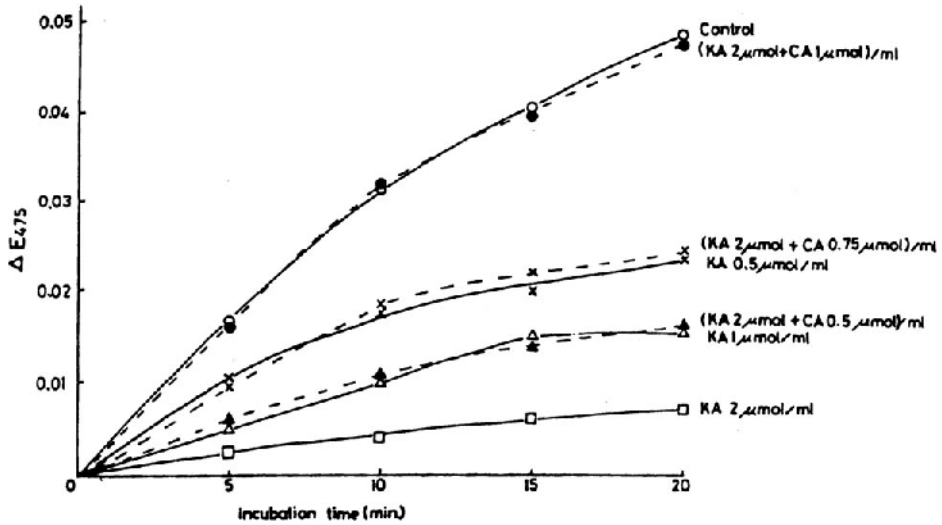

Figure 6 Suppression of melanogenesis. Reduction of tyrosinase (gold fish) inhibitory effect of kojic acid after pretreatment with cupric acetate. KA: Kojic acid; CA: cupric acid.

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Figure 7 Streptomyces fervens produces melanin, and its melanin synthesis was inhibited by kojic acid at dose response, when the concentration of kojic acid increased from left to right. The left tube shows the color without kojic acid. Streptomyces was alive, even though melanin synthesis was inhibited.

Table 4 In Vitro and Animal Assays for Depigmentation Agents

Assays with which melanogenesis inhibition was confirmed	Depigmentation agents
1. Tyrosinase inhibition test 2. Melanin reduction of B-16 melanoma cells	Kojic acid Hydroquinone Arbutin Ellagic acid 4n-butylresorcinol Ascorbic acid Liquiritin
3. Reduction of melanin pigments of *Streptomyces ferbens*	Kojic acid Hydroquinone
4. Reduction of melanin pigments of black goldfish	Ascorbic acid Kojic acid (Fig. 3)
5. Reduction of melanin pigments of pigmented mammals (C57 black mouse, Yucatan pig, etc.)	APTA[a] (topical application or intraperitoneal injection)

[a] n-2,4-Acetoxyphenyl thioethyl acetamide.

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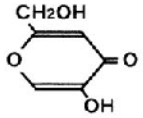
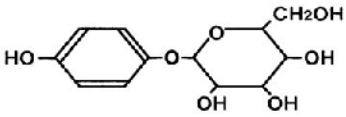
Table 5 Chemical Structures of Main Depigmentation Agents

1. Hydroquinone

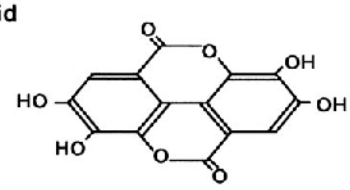
2. Kojic acid



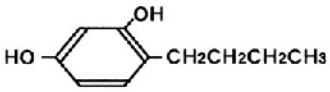
3. Arbutin



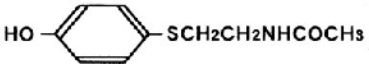
4. Ellagic acid



5. Rucinol
(4-n-Butylresorcinol)



6. N-Ac-4-S CAP
(N-2,4-acetoxyphenyl thioethyl acetamid)



melanin, turning the color of the medium to black again. Various assays to detect depigmentation agents (9–12) are listed in Table 4, and the chemical structures of main depigmentation agents are shown in Table 5.

Cultured B-16 melanoma cells are also excellent material for visually confirming the melanogenesis inhibition in vitro. A recommended method is to culture B-16 cells in Eagle's MEM with 10% fetal bovine serum, and depigmentation agents are added in the culture medium at different concentrations. After 5 days of the culture, the cells are fixed by formalin and stained by ammonical silver nitrate, then premelanosome can be visually stained in black. When the

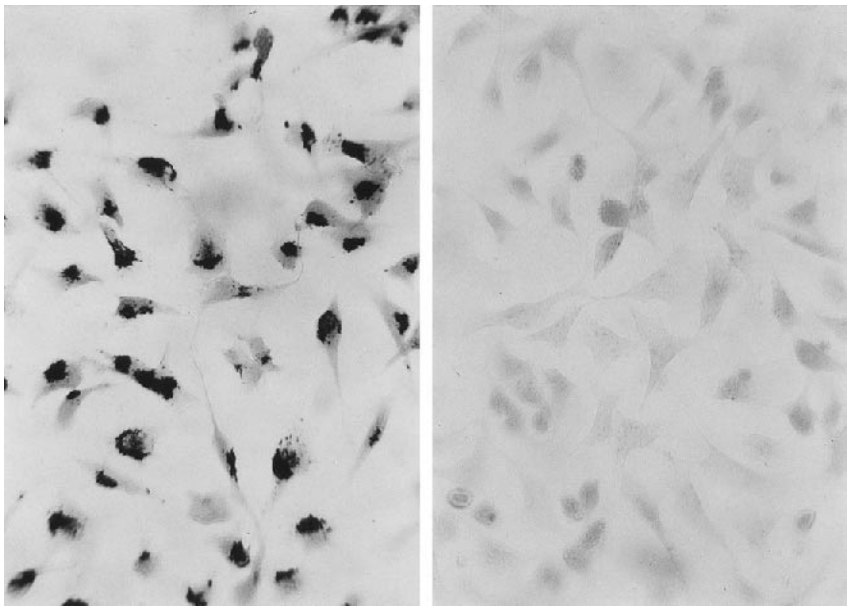


Figure 8 Assay of melanogenesis inhibition using B-16 melanoma cells. Right side shows inhibition effect of kojic acid put into the culture medium at 2.5 mM concentration. Premelanosome reaction is negative. Left side shows control without kojic acid, and premelanosome is clearly seen.

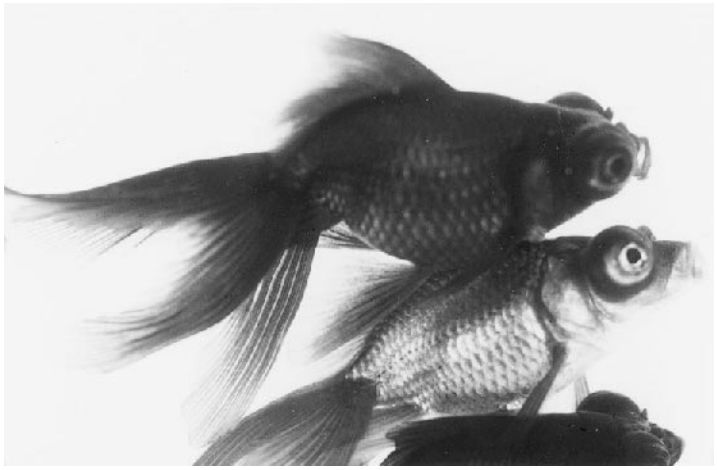


Figure 9 Black gold fish (upper and bottom as controls) changed color form black to brown, when kojic acid was added in the water at 0.25% for a month (middle fish).

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cells are alive, and such premelanosome stain is negative with the presence of depigmentation agents, melanogenesis is recognized as having been successfully inhibited (Fig. 8).

More dramatic effects of melanogenesis inhibition can be recognized when a depigmentation agent is added to the water in which black goldfish are kept. The addition of kojic acid required a month or two for the black goldfish to turn to yellowish brown; since they were alive and vivid, this demonstrated that only melanogenesis was inhibited, not systemic metabolism (Fig. 9).

CLINICAL EVALUATION

Depigmentation agents can be screened in vivo by tyrosinase inhibition tests or various other methods that clearly demonstrate the inhibition of melanogenesis; however, what is most important is that not only they show definite melanogenesis inhibition in vitro, but also they improve the hyperpigmentation of melasma in clinical evaluation. When there is no clinical effect of depigmentation, they are of course useless, even though they showed excellent results in vivo trials. Laser is not effective to melasma, and is very effective to solar lentigo and to nevus of Ota to which depigmentation agents are less effective or ineffective. Therefore, the best target for depigmentation agents is apparently melasma.

First, for that purpose, depigmentation agents should be mixed in vehicles, normally creams or lotions, without any alteration of the color or the effectiveness. They should be put into production without producing impurities. They should pass acute, subacute, and chronic toxicity tests, skin and eye irritation tests, skin sensitization tests (maximization and similar tests), oncogenicity tests (Ames test, micronuclei tests, carcinogenicity tests), teratogenicity tests, and stability tests. These tests are all required to develop new drugs and likewise with depigmentation agents. It is because depigmentation agents require several months to exhibit their effects and consumers may use them for several months or even several years.

Double-blind clinical tests for melasma usually are not appropriate because as it takes more than 3 months for the effect to be recognized. Actually, depigmentation agents like kojic acid, hydroquinone, and arbutin can improve the brown hyperpigmentation of melasma by continual usage for 3–12 months. Theoretically it is possible to give active depigmentation agents to one group while a second group is given a placebo cream for 3 to 12 months (13,14); there should be no significant differences between the backgrounds of the melasma patients as to age, severity, and sun exposure. It is ethically acceptable to use a placebo when another, effective treatment is given. However, when melasma patients are involved in the clinical trial, they have the right to see improvement in a short period of time. Therefore, the long-term use of placebo cream was abandoned

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because it apparently deceived patients who anticipated the effect. Double-blind tests are alright when the test ends in a week or so (as with corticosteroid ointments or antibiotics), especially when some another reliable basic treatment is given or the placebo is a competing drug having a definite effect.

Hydroquinone cream is not suitable as an active placebo because the brown color change after a few months indicate that it is hydroquinone: this is an open test (6), not a blind test.

With cosmeceuticals, double-blind tests have not always been demanded, presumably because they were not as strong as drugs and had mild effects not detectable in a short period. When some medical effects are exhibited after long-time usage, double-blind tests are difficult and, in some instances, not ethical when the patients are to be given a placebo with no effect for months. Therefore, double-blind tests should be introduced with care with cosmeceuticals with mild and slow effects.

The evaluation of the treatment of pigmentary disorders of the face is not easy. With melasma, the brown pigmentation fades so slowly that patients often do not recognize the effects of depigmentation agents after 6 months of continual, twice-a-day application. The best way to evaluate is to take color photographs of the faces of melasma patients from three angles—front, 45° right, and 45° left. When the same camera, flashlight, and color film are used, the effect of depigmentation agents can surely be recognized (7,13,14). First the color of the melasma turns from brown to yellowish brown or normal skin color, and second, the contrast at the border of the melasma becomes obscure. When colorimetry is used, it is possible to recognize the change of tint, but when the place of measurement differs at times of measurement, correct change of color is difficult to be obtained. Mapping the human cheeks and forehead to determine the same spots at each time of measurement is usually difficult.

On the other hand, pattern recognition using color photographs from the same three angles of the face is much easier (13,14). When past color photographs from the same three angles of the patient's face are shown when the patient comes for evaluation, the effect of whitening is easily recognized. At the very least, classification (''cured, almost cured, remarkably effective, effective, slightly effective, no effect, and exacerbation'') is possible. Tables 6 and 7 and Figures 10 and 11 illustrate such evaluations.

Similar evaluation is possible with solar lentigo, ephelid, and pgimented cosmetic dermatitis; however, at the beginning of 21st century, the best treatment for solar lentigo is laser. Solar lentigo is due to the local proliferation of melanocytes; therefore, the destruciton of melanocytes without giving serious damage to epidermal cells is ideal. Fortunately, lasers can do this, and iatrogenic vitiligo is not formed by this treatment.

Pigmented cosmetic dermatitis is sometimes similar to melasma, when reticular hyperpigmentation is lacking and moderate diffuse brown hyperpigmenta-

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Table 6 Effect of 1% Kojic Acid Cream I on Melasma Patients (1982)

Effect	Cases treated	Duration of treatment (months, mean ± SD)	%
Complete cure	0	—	0.0
Remarkably improved	37	13.9 ± 4.3	95.5
Improved	26	9.5 ± 5.5	
No effect	0	—	0.0
Worsened	3	5.3 ± 4.9	4.5
Total	66	11.8 ± 5.4	100.0

Source: Ref. 13.

tion is the main symptom. Biopsy shows basal liquefaction of the epidermis along with incontinentia pigmenti histologica and small amount of cellular infiltration composed of lymphocytes and histiocytes in the upper dermis, not like the basal hyperpigmentation of the basal layer cells of melasma. The most important and essential treatment for pigmented cosmetic dermatitis is not the use of depigmentation agents, but of patch testing of cosmetic series allergens including phenylazo-naphthol, D&C Red 31, D&C Yellow No. 11, benzyl salicylate, jasmin absolute, ylangylang oil, geraniol, sandalwood oil, artificial sandalwood, cinnamic alcohol, fragrance mix 1 and 2, etc. (15). The reading should be performed on the second, third, and seventh day so as not to overlook slow, but strong, allergic reactions. The exclusive use of allergen-free soaps and cosmetics for a year or

Table 7 Effect of 1% Kojic Acid Cream II with an Improved Base Cream on Melasma Patients (1994)

Effect	Cases treated	Duration of treatment (months, mean)	%
Complete cure	0	—	0.0
Remarkably improved	48	11.5	80.9
Improved	58	11.1	
No effect	25	12.1	19.1
Worsened	0	—	0.0
Total	131	11.4	100.0

Side effect: Those who were contact sensitized by having previously used kojic acid cream containing betacyclodextrin also developed erythema and itching by the usage of 1% kojic acid cream II. The rate of the dermatitis was 2 out of 131 patients in the table (1.5%). Those who had not used betacyclodextrin-containing kojic acid cream had not produced contact dermatitis.

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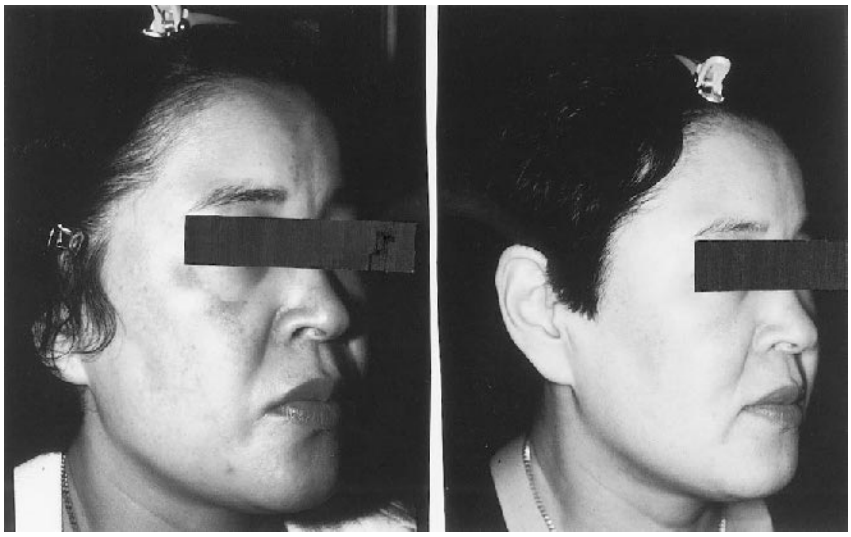
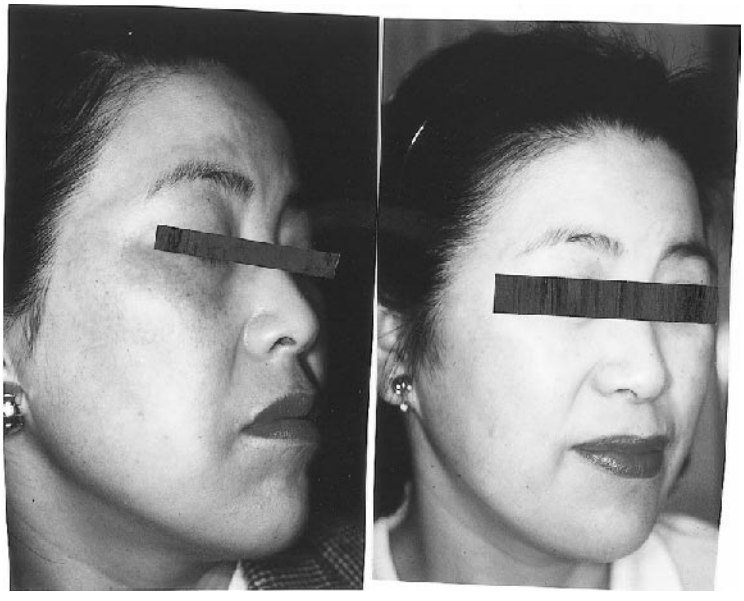


(a) (b)

Figure 10 Hyperpigmentation in a 42-year-old melasma patient (a) decreased remarkably by the application of 1% kojic acid cream twice a day for a year (b).



(a) (b)

Figure 11 Hyperpigmentation in a 48-year-old melasma patient (a) decreased remarkably by the application of 1% kojic acid cream twice a day for one-and-a-half years (b).

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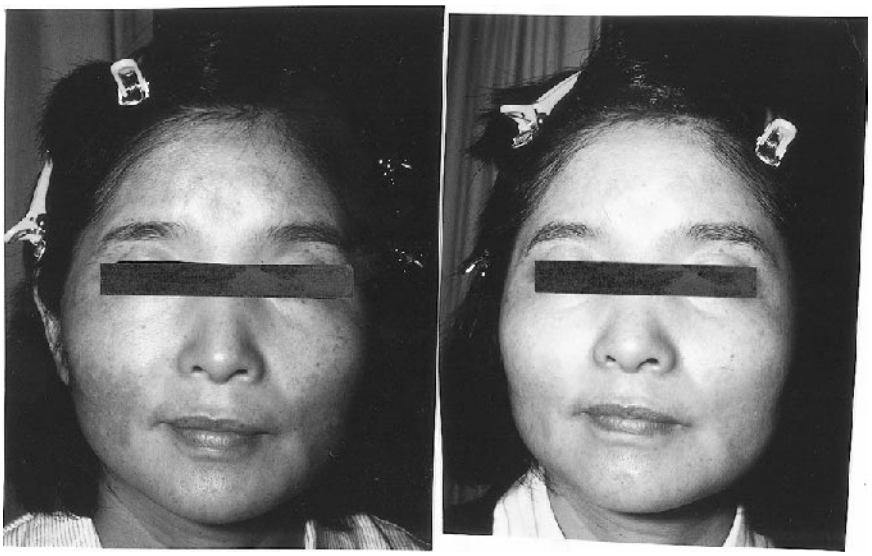
more restores the normal skin color of the patient (15,16). It is impossible to treat the disease with corticosteroid ointments, even though it is a kind of allergic contact dermatitis, because only a small amount of cosmetic allergens invade the skin everyday and these are enough to provoke the disease and maintain the hyperpigmentation. With this disease, allergen control (15) is the treatment of choice; however, the additional use of a depigmentation agent accerelates the cure, presumably because the long-term inflammation at the basal layer of the epidermis enhances the melanin production and increases brown hyperpigmentation. An important fact is that sometimes melasma is complicated by pigmented cosmetic dermatitis (which is shown by biopsy with the presence of incontinentia pigmenti histologica and the inflammatory infiltrates in the upper dermis, by occasional slight erythema with itching on the face, and by positive patch test results



(a) (b)

Figure 12 A 44-year-old female who suffered from melasma of the face complicated by pigmented cosmetic dermatitis (a). In addition to noninflammatory diffuse brown hyperpigmentation, she occasionally produced irregular brown hyperpigmentation of the face. Trepan biopsy showed not only basal hyperpigmentation of the epidermis, but also cellular infiltration composed of lymphocytes and histiocytes in the upper dermis. Patch test revealed that she was strongly sensitized to d-hydroxycitronellal. Allergen control by the exclusive use of allergen-free cosmetics and soaps to avoid contact with d-hydroxycitronellal could remarkably cure the inflammatory hyperpigmentation, and the remaining diffuse pigmentation of melasma was almost cured by the usage of 1% kojic acid cream for 10 months (b).

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of common cosmetic allergens). It is understandable that when melasma patients try to conceal the pigmentation by frequent use of various cosmetics, some of them become sensitized to cosmetic components, which results in the complication of pigmented cosmetic dermatitis. Such a case is shown in Figure 12.

THE CASE OF KOJIC ACID

In 1977, a project was started to find out the cause of melasma and its reliable treatment. From 1970 to 1974, most of the causative contact cosmetic allergens that produce pigmented cosmetic dermatitis had been discovered; by 1977, the disease, which had been incurable prior to 1971, was cured, not by medication but by the exclusive use of allergen-free cosmetics and soaps. This usage of allergen-free cosmetics and soaps was designated as the allergen control system (ACS) (15). The effect of ACS had been so dramatic that a number of melasma patients whose outlook was somewhat similar to pigmented cosmetic dermatitis visited Saiseikai Central Hospital in Tokyo everyday, where ACS was invented and reported on by the mass media.

The introduction of system engineering to develop a subsystem to find the causes and how to eliminate them was key to solving the problem of pigmented cosmetic dermatitis. A new team was formed to solve the problem of melasma adopting a similar system engineering prototype; a team investigated the role of female hormones analyzing the plasma of the both melasma patients and age-matched melasma-free women, at the seventh days of both the ovarial and luteal phases (1). The second team investigated photohypersensitivity by an automatic UVA and B irradiator with melasma patients. The third group started to develop a cream containing a melanogenesis inhibitor, a depigmentation agent. A plan to develop 1% hydroquinone cream was rejected by the Ministry of Health and Welfare (MHW) of Japan because, at that time, the erroneous idea that the serious and persistent leukomelanoderma caused by a depigmentation agent (hydroquinone monobenzyl ether cream banned in 1957) was an effect of hydroquinone released from hydroquinone monobenzylether. Therefore, among the known chemicals that were tyrosinase inhibitors, kojic acid was selected as the new depigmentation agent, because of its extremely long history of safe ingestion. In Japanese, kojic means ferment and had been used to brew Japanese liquor (Sake) made from rice. Pure kojic acid could be produced from glucose by fermentation and various assays to determine the mechanisms of depigmentation along with the necessary safety evaluation tests were performed. The results are shown in Figure 13 and Table 8, showing its confirmed mechanism of action and its safety.

The initial clinical evaluation of kojic acid cream showed that 1% cream was better than 2.3% (saturated) cream, because with the latter, crystallized kojic

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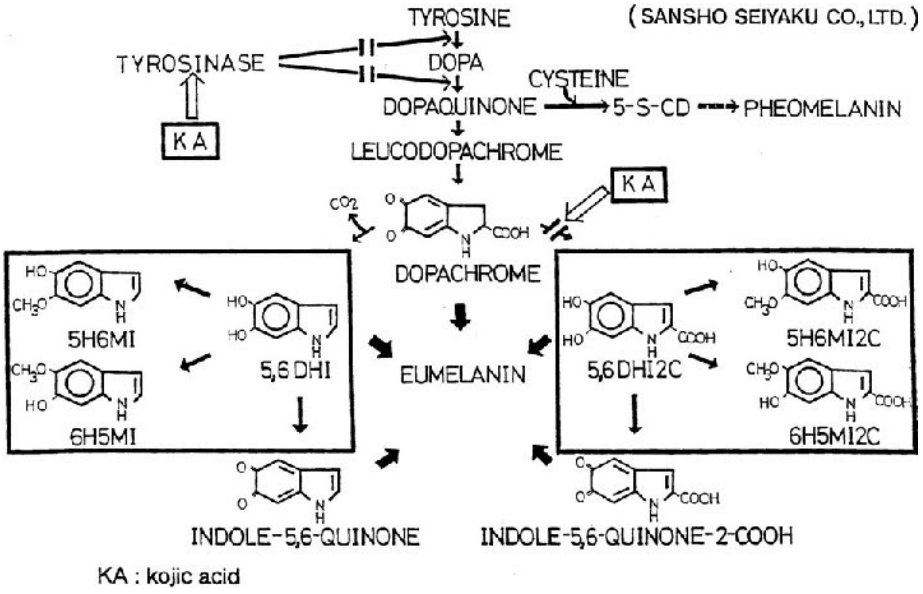

Figure 13 Inhibitory action of kojic acid on melanin polymer formation.

acid gradually appeared and the effect of the improvement was inferior to 1% cream, with which kojic acid melted very well to the vehicle. At this time, the first and the second trials had almost ended, having shown that the cause of melasma was most likely the increase in plasma P4 levels at the luteal phase, and also that 20% of melasma patients were strongly hypersensitive to UVB rays.

Sun protection was introduced to those patients who showed such photohypersensitivity. Some melasma patients were remarkably improved by the continual daily application of 1% kojic acid cream for 6 months, however, after, day of exposure to sunlight (through such activities as golf, fishing, mountaineering, etc.), the melasma reappeared.

The effect of whitening was steady but too slow with this initial 1% preparation of kojic acid. ^{14}C-labeled kojic acid cream was observed to be quickly absorbed from the skin to the liver, intestines, and kidneys in mice. When the absorption was thus quick, the depigmentation agent did not stay at the epidermis where it had its target organ, melanocytes, for a long enough time to inhibit melanogenesis. Therefore, the second preparation conceived was 1% kojic acid cream wherein kojic acid was mixed with betacyclodextrin to slow absorption into the dermis. This successfully sped up the whitening effect; however, contact

Table 8 Toxicity of Kojic Acid (KA)

1. LD_{50}

	Mice	Rats
Subcutaneous	2050∼2080 mg/kg	3010∼3080 mg/kg
Oral	2650∼2920 mg/kg	2260∼3040 mg/kg

2. Chronic toxicity (rats)

Oral, 125, 250, 500, 1000 mg/kg for 26 weeks $\frac{\text{death}}{18}$

3. Teratogenicity

Mice	Rabbits
None	None

4. Mutagenicity

Ames test	(−) up to 1000 μg/plate
Micronuclei test	Negative
Dominant lethal test (mice)	Negative

5. Skin irritation test

Draize method (rabbits) Negative $\left(\frac{0}{6}\right)$
50% KA aq., Patch test for 24 h.

Chronic irritation test (rabbits) Negative $\left(\frac{0}{9}\right)$
Patch test for 6 h ×30 days

Phototoxicity test (guinea pigs): (−)
5.0% KA ethanol + UVA × 5 days

6. Maximization test (guinea pigs): $\frac{0}{10}$

7. Human skin closed patch test for 48 h

3% KA aq.	$\frac{1}{30}$	?(+),	$\frac{29}{30}$	Negative
1% KA aq.	$\frac{2}{30}$	?(+),	$\frac{28}{30}$	Negative

sensitization to kojic acid occurred (17). Betacyclodextrin turned out to be a new adjuvant and, consequently, it was removed; the base cream was improved to delay the absorption without using betacyclodextrin. Contact hypersensitivity to kojic acid is rare today. Effects as in Table 7 have been followed up every year, and 30 cases of melasma who had used 1% kojic acid cream for more than 2 years were examined (CBC, liver function tests, and other systemic abnormalities including carcinogenesis). No abnormal results were demonstrated, except in one person with meningioma, which was considered as coincidental. Such follow-up is always necessary whenever a new drug or cosmeceutical is introduced. Today,

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new depigmentation agents, kojic acid, arbutin, and rucinol, are commercially distributed as cosmeceuticals (the Japanese term is quasidrug). Several others may be introduced in the future (18,19).

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11
Antioxidant Defense Systems in Skin

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INTRODUCTION

As the outermost organ of the body, the skin is frequently and directly exposed to a prooxidative environment, including ultraviolet radiation, drugs, and air pollutants (1). Besides external inducers of oxidative attack, the skin has to cope with endogenous generation of reactive oxygen species (ROS) and other free radicals, which are continuously produced during physiological cellular metabolism. To counteract the harmful effects of ROS, the skin is equipped with antioxidant systems, which maintain an equilibrium between prooxidants and antioxidants.

In the course of skin evolution, a variety of primary (preventive, e.g., vitamin C) and secondary (interceptive, e.g., vitamin E) antioxidant mechanisms have been developed, which form an ''antioxidative network'' of closely interlinked components (Fig. 2). While some antioxidants can be synthesized by humans (e.g., glutathione or ubiquinol-10), others have to be supplied by intake (e.g., antioxidant vitamins C and E, and trace metals). Antioxidants intervene at different levels of oxidative processes: (1) scavenging free radicals; (2) scavenging lipid peroxyl radicals; (3) binding metal ions; or (4) removing oxidatively damaged biomolecules (2). However, the antioxidant defense in cutaneous tissues can be overwhelmed either by an increased exposure to exogenous (e.g., UV exposure) or endogenous (e.g., inflammatory disorders) sources of ROS, or by

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a primarily depleted antioxidant defense (e.g., malnutrition) facing a normal level of prooxidative challenge. Such a disturbance of the prooxidant/antioxidant balance may result in oxidative damage of biomolecules, such as lipids, proteins, and DNA, and has been termed ''oxidative stress'' (3,4). In skin, the induction of oxidative damage by environmental stimuli such as UVA, UVB, and ozone was demonstrated to occur in lipids (5–7), proteins (8), and DNA (9,10).

The chapter summarizes the currently available knowledge on (1) the presence and physiological distribution of natural antioxidants in skin; (2) their response to oxidative environmental stressors; and (3) the photoprotective potential of topically applied antioxidants.

CONSTITUTIVE SKIN ANTIOXIDANTS

Water-Soluble Antioxidants

Ascorbate

Antioxidant Properties Ascorbate, a ketolactone, is also known as vitamin C (see Fig. 1). While most mammals are able to synthesize ascorbate from glucose-derived glucuronic acid, guinea pigs, monkeys, and humans lack the enzyme gulonolactase and therefore require the dietary intake of this vitamin. Dietary ascorbate is absorbed and distributed throughout the body within a few hours. The biochemical importance of vitamin C is primarily based on its reducing potential, as is required in a number of hydroxylation reactions. Several hydroxylases involved in collagen synthesis require ascorbate as a reductant (11). Due to its high reduction potential, ascorbate is an efficient scavenger of superoxide anion radicals, hydroxyl radicals, hypochlorite, singlet oxygen, thiyl radicals, and water-soluble peroxyl radicals (2,12,13). Oxidation of ascorbate results in the formation of dehydroascorbate via the ascorbyl radical, which can be recycled back to ascorbate in the presence of thiols (Fig. 2), or irreversibly decomposes

Figure 1 Chemical Structures of Selected Antioxidants. *1* L-ascorbic acid (176.1 g mol^{-1}, $pKa_1 = 4.2$, $pKa_2 = 11.6$), *2* uric acid (168.1 g mol^{-1}, pKa_1 (37°C) = 5.2 [197]), *3* D-α-lipoic acid (206.3 g mol^{-1}, pKa = 5.4), *4* tocopherols (α: $R_1 = R_2 = CH_3$, 430.7 g mol^{-1}; β: $R_1 = CH_3$, $R_2 = H$, 416.7 g mol^{-1}; γ: $R_1 = H$, $R_2 = CH_3$, 416.7 g mol^{-1}; δ: $R_1 = R_2 = H$, 402.7 g mol^{-1}), *5* tocotrienols (α: $R_1 = R_2 = CH_3$, 424.7 g mol^{-1}; β: $R_1 = CH_3$, $R_2 = H$, 410.6 g mol^{-1}; γ: $R_1 = H$, $R_2 = CH_3$, 410.6 g mol^{-1}; δ: $R_1 = R_2 = H$, 396.6 g mol^{-1}), *6* ubiquinone ($n = 9$: 795.3 g mol^{-1}; $n = 10$: 863.4 g mol^{-1}), *7* ubiquinol ($n = 9$: 797.3 g mol^{-1}; $n = 10$: 865.4 g mol^{-1}), *8* vitamin A precursors (R = H: β-carotene, 536.9 g mol^{-1}; R = OH: cryptoxanthin, 552.9 g mol^{-1}), *9* vitamin A (all-trans-retinol: $R = CH_2$-OH, 286.5 g mol^{-1}; all-trans-retinoic acid: R = COOH, 300.4 g mol^{-1}).

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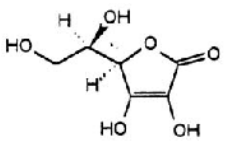

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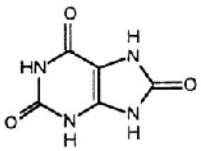

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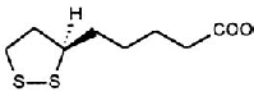

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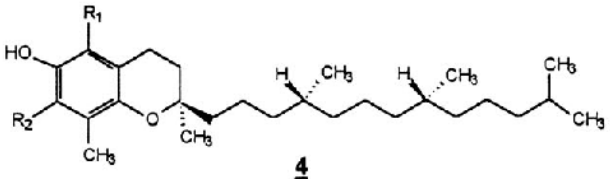

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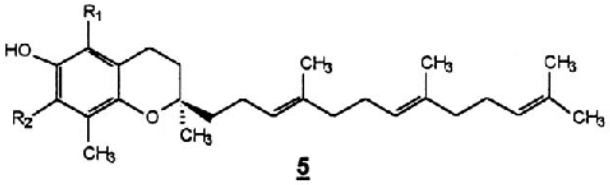

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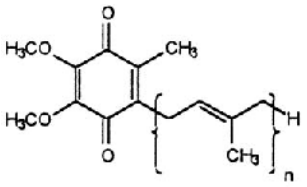

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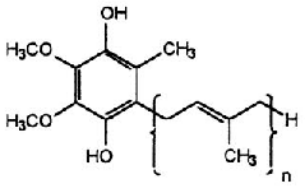

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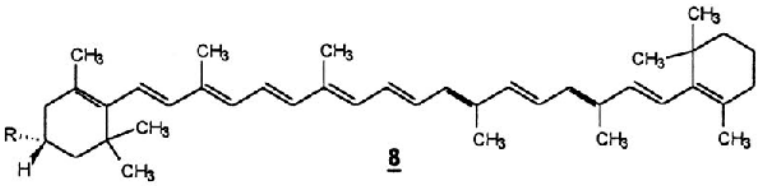

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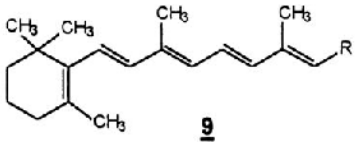

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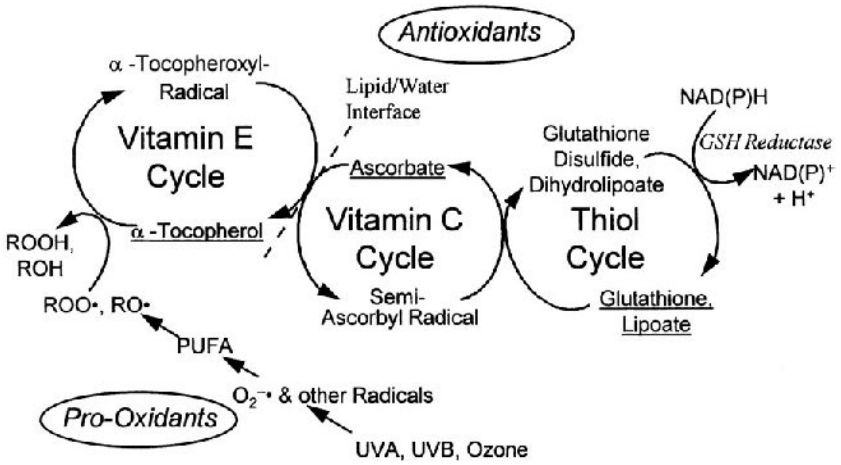

Figure 2 Activation of the Antioxidant Network by Environmental Oxidative Stressors O_2^{-}•: Superoxide anion radical; PUFA: polyunsaturated fatty acids; ROO•, RO•: lipid (per-) oxy radicals; ROOH, ROH: Lipidhydro(per)oxides. Please note that some of the depicted recycling mechanisms have been found in other than cutaneous systems (see ''Antioxidant Properties'').

to the unstable diketogulonic acid. Although ascorbate is not able to scavenge lipophilic radicals directly, in the presence of vitamin E it synergistically reduces lipid peroxyl radicals by reacting with tocopheroxyl radicals. This leads to regeneration of active tocopherol (14) (Fig. 2). Ascorbate has also been reported to show prooxidant properties. Mixtures of copper or iron salts with ascorbate are well known to stimulate lipid peroxidation in vitro (15). With the exception of pathological metal overload disease states, however, the prooxidative potential of ascorbate is not considered to be of relevance in vivo (16).

Prevalence in Skin In skin, the data available on ascorbate concentrations are limited and variable due to differences in species, skin layer analyzed, and method of analysis (Table 1). Importantly, however, vitamin C is present at significant levels in both the dermis and epidermis of animals and humans. In hairless mice, vitamin C levels are only slightly higher in the epidermis than in the dermis (5,17). In human skin, which is dependent on dietary vitamin C, the epidermis apparently contains approximately fivefold higher levels than the dermis (18). This difference in dermal and epidermal vitamin C levels may reflect an increased utilization in the dermis for the regulation of collagen and elastin biosynthesis (19), or facilitated transport mechanisms for vitamin C from the dermal

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Table 1 Physiological Levels of Ascorbate in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Total skin	Rat	0.2 g/kg tissue	Salomon and Stubbs (198)	1961
Total skin	Human	41 μg/g dry weight	Stüttgen and Schaefer (199)	1974
Total skin	Mouse	6–7 nmol/mg protein	Fuchs et al. (101)	1989
Epidermis	Mouse	1321 ± 77 nmol/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	1064 ± 54 nmol/g tissue	Shindo et al. (17)	1993
Epidermis	Human	3798 ± 1016 nmol/g tissue	Shindo et al. (18)	1994
Dermis	Human	723 ± 320 nmol/g tissue	Shindo et al. (18)	1994

vasculature to the epidermis. The epidermis is not only more directly exposed to the environment than the underlying dermis and therefore might have a higher demand of antioxidant protection, but also requires the presence of ascorbate for efficient formation of the stratum corneum barrier (20). Isolated human stratum corneum was reported to contain only very low ascorbate levels, as compared with levels in subjacent epidermal layers (6). The latter phenomenon is most likely due to both the hydrophobicity and, due to its location, the high degree of environmental exposure of the stratum corneum.

Glutathione

Antioxidant Properties Glutathione (γ–glutamyl-cysteinyl-glycine; GSH), present intracellularly at millimolar concentrations, is an important water-soluble antioxidant and reducing compound. Oral GSH is poorly absorbed and is not required to be provided by dietary intake (21). In cells, glutathione is synthesized from glutamate, cysteine, and glycine (22). It acts as a substrate for numerous reducing enzymes, among them glutathione peroxidase and the phospholipid hydroperoxide glutathione peroxidase. Therefore, the absence of glutathione may lead to an accumulation of lipid hydroperoxides (2). Importantly, glutathione also protects cells by reacting directly with reactive oxygen species such as singlet oxygen ($^1O_2$), hydroxyl radicals ($HO^\bullet$), and superoxide radicals ($O^\bullet{}_2^-$), resulting in the formation of thiyl radical ($GS^\bullet$), and subsequently glutathione disulfide (GSSG). The latter can be recycled to GSH by the NADPH-dependent enzyme glutathione reductase (Fig. 2). The ratio of GSH/GSSG in tissues in normally high (i.e., >100) (23). In many biological systems the GSH/GSSG ratio is lowered upon prooxidative conditions and therefore is frequently used as an indicator of oxidative stress. In mice, ascorbate supplementation increases GSH levels in lung epithelial tissue (24), and glutathione deficiency increases hepatic ascorbic acid synthesis (25), suggesting that the antioxidant actions of

glutathione and ascorbate are closely linked. In humans, who are dependent on dietary vitamin C intake, this link remains to be clarified.

Prevalence in Skin Although a number of studies are available on glutathione (GSH) and glutathione disulfide (GSSG), absolute values obtained for levels in total skin, epidermis, and dermis are highly variable (Table 2). However, comparing the relative levels, most studies demonstrated higher glutathione levels in the epidermis than in the dermis. Furthermore, the epidermis reveals a higher ratio of GSH/GSSG than the dermis, indicating either a lower oxidative challenge or a better antioxidative protection. Since the epidermis is more directly exposed to the environment, it seems also possible that the pathways leading to the endogenous formation of epidermal glutathione are upregulated by chronic environmental factors, as was shown for glutathione peroxidase in ozone-exposed lung epithelium (26). It must be considered that the cell turnover rate in the epidermis is very high, as well as cellular differentiation processes; since GSH is an important substrate for essential enzymes and GSSG can inactivate enzymes by forming disulfides (27), a high GSH/GSSG ratio could be essential for the stratified and keratinized epidermis.

Urate

Antioxidant Properties Uric acid (deprotonated form: urate) is a small water-soluble molecule (Fig. 1) that accumulates in human tissues as the end-product of purine metabolism. In blood plasma, urate has been shown to be a powerful scavenger of singlet oxygen, peroxyl-, and hydroxyl radicals (28). Further studies have demonstrated that urate scavenges ozone (15) and hypochlorous acid (29). In addition to its radical-scavenging potential, urate was proposed to stabilize reduced vitamin C in serum. This stabilizing effect appears to be due to inhibition of iron-catalyzed oxidation of ascorbate, which largely results from the formation of a stable, noncatalytic urate–iron complex (30). Unlike radical-scavenging reactions, this protective effect provided by iron chelation is not associated with depletion of urate. Direct free-radical attack upon urate generates allantoin, which has therefore been proposed as a marker molecule for free-radical reactions in vivo (31).

Prevalence in Skin Only little data are available on urate levels in cutaneous tissues. Lopez-Torres et al. reported values of 147 ± 5 nmol g^{-1} tissue in the epidermis, and 75 ± 9 nmol g^{-1} in the dermis of hairless mice (32). In humans, Shindo et al. reported levels of 1071 ± 242 nmol g^{-1} tissue in the epidermis, and 182 ± 24 nmol g^{-1} tissue in the dermis, respectively (18). Thus, as found for other antioxidants, the highest cutaneous urate levels are present in epidermal tissue.

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Table 2 Physiological Levels of Glutathione in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Human	1.8 µmol/g tissue (GSH) 0.09 µmol/g tissue (GSSG)	Halprin and Ohkawara (200)	1967
Total skin	Guinea pig	0.7–1.1 µmol/g tissue (GSH) 1.4–1.5 µmol/g tissue (GSSG)	Benedetto et al. (201)	1981
Epidermis	Mouse	0.75 µmol/g tissue (GSH)	Wheeler et al. (65)	1986
Dermis	Mouse	0.32 µmol/g tissue (GSH)	Wheeler et al. (65)	1986
Epidermis	Human	1.2 µmol/g tissue (GSH)	Connor and Wheeler (66)	1987
Total skin	Mouse	3.9–6.3 µmol/g protein (GSH) 1–1.5 µmol/g protein (GSSG)	Fuchs et al. (27,101)	1989
Epidermis	Mouse	1.16 µmol/g tissue (GSH) 0.07 µmol/g tissue (GSSG)	Shindo et al. (17)	1993
Dermis	Mouse	0.59 µmol/g tissue (GSH) 0.16 µmol/g tissue (GSSG)	Shindo et al. (17)	1993
Epidermis	Human	0.46 µmol/g tissue (GSH) 0.02 µmol/g tissue (GSSG)	Shindo et al. (18)	1994
Dermis	Human	0.08 µmol/g tissue (GSH) 0.01 µmol/g tissue (GSSG)	Shindo et al. (18)	1994

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Lipid-Soluble Antioxidants

Vitamin E

Antioxidant Properties Vitamin E is the major lipophilic antioxidant in plasma, membranes, and tissues (33). The term ''vitamin E'' collectively refers to the eight naturally occurring molecules (four tocopherols and four tocotrienols), which exhibit vitamin E activity. Tocotrienols differ from tocopherols in that they have an isoprenoid instead of a phytyl side chain (see Fig. 1); the four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol nucleus (α– has 3, β– and γ– have 2, and δ has 1). In humans, α–tocopherol is the most abundant vitamin E homologue, followed by γ–tocopherol.

Vitamin E is among the early recognized biological antioxidants, and its redox and free-radical chemistry are well documented (33). Vitamin E acts as an antioxidant by scavenging free radicals, which can, either directly or indirectly, initiate ($HO^{\bullet}$, and $O^{\bullet}_2{}^{-}$) or propagate (lipid peroxyl radicals) lipid chain reactions (34). Vitamin E can also react with nitric oxide (35). The major antioxidant role of vitamin E is generally considered to be the arrest of chain propagation by scavenging lipid peroxyl radicals. The initial oxidation product of tocopherol is the metastable tocopheroxyl radical (Fig. 2), which can be either reduced to tocopherol by coantioxidants, or reacts with another lipid peroxyl radical, yielding tocopherol-quinone (36). Thus, one molecule of tocopherol is able to scavenge two peroxyl radical molecules. Since the physiological molar ratio of tocopherols to polyunsaturated phospholipids, first-line targets of oxidative attack, is less than about 1:1000 in most biological membranes, regeneration of tocopherol is essential for its high antioxidant efficacy in vivo. As mentioned above, several hydrophilic coantioxidants, such as ascorbate and glutathione, can regenerate vitamin E from the tocopheroxyl radical and thus enhance the antioxidant capacity of vitamin E (14).

Furthermore, there is some in vitro evidence that ubiquinol-10 protects α–tocopherol from photo-oxidation by recycling mechanisms (37). In vitro, unphysiologically high concentrations of α–tocopherol were reported to induce prooxidative effects leading to acceleration of lipid peroxidation (38,39). In human skin in vivo, however, such adverse health effects have not been reported.

Prevalence in Skin As demonstrated in other body tissues, α-tocopherol is the predominant vitamin E homologue in murine and human skin (Table 3) (5,6,18). In addition, γ–tocopherol is present in murine and human epidermis, dermis, and stratum corneum. The α–tocopherol/γ–tocopherol molar ratio in the human dermis and epidermis is approx. 10:1. Notably, a vitamin E gradient has recently been demonstrated in human upper arm stratum corneum. The highest α–tocopherol levels were found in the lower stratum corneum, whereas the lowest levels were present in the upper layers. The α–tocopherol/γ–tocopherol ratio

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Table 3 Physiological Levels of α- and γ-Tocopherol in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Total skin	Mouse	200 pmol α-tocopherol/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	4.8 ± 0.5 nmol α-tocopherol/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	3.3 ± 0.3 nmol α-tocopherol/g tissue	Shindo et al. (17)	1993
Epidermis	Human	31 ± 3.8 nmol α-tocopherol/g tissue 3.3 ± 1 nmol γ-tocopherol/g tissue	Shindo et al. (18)	1994
Dermis	Human	16.2 ± 1.1 nmol α-tocopherol/g tissue 1.8 ± 0.2 nmol γ-tocopherol/g tissue	Shindo et al. (18)	1994
Stratum corneum	Mouse	8.4 ± 1.3 nmol α-tocopherol/g tissue 2.9 ± 0.9 nmol γ-tocopherol/g tissue	Thiele et al. (5)	1997
Stratum corneum	Human	33 ± 4 nmol α-tocopherol/g tissue 4.8 ± 0.8 nmol γ-tocopherol/g tissue	Thiele et al. (6)	1998

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decreased from about 10:1 in the lower layers to about 3:1 in the upper stratum corneum. The α–tocopherol levels in human dermis and epidermis were severalfold higher than in corresponding layers of hairless mouse skin (17,18). Consistently, human stratum corneum contains almost tenfold higher α–tocopherol levels than measured in murine stratum corneum (5,6). As observed for hydrophilic antioxidants, higher vitamin E levels were found in murine and human epidermis, as compared with dermal levels. It remains to be clarified whether the uptake and transport of α–tocopherol in the epidermis is an unspecific and passive process or, as described for human hepatocytes (33), is regulated by a mechanism involving a specific binding enzyme (α–tocopherol transfer protein).

Ubiquinols/Ubiquinones (''Coenzyme Q'')

Antioxidant Properties The terms ''coenzyme Q,'' as well as ''ubiquinone,'' are commonly used for the redox couple ubiquinol/ubiquinone (see Fig. 1). Ubiquinones are lipid-soluble quinone derivatives with an isoprenoid side chain. In nature, ubiquinone homologues containing 1 to 12 isoprene units occur; the predominant form of ubiquinone in humans is ubiquinone-10 (contains 10 isoprene units), and in mice ubiquinone-9. In liver cells, about 40 to 50% of the total cellular ubiquinone is located in the mitochondria, 25 to 30% in the nucleus, 15 to 20% in the endoplasmic reticulum, and only 5 to 10% in the cytosol (40). In vitro, the reduced forms of ubiquinones, the ubiquinols, are by two to three orders of magnitude more potent antioxidants (41). The role of ubiquinol/ubiquinone as a redox carrier in the respiratory chain is well established, participating in the transfer of protons across the inner mitochondrial membrane (42). Ubiquinols can react with reactive oxygen species and thus prevent direct damage to biomolecules and initiation of lipid peroxidation. Although ubiquinones cannot prevent autocatalytic free-radical reactions by donating a phenolic hydrogen atom (unlike ubiquinols and tocopherols), it scavenges singlet oxygen and inhibits lipid peroxidation in model membranes (43). Furthermore, there is some in vitro evidence that ubiquinol-10 protects α–tocopherol against superoxide-driven oxidation (37). In low-density lipoproteins, its protective potential against lipid peroxidation was shown to exceed that of α–tocopherol (44). However, it must be noted that the antioxidant properties reported for ubiquinones are strongly dependent on the length of the side chain and the model systems used. A growing scientific and commercial interest in ubiquinones has led to its incorporation into skin-care products; however, further research is needed to better understand its protective antioxidant mechanisms in human skin.

Prevalence in Skin In both mouse and human skin, the highest ubiquinol levels were found in the epidermis. In human skin, the majority of ubiquinone is present in its oxidized form (ubiquinone-10) (Table 4). This is in accordance with the ratios determined in brain and lung tissues, but different from those in

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Table 4 Physiological Levels of Ubiquinone/Ubiquinol in Skin

Skin layer	Species	Concentration	References	Year
Total skin	Mouse	20–48 pmol ubiquinol-9/mg protein 98–136 pmol ubiquinone-9/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	1.9 ± 0.2 nmol ubiquinol-9/g tissue 15.2 ± 1.1 nmol ubiquinone-9/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	1.2 ± 0.2 nmol ubiquinol-9/g tissue 10.0 ± 0.7 nmol ubiquinone-9/g tissue	Shindo et al. (17)	1993
Epidermis	Human	3.5 ± 0.8 nmol ubiquinol-10/g tissue 4.1 ± 0.6 nmol ubiquinone-10/g tissue	Shindo et al. (18)	1994
Dermis	Human	0.4 ± 0.1 nmol ubiquinol-10/g tissue 2.9 ± 0.8 nmol ubiquinone-10/g tissue	Shindo et al. (18)	1994
Stratum corneum	Mouse	ubiquinol-9 and ubiquinone-9: not detectable (<0.1 pmol/mg)	Thiele et al. (5)	1997
Stratum corneum	Human	ubiquinol-10 and ubiquinone-10: not detectable (<0.1 pmol/mg)	Thiele et al. (6)	1998

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heart, kidney, liver, and blood plasma, where the majority of ubiquinone is present in the reduced form (45). Interestingly, all three organs—skin, brain, and lung—are well known to be challenged by a high load of oxidative stress. Despite its high lipid content, the stratum corneum appears to be very low in ubiquinol/ubiquinone levels (5,6). Most likely, this results from the loss of nuclei and organelles, both rich in ubiquinones, during the terminal differentiation process of keratinocytes into the stratum corneum barrier.

Carotenoids and Vitamin A

Antioxidant Properties Dietary vitamin A is available in the form of provitamin A compounds (e.g., α– and β–carotene, and cryptoxanthin) (see Fig. 1), or directly from animal food (liver, milk, egg, and fish) (46). In comparison with α–tocopherol, β–carotene membrane levels are severalfold lower; however, β–carotene accumulates significantly in skin and may achieve levels far exceeding those of α–tocopherol in subjects on a β–carotene-supplemented diet (47). There are at least three known mechanisms by which carotenoids protect cells from oxidative stress: (1) by quenching triplet-state sensitizers; (2) by quenching singlet oxygen; and (3) by scavenging peroxyl radicals (48,49). Triplet sensitizers, such as flavins and porphyrins, may abstract a hydrogen atom or an electron from various molecules; this can lead to further radical-mediated damage (type I) or formation of singlet oxygen (type II) by reaction with ground-state oxygen. The quenching of singlet oxygen by carotenoids is almost entirely an energy transfer process yielding ground-state oxygen and a triplet excited carotenoid (2). The role of carotenoids within the ''antioxidant network'' is not clear. It has been demonstrated in liver homogenates that dietary carotenoids increase the resistance to lipid peroxidation primarily by enhancing α–tocopherol membrane levels, while direct antioxidant effects provided by carotenoids were less protective (50). Several forms of vitamin A (13-cis-retinoic acid, all-trans-retinoic acid, all-trans-retinol) (see Fig. 1), however, were shown to effectively inhibit lipid peroxidation in liver microsomes (2,51). Carotenoids protect biological systems against triplet sensitizers and singlet-oxygen-mediated oxidative damage largely without being sacrificed. Both carotenoids and retinoids act at physiological oxygen tension as peroxyl radical scavengers, thus preventing oxidative damage (49). As opposed to the reducing antioxidants ascorbate and dihydrolipoic acid, β–carotene was not effective in recycling α–tocopherol in mouse skin homogenates irradiated with solar-simulated UV irradiation (52).

Prevalence in Skin Data available on carotenoid and vitamin A levels in skin are very limited. Vahlquist et al. revealed that the levels of β–carotene in human skin (epidermis: 2.2 $\mu g\ g^{-1}$ protein; dermis: 0.7–0.8 $\mu g\ g^{-1}$ protein; subcutis: 18.9 $\mu g\ g^{-1}$ protein) is severalfold higher than that of vitamin A (retinol; epidermis: 0.3 $\mu g\ g^{-1}$ protein; dermis: 0.2–0.4 $\mu g\ g^{-1}$ protein; subcutis: 6.4 $\mu g\ g^{-1}$

protein) (53). Furthermore, the same investigators detected carotene and retinol in skin surface lipids, but no data are yet available on stratum corneum levels of these compounds (53). Recently, Stahl et al. detected relatively high basal levels of carotenoids in human skin of the forehead (0.40 ± 0.09 nmol g^{-1}), back (0.22 ± 0.13 nmol g^{-1}), and palmar hand (0.32 ± 0.08 nmol g^{-1}), while significantly lower levels were present in the skin of the dorsal hand (0.03 ± 0.10 nmol g^{-1}) and the inside of the forearm (0.07 ± 0.05 nmol g^{-1}). Furthermore, skin carotenoid levels were increased after oral carotenoid supplementation (with daily doses of 20–25 mg carotenoids), and correlated well with increased serum carotenoid levels (54). Higher levels of β–carotene (1.4 ± 0.7 nmol g^{-1} tissue) and lycopene (1.6 ± 0.6 nmol g^{-1} tissue) in human skin samples were found when the subcutaneous fat was included in the whole skin samples (55). Thus, as was reported for other skin antioxidants, β–carotene levels are higher in the epidermis than in the dermis. This difference seems to be less pronounced for vitamin A (53,56).

Enzymatic Antioxidant Systems

The Enzymatic Glutathione System

Antioxidant Properties The major components of the enzymatic glutathione system are glutathione (GSH) peroxidase (GSH-Px), GSSG reductase, phospholipid hydroperoxide GSH-peroxidase, and GSH-S-transferase. GSH-Px is a selenoenzyme consisting of four identical subunits, each of which contains a selenocysteine residue in its active site. In eukaryotes, the majority of its enzymatic activity is localized in the cytosol, and, to a lesser extent, in mitochondria (57,58). GSH-Px reduces H_2O_2 and lipid hydroperoxides at the expense of two molecules of GSH, which are oxidized to GSSG. GSSG-reductase, a dimeric enzyme containing FAD in its active site, catalyzes the reduction of GSSG using reducing equivalents such as NADPH (59) (Fig. 2). Nonselenium-dependent GSH-peroxidases (GSH-S-transferases) and phospholipid hydroperoxide GSH-peroxidase are able to catalyze the reduction of lipid hydroperoxides, but not of hydrogen peroxide (60). A developing body of data indicates that polymorphism at GSH-S-transferases (GSTs) genes influences skin cancer susceptibility. It was proposed that GSTs influence tumorigenesis because these enzymes detoxify the products of UV-induced oxidative stress (61), and that heritable deficiency of specific GSTs may be a genetic determinant of individual skin sensitivity toward UV irradiation (62). Recently, increased tumorigenesis has been demonstrated in mice lacking π-class GSTs (63).

Prevalence in Skin As compared to liver, kidney, and brain, skin GSH-Px and GSH-reductase activities are markedly lower (47). The baseline levels measured in epidermis and dermis vary considerably between different studies

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and therefore do not point to a clear preferential distribution of GSH-Px in skin (see Table 5). GSH-S-transferase is expressed during all stages of differentiation of cultured human keratinocytes, but was reported to lack substrate specificity and catalytic activity for reduction of lipid hydroperoxides (64). GSH-S-transferase and GSH-reductase activities have been detected at similar levels in murine epidermis (19.6–53.3 U/mg protein, and 22.5–31.6 U/mg protein, respectively) and dermis (33.8–64.8 U/mg protein, and 14.3–27.6 U/mg protein, respectively) (65,66). While little is known about absolute levels of GSTs in distinct layers of human skin, π-, μ-, and α-class GSTs have been localized immunohistologically in normal skin, naevi, and melanoma (67): π-GSTs were found in the stratum basalis and, to a lesser extent, in the superficial epidermal layers. Distribution of GST π in the epidermis showed that only the stratum basale, where melanocytes are located, stained well. The α-GSTs were relatively abundant in the upper strata and to a lesser extent, in the basal layers.

Superoxide Dismutases

Antioxidant Properties Superoxide dismutase (SOD) catalyzes in the dismutation reaction of superoxide radicals ($O^{\bullet}_2{}^{-}$) to H_2O_2. SODs are found in virtually all eukaryotic cells. Three types of human SOD have been purified: Cu/Zn-SOD (a cytosolic enzyme); Mn-SOD (a mitochondrial enzyme); and an extracellular SOD (EC-SOD, a tetrameric glycoprotein which contains Cu^{II} and Zn^{II}) (68,69). Cu/Zn-SOD consists of two protein subunits, each of which has an active site containing one Cu^{II} and one Zn^{II}. The Cu ion serves as an active redox site, while the Zn ion maintains the protein structure (68). The Mn-SOD consists of four subunits, each containing Mn^{II}, and is more labile than Cu/Zn-SOD. The presence of SOD in various compartments of the body may facilitate immediate dismutation of $O^{\bullet}_2{}^{-}$ at the site where it is generated.

Table 5 Activities of GSH-Px in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Mouse	80.2 U/mg protein	Wheeler et al. (65)	1986
Dermis		37.0 U/mg protein		
Epidermis	Mouse	80.2 U/mg protein	Connor and Wheeler (66)	1987
Dermis		36.5 U/mg protein		
Total skin	Mouse	35 U/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	11.7 ± 1.4 U/mg protein	Shindo et al. (17)	1993
Dermis		27.5 ± 2.5 U/mg protein		
Epidermis	Human	17.8 ± 1.0 U/mg protein	Shindo et al. (18)	1994
Dermis		15.0 + 1.3 U/mg protein		

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Prevalence in Skin Many investigators measured SOD activities in epidermal and dermal tissues, mostly using unspecific spectrophotometric activity assays determining total SOD activity (70). The reported activity levels are highly variable and do not allow clear conclusions about its preferential distribution within layers of skin (Table 6). Both in human and in pig epidermis, the Cu/Zn-SOD activity seems to be five- to tenfold higher than that of Mn-SOD (71,72). As compared with other body tissues, SOD activity is relatively low in skin (47).

Catalase

Antioxidant Properties Catalase is a tetrameric enzyme that is expressed in all major body organs. Each of its four subunits contains a heme-group in its active site and one tightly bound molecule of NADPH (73). Highest catalase activities are found in the peroxisomes, where it constitutes about 50% of the peroxisomal protein. The major role of catalase as an antioxidant is its ability to detoxify H_2O_2 by decomposing two H_2O_2 molecules to two molecules of water and one of oxygen.

Prevalence in Skin Epidermal activities were first measured in mice by Solanki et al., who reported 78–175 U/mg protein (74). Shindo et al. measured activities of 30.4 ± 4.3 U/mg protein in murine epidermis, and 33.3 U/mg protein in murine dermis, respectively. The same authors reported higher catalase activities in human epidermis (62 ± 6 U/mg protein), but lower activities in human dermis (14.6 ± 2.9 U/mg protein).

Effect of Environmental Stressors on Skin Antioxidants

UVB and UVA irradiation induce the formation of ROS in cell cultures (75,76), skin homogenates (52,77,78), and intact murine and human skin (79,80). Evalua-

Table 6 Physiological Activities of Superoxide Dismutase in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Human	12.0 U/mg protein	Kim and Lee (202)	1987
Epidermis	Pig	11.4 U/mg protein	Ohkuma et al. (72)	1987
Dermis	Human	10.5 U/mg protein	Kim and Lee (202)	1987
Epidermis	Mouse	0.6 U/mg protein	Carrao and Pathak (203)	1988
Epidermis	Guinea pig	0.5 U/mg protein	Carrao and Pathak (203)	1988
Total skin	Mouse	3.0–4.3 U/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	11.7 ± 1.4 U/mg protein	Shindo et al. (17)	1993
Dermis	Mouse	27.5 ± 2.5 U/mg protein	Shindo et al. (17)	1993
Epidermis	Human	17.8 ± 1.0 U/mg protein	Shindo et al. (18)	1994
Dermis	Human	15.0 + 1.3 U/mg protein	Shindo et al. (18)	1994

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tion of the protective mechanisms of skin have included measurements of baseline levels of antioxidants in the dermis and epidermis (17,18) and the antioxidant response to UVB and UVA light in these layers (27,81). Terrestrial UVR consists of UVB (280–320 nm) and UVA (UVA-II: 320–340 nm, UVA-I: 340–400 nm). Radiation of less than 280 nm (UVC) does not reach the Earth's surface, since they are absorbed by stratospheric ozone.

While ozone (O_3) in the upper atmosphere (stratosphere) occurs naturally and protects skin by filtering out harmful solar ultraviolet radiation, O_3 at ground level (troposphere) is a noxious, highly reactive oxidant pollutant. The precursors of photochemical oxidants are volatile organic compounds (e.g., vapor-phase hydrocarbons and halogenated organics), oxides of nitrogen (NO_x), NO and other radicals, O_2, and sunlight (82). As a major pollutant in photochemical smog, O_3 occurs at concentrations between 0.1 and 0.8 ppm and represents a severe urban air quality problem (83). In addition to photochemical smog, O_3 is generated during operation of high-voltage devices and dermatological phototherapy equipment (83). There is ample evidence that acute (2–6 h) and chronic in vivo exposure to O_3 causes airway inflammation and affects pulmonary function in humans (84–86). The biological effects of O_3 are attributed to its ability to cause ozonation, oxidation, and peroxidation of biomolecules, both directly and via secondary reactive reactions. Hydrogen peroxide, hydroperoxides, hydroxyl radical, superoxide anion, and singlet oxygen have been proposed as intermediates in these secondary reactions (83,87–90). Analogous to the respiratory tract and the surface tissues of plants, a primary function of the skin is to provide a protective barrier against noxious environmental agents including oxidative air pollutants.

Numerous studies have documented the effects of O_3 on the respiratory tract in animals and humans (83,91,92) and on plants (93–96). In contrast, only little is known about the effect of O_3 on cutaneous tissues. Recently, a series of studies were published investigating the impact of O_3 on skin antioxidants (1,5,7,97). Since O_3 levels are frequently highest in areas where exposure to ultraviolet radiation is also high, the concomitant exposure to O_3 and ultraviolet radiation in photochemical smog could be of relevance for skin pathologies, as has been implicated for plants (93,98).

Hydrophilic Skin Antioxidants

Ascorbate and Urate High acute doses of solar simulated UVA/UVB (SSUV) have been demonstrated to deplete ascorbate and urate in cultured human skin equivalents. The SSUV dose needed to deplete these hydrophilic antioxidants was much higher than those necessary to deplete lipophilic antioxidants ubiquinol-10 and α–tocopherol (99). In hairless mice, however, Shindo et al. (100) observed depletion of ascorbate already at lower SSUV doses than those needed to deplete lipophilic antioxidants or GSH. A single acute ozone exposure

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depletes ascorbate in the upper epidermis of hairless mice, but not in lower skin layers (7).

Glutathione Fuchs et al. (27,101) reported that single exposures to UVB, but not to UVA, deplete GSH and increase GSSG in excised mouse skin, while ascorbate levels remained unchanged. However, UVA irradiation of human fibroblasts depleted intracellular glutathione levels (102). Treatment of hairless mice with 8-methoxypsoralene plus UVA (PUVA) resulted in a significant depletion of cutaneous glutathione after 24 to 48 h (65). Epidermal GSH levels of UVB-treated hairless mice were depleted by 40% within minutes after exposure and returned to regular levels after half an hour (66).

Lipophilic Skin Antioxidants

Vitamin E Recently, it was demonstrated that a single suberythemogenic dose of SSUV light (UVA and UVB; 0.75 MED) depletes human stratum corneum α–tocopherol by almost 50%, and murine stratum corneum α–tocopherol by 85% (6). These findings were in contrast to previous studies investigating the effects of SSUV light on dermal and epidermal antioxidants, in which doses equivalent to 3 MED or more were necessary to detect a significant depletion of α–tocopherol (17,81,103). Hence, it was concluded that α–tocopherol depletion in the stratum corneum is a very early and sensitive event of photooxidative damage in skin (6). The high susceptibility of stratum corneum vitamin E to SSUV may be, at least in part, due to a lack of coantioxidants in the stratum corneum. Ubiquinol-10 was undetectable in human stratum corneum at levels found in epidermis and dermis (6). Additionally, ascorbate, the major hydrophilic coantioxidant that is capable of recycling photooxidized α–tocopherol (52,78) is present only at very low levels in murine and human stratum corneum, as compared to epidermal and dermal tissue (Thiele et al., unpublished observations).

Vitamin E may be depleted (1) directly, by absorption of UVB-radiation; and/or (2) indirectly, by excited-state singlet oxygen or reactive oxygen intermediates that are generated by photosensitizers upon UV absorption also in the UVA range. Since both UVB and UVA alone have been shown to deplete murine α–tocopherol (6), both mechanisms may be relevant. The absorption maxima of α– and γ–tocopherol fall between 290 and 295 nm (104,105) and thus extend well into the solar UV spectrum. Interestingly, a large part of terrestrial UVB (around 290–300 nm) is absorbed in the human stratum corneum (106). Furthermore, depletion of α–tocopherol by UVR is maximal at wavelengths in the range of its absorption maximum in skin homogenates of hairless mice (52). This congruency suggests that α–tocopherol is directly destroyed upon short-wavelength UVB absorption. Indeed, tocopheroxyl radical formation occurs in UVB-irradiated skin homogenates (52). Direct depletion of α–tocopherol and formation of its radical

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may also affect other endogenous antioxidant pools. As mentioned previously, α–tocopherol is readily regenerated from its radical at the expense of reductants like ascorbate (52,107) (Fig. 2), which itself can be regenerated by glutathione (25). In addition to direct depletion by UVB, skin α–tocopherol levels may also be consumed as a consequence of its chain-breaking antioxidant action. The absorption of UVB and UVA photons by endogenous photosensitizers (e.g., porphyrins, riboflavin, quinones, and bilirubin) results in its electronically excited state (108,109). The excited sensitizer subsequently reacts with another substrate (type I reaction) to form radicals or radical ions, or with oxygen (type II reaction) to generate singlet oxygen (110). Photosensitizers, such as melanin, are present in variable amounts in the stratum corneum (111). Hence, their wavelength-dependent potential to generate or to quench free radicals, and to absorb UVR, may modulate α–tocopherol depletion during and after solar exposure.

Recently, Thiele et al. investigated the effects of the air pollutant ozone on skin antioxidants: while no depletion of vitamin E was observed in whole skin (97), α–tocopherol depletion was detected in the outer epidermis when skin layers were analyzed separately (7). It was concluded that ozone itself is too reactive to penetrate deeply into skin and reacts rapidly with skin barrier lipids and proteins (8). Consequently, it was demonstrated that the stratum corneum is the most susceptible skin layer for ozone-induced vitamin E depletion (5).

Ubiquinol/Ubiquinone Ubiquinol-9 has been shown to be the most susceptible nonenzymatic antioxidant in murine skin, with respect to SSUV-induced (280–400 nm) depletion in vivo (17). Similar results were obtained for ubiquinol-10 in SSUV-irradiated human cell culture models (99). Exposure of purified ubiquinol-9 and α–tocopherol to SSUV in vitro resulted in the depletion of both compounds, which have similar absorption maxima around 295 nm (81). Since ubiquinol depletion precedes that of α–tocopherol in UVR-challenged skin in vivo, it is thought that ubiquinol protects vitamin E, as demonstrated in vitro (37).

Vitamin A/Carotenoids A single exposure of human volar forearm skin to SSUV (3 MED) was found to lower the skin lycopene (Ψ,Ψ–carotene) level by 31 to 46%, whereas the same UV dose did not induce significant changes in the skin β–carotene level (55). However, repeated exposures of human volunteers to solar light (total UV dose of about 10 kJ cm^{-2}) depleted also β–carotene levels in skin (112).

Enzymatic Skin Antioxidants

Catalase, SOD, GSH-Px, GSSG Reductase It was demonstrated by Aronoff et al. more than three decades ago that photooxidation of a single porphyrin ring in catalase results in complete inhibition of its activity (113). Superoxide anion radicals (114) and ozone (115) have also been shown to inactivate catalase

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activity. Punnonen et al. demonstrated that UVB (116), as well as UVA or PUVA therapy (117) decreases the activity of both catalase and SOD in cultured human keratinocytes. In cultured human fibroblasts, a single UVA exposure decreased catalase activities immediately, while GSH-Px and GSSG reductase remained unaffected, and SOD activity decreased only 3 days after exposure (118). In vivo exposures of hairless mouse skin using SSUV light demonstrated that dermal and epidermal catalase is more susceptible to photoinactivation than SOD, and far more than GSH-Px and GSSG reductase (17,81). In vitro, purified catalase was demonstrated to be directly inactivated by SSUV light, while SOD activity remained unaffected (81). Hence, while direct photodestruction appears to account for catalase inactivation, other mechanisms, possibly involving free-radical-mediated oxidative protein damage, may account for the observed UV-induced loss of skin SOD activity in vivo. Notably, chronic UVB irradiation was recently shown to upregulate human epidermal SOD activity in vivo, whereas the activities of other antioxidant enzymes remained unchanged (119).

ROLE OF ANTIOXIDANTS IN THE PHOTOPROTECTION OF SKIN

Topical Application of Antioxidants

UVR-induced skin damage includes acute reactions, such as erythema, edema, and pain, followed by exfoliation, tanning, and epidermal thickening. Premature skin aging (photoaging) and carcinogenesis are generally believed to be consequences of chronic UVR exposure (120). ROS and other free radicals, particularly the highly damaging hydroxyl radical, deplete the skin of its antioxidant defense and, when the latter is overwhelmed, can damage biomolecules such as lipids, proteins, and nucleic acids (27,81,101). Therefore, apart from using chemical and/or physical sunscreens to diminish the intensity of UVR reaching the skin, preventing ROS from reacting with these biomolecules by strengthening the skin's antioxidative capacity is an emerging approach in limiting UVR-induced skin damage (121–123). Topical application of antioxidants, such as vitamin E, provides an efficient means of increasing antioxidant tissue levels in epidermis and dermis (103,124). The stratum corneum, which was shown to be the most susceptible skin layer for UVR-induced depletion of vitamin E (6), may particularly benefit from an increased antioxidant capacity.

A selected overview of animal and human studies investigating acute and chronic photoprotection of skin by topical administration of antioxidants is given in Tables 7 to 10.

Vitamin E

The photoprotective effect of vitamin E (α–tocopherol) and its acetyl ester have been studied extensively (see Table 7). Numerous topical studies demonstrated

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Table 7 Photoprotective Effects of Topically Applied Vitamin E (α–Tocopherol) and Derivatives In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin E Vitamin E acetate	Rabbit	Erythema (MED)	Vitamin E protective; vitamin E acetate not protective	BHT also protective; vitamin E also protective when applied after UVR exposure	Roshchupkin et al., 1979 (125)
Vitamin E	Human	Mechanoelectrical properties of skin	Protection against UVR- and PUVA-induced damage		Potapenko et al., 1983 (139)
Vitamin E Vitamin E derivatives with shorter hydrocarbon chains Vitamin E acetate	Human, rabbit	PUVA-induced erythema and changes in mechanoelectrical properties of skin	Vitamin E and derivatives with shorter hydrocarbon chain protective; vitamin E acetate not protective	No protection of vitamin E and derivatives when applied after UVR exposure	Potapenko et al., 1984 (38)
Vitamin E	Mouse	Lipid peroxidation	Protective	Vitamin A, BHT, and β–carotene also protective	Khettab et al., 1988 (130)
Vitamin E	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective		Bissett et al., 1989 (136)
Vitamin E	Human	Erythema (MED)	Protective	SPF determination	Möller et al., 1989 (126)
Vitamin E Trolox® Vitamin E acetate Vitamin E succinate Vitamin E linoleate Vitamin E nicotinate	Mouse	Skin wrinkling and sagging, skin tumor incidence, and histology	Vitamin E esters not as protective as vitamin E or vitamin E analog Trolox®; no protection against UVA-induced skin sagging	Glutathione, β–carotene, BHT, and mannitol not protective	Bissett et al., 1990 (137)
Vitamin E	Mouse	Skin tumor incidence and immunosuppression	Protective	Prolonged pretreatment	Gensler et al., 1991 (131)

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Vitamin E Vitamin E acetate	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	Vitamin E protective after single application; vitamin E acetate only protective after prolonged application	Limited conversion of vitamin E acetate into vitamin E after single application	Schoonderwoerd et al., 1991 (133)
Vitamin E acetate	Mouse	Lipid peroxidation and DNA synthesis rate	Protective		Record et al., 1991 (140)
Vitamin E	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective	Additive protection in combination with anti-inflammatory agents	Bissett et al., 1992 (135)
Vitamin E acetate	Mouse	Erythema, edema, and skin sensitivity	Protective	Treatment immediately after UVR-exposure	Trevithick et al., 1992 (141)
Vitamin E acetate	Mouse	Edema and histology	Protective	Delayed treatment after UVR-exposure; increased skin vitamin E concentration	Trevithick et al., 1993 (142)
Vitamin E Vitamin E acetate Vitamin E sorbate	Mouse	Skin wrinkling	Vitamin E and sorbate ester protective; vitamin E acetate ester only modestly protective	Sorbate ester more protective than free vitamin E	Jurkiewicz et al., 1995 (80)
Vitamin E Vitamin E acetate	Human	Erythema (skin color)	Moderate protection of vitamin E and vitamin E acetate when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Vitamin E Vitamin E acetate	Rat	UVA-induced binding of 8-MOP to epidermal biomacromolecules	Vitamin E protective; vitamin E acetate only protective after prolonged application	Conversion of vitamin E acetate into vitamin E slow	Beijerbergen van Henegouwen et al., 1995 (132)
Vitamin E acetate Vitamin E succinate	Mouse	Skin tumor incidence and immunosuppression	No protection		Gensler et al., 1996 (143)

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Table 7 Continued

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin E	Yorkshire pig	Sunburn cell formation	Protection against UVR-induced damage	Minimal protection in reducing PUVA-induced damage	Darr et al., 1996 (128)
Vitamin E	Mouse	Immunosuppression and lipid peroxidation	Protective	No protection when applied after UVR exposure	Yuen et al., 1997 (105)
Vitamin E	Mouse	Histology (sunburn cell formation and skin thickness)	Protective		Ritter et al., 1997 (129)
Vitamin E Vitamin E acetate Vitamin E methyl ether	Mouse	Formation of DNA photoadducts	Vitamin E derivatives less protective than vitamin E	Sunscreening properties of vitamin E	McVean et al., 1997 (10)
Vitamin E	Mouse	Chemiluminescence after UVA exposure	Protective	β–Carotene also protective	Evelson et al., 1997 (134)
Vitamin E	Mouse	Lipid peroxidation	Protective	Skin's enzymatic and nonenzymatic antioxidant capacity investigated	Lopez-Torres et al., 1998 (124)
Vitamin E	Human	Erythema (skin color and skin blood flow)	Moderate protection	No protection when applied after UVR exposure; SPF (determined in vitro) = 1	Dreher et al., 1998 (127,180)

Abbreviations: BHT, butylated hydroxytoluene; CPZ, chlorpromazine; MED, minimal erythema dose; 8-MOP, 8-methoxypsoralen; PUVA, 8-methoxypsoralen and UVA treatment; SPF, sun protection factor.

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significantly reduced acute skin responses when vitamin E was applied before UVR exposure, such as erythema and edema (125–127), sunburn cell formation (128,129), lipid peroxidation (105,124,130), DNA adduct formation (10), immunosuppression (105,131), as well as UVA-induced binding of photosensitizers (132,133) and chemiluminescence (134). Chronic skin reactions due to prolonged UVR exposure, such as skin wrinkling (80,135–137), and skin tumor incidence (131,135–137) were also diminished by topical vitamin E. However, most studies used animal models, while only few studies exist demonstrating photoprotection by topical application of vitamin E in humans (126,127,138,139).

Vitamin E esters, particularly vitamin E acetate, were also shown to be promising agents in reducing UVR-induced skin damage (80,132,133,137,140–142) (see Table 7). However, their photoprotective effects appeared to be less pronounced as compared to vitamin E; moreover, some studies failed to detect photoprotection provided by vitamin E esters (125,138,143). Since the free aromatic hydroxyl group is responsible for the antioxidant properties of vitamin E, vitamin E esters need to be hydrolyzed during skin absorption to show activity. Vitamin E acetate was shown to be absorbed and penetrate skin easily (144–146). A skin bioavailability study demonstrated that vitamin E and vitamin E acetate behave similarly with regard to penetration of rat epidermis (132). The authors concluded that the aromatic hydroxyl group in vitamin E is not dissociated in the skin penetration limiting layer, the stratum corneum. Consequently, the difference between physicochemical parameters determining skin transport for vitamin E and its esters seem negligible. Notably, the bioconversion of vitamin E acetate to its active antioxidative form, α–tocopherol, was found to be slow and to occur only to a minor extent in vivo (132,147). Hence, the less pronounced or missing photoprotective effects of topically applied vitamin E acetate after a single application might be explained by a limited bioavailability of the ester-cleaved form during oxidative stress at the site of action (e.g., superficial skin layers). As was further shown by the same authors, photoprotection was obtained only after several topical applications of vitamin E acetate. A photocarcinogenesis study by Gensler et al. (143) even demonstrated an increased skin tumor incidence after topical application of vitamin E esters compared to nontreated, but UVR-exposed, hairless mice. Confirming the results obtained by Beijersbergen van Henegouwen et al. (132), Gensler and coworkers found an accumulation of vitamin E acetate in the skin after prolonged topical application, whereas the level of free α–tocopherol remained relatively low. A human study further demonstrated that topically applied α–tocopherol acetate, though substantially absorbed into skin, is not significantly metabolized to the hydrolyzed form, even after long-term administration (147).

In addition to the antioxidative properties of vitamin E, further photoprotective mechanisms have been discussed. Recent studies on vitamin E using a liposome dispersion model to estimate the photooxidation of biomolecules (148), or

measuring DNA-adduct formation in vivo (10), indicated that vitamin E may also have substantial sunscreening properties. On the other hand, a determination of the sun protection factor (SPF) of a vitamin E lotion (2 w%) in vitro resulted in no significant sunscreening effect when administered at a dose of 2 mg cm^{-2} (127). Additionally, interactions of vitamin E with the metabolism of arachidonic acid have been described. Vitamin E was shown to modulate the activity of cyclooxygenase and to depress the biosynthesis rate of prostaglandin E_2, possibly by inhibiting the release of arachidonic acid by phospholipase A_2 (33,149). Interactions with the eicosanoid system may result in an anti-inflammatory effect and thus complement antioxidative photoprotection in skin.

Vitamin C

Few studies have reported photoprotective effects for vitamin C (see Table 8). Using a porcine skin model, Darr and associates proposed that topically applied vitamin C is only effective when formulated at high concentration in an appropriate vehicle (150). Vitamin C is highly unstable and is only poorly absorbed into the skin, possibly explaining its modest photoprotective effect when applied topically (151). Hence, more lipophilic and more stable vitamin C esters, such as its palmitatyl, succinyl, or phosphoryl ester (151–153), might be promising derivatives providing increased photoprotection, as compared to vitamin C. As described for vitamin E esters, such compounds must be hydrolyzed to vitamin C to be effective as antioxidants.

Other Antioxidants

Besides vitamin E and vitamin C, several other compounds with antioxidative potential have been suggested to lower photodamage when topically applied (see Table 9). Administration of different plant extracts, particularly flavonoids, were reported to diminish acute and chronic skin damage after UVR exposure (154–159). Flavonoids (e.g., apigenin, catechin, epicatechin, α–glycosylrutin, and silymarin) are polyphenolic compounds that occur in plants and, due to their free phenolic groups, exhibit antioxidative capacity. Furthermore, flavonoids may possibly also have anti-inflammatory properties (160). Thiols, such as N-acetylcysteine and derivatives, are another important group of potent radical scavengers (161,162). It was demonstrated in several rat studies that topical administration of thiols diminishes UVA-induced binding of photosensitizers to epidermal lipids and DNA (163,164) and afforded some protection against the damaging effects of UVB on epidermal DNA (165). Treatment with cysteine derivatives, like N-acetyl-cysteine, resulted in increased intracellular glutathione (GSH) levels in human keratinocytes (166). Thus, thiol-induced stimulation of GSH biosynthesis may be a key mechanism accounting for the observed photoprotective effects. Exogenously applied GSH penetrates the cell membrane and the skin only poorly

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Table 8 Photoprotective Effects of Topically Applied Vitamin C (Ascorbic Acid) and Derivatives In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin C Vitamin C palmitate	Mouse	Skin wrinkling and sagging, skin tumor incidence, and histology	Vitamin C palmitate less protective than vitamin C; no protection against UVA-induced skin sagging		Bissett et al., 1990 (137)
Vitamin C	Yorkshire pig	Erythema (skin blood flow) and sunburn cell formation	Protection against UVR- and PUVA-induced damage	High vitamin C concentration	Darr et al., 1992 (150)
Vitamin C	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective	Additive protection in combination with anti-inflammatory agents	Bissett et al., 1992 (135)
Vitamin C palmitate	Human	Erythema (skin color)	Poor protection when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Vitamin C	Yorkshire pig	Sunburn cell formation	No protection against UVR-induced damage, protective against PUVA-induced damage	Additive protection in combination with sunscreens	Darr et al., 1996 (128)
Vitamin C	Human	Erythema (skin color and skin blood flow)	Poor protection	SPF (determined in vitro) = 1	Dreher et al., 1998 (127)

For abbreviations see Table 7.

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Table 9 Photoprotective Effects of Topically Applied Plant Extracts, Flavonoids, N-Acetyl-Cysteine and Derivatives, and Other Antioxidants In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Green tea extract	Mouse	Skin tumor incidence	Protective	Green tea contains catechin and epicatechin derivatives	Wang et al., 1991 (154)
Polypodium leucotomos (tropical fern) extract	Guinea pigs, human	Erythema (skin color)	Protection against UVR- and PUVA-induced damage	Extract with immunomodulating properties	González et al., 1996 (155)
Polypodium leucotomos (tropical fern) extract	Human	Erythema (MED), immediate pigment darkening, delayed tanning, minimal phototoxic dose, and histology	Protection against UVR- and PUVA-induced damage		González et al., 1997 (156)
Epigallocatechin-3-gallate	Mouse	Skin tumor incidence	Protective	Not immunosuppressive; isolated from green tea	Gensler et al., 1996 (157)
Apigenin	Mouse	Skin tumor incidence	Protective		Birt et al., 1997 (158)
Silymarin	Mouse	Edema, sunburn and apoptotic cell formation, and skin tumor incidence	Protective	Isolated from milk thistle plant	Katiyar et al., 1997 (159)
N-acetyl-cysteine Captopril Other thiols	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	N-acetyl-cysteine and captopril most protective thiols	Vitamin E less protective	Van den Broeke et al., 1993 (164)
N-acetyl-cysteine	Rat	DNA synthesis rate	Protective		Van den Broeke et al., 1994 (165)

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N-acetyl-cysteine Several cysteine derivatives	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	Protective	High epidermal bioavailability of N-acetyl-cysteine	Van den Broeke et al., 1995 (163)
Melatonin	Human	Erythema (skin color)	Protective	Also protective when applied after UVR exposure	Bangha et al., 1996 (204)
Melatonin	Human	Erythema (skin color)	Protective	No protection when applied after UVR irradiation; melatonin without sunscreening properties	Bangha et al., 1997 (170)
Melatonin	Human	Erythema (skin color and skin blood flow)	Protective	No protection when applied after UVR irradiation; melatonin with sunscreening properties	Dreher et al., 1998 (127,180)
Superoxide dismutase	Guinea pig	PUVA-induced erythema and edema	Protective	β–Carotene also protective; vitamin E, vitamin E acetate, and glutathione not protective	Carraro et al., 1988 (174)
Superoxide dismutase	Guinea pig	Erythema	Not protective		Hamanaka et al., 1990 (177)
Superoxide dismutase	Human	Erythema (skin color)	Protective when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Superoxide dismutase	Mice, human	PUVA-induced erythema and edema	Protective	Prolonged pretreatment	Alaoui et al., 1994 (175) Filipe et al., 1997 (176)
2,4-Hexadienol	Mouse	Skin wrinkling and sagging, and skin tumor incidence	Protective, not protective against UVA-induced skin sagging	Also other conjugated dienes tested	Bissett et al., 1990 (205)

For abbreviations, see Table 7.

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and does not prevent photodamage in mice when applied topically (137) or injected intraperitoneally (167).

A photoprotective effect for the redox couple α-lipoate/dihydrolipoate (also referred to as ''α-lipoic acid'') has been proposed for skin (168). Dihydrolipoate, the reduced form of lipoic acid, is a reductant with a more negative redox potential (−0.32 V for the couple lipoate/dihydrolipoate) than ascorbate (0.08 V for the couple dehydroascorbate/ascorbate), which is thus able to regenerate ascorbate from its oxidation products (see Fig. 2). In liposomes irradiated with solar-simulated UV light, dihydrolipoate in combination with ascorbate was shown to strongly enhance the recycling of α–tocopherol (52). It was demonstrated in hairless mice that α-lipoate readily penetrates skin and thereafter is reduced to its more potent antioxidant form, dihydrolipoate (169). Fuchs et al. reported anti-inflammatory properties of dihydrolipoate in dermatitis induced by reactive oxidants in hairless mice (168).

Regarding the pineal hormone melatonin (N-acetyl-5-methoxytryptamine), Bangha and coworkers showed a suppression of UVR-induced erythema by topical melatonin in humans (170). Besides melatonin's antioxidant (171) and dose-dependent sunscreening properties (127,170), it may also act in an immunomodulatory way (172,173). Photoprotective effects were also reported for topical application of several other substances with antioxidant properties. Interestingly, topical administration of *superoxide dismutase* SOD resulted in reduction of PUVA-induced skin reactions after single application in guinea pigs (174), or after prolonged pretreatment of murine (175) and human skin (176), respectively. In contrast, Hamanaka and associates did not observe significantly lowered UVB-induced erythema reaction after topical administration of SOD in guinea pigs (177). However, they demonstrated that, while cutaneous SOD activity was decreased in nontreated control animals after UVB exposure, topical SOD diminished this decrease in activity. Due to its high molecular weight, SOD is unlikely to penetrate into deeper skin layers. Yet, it was shown to be capable of inhibiting PUVA-induced erythema, suggesting that oxidative processes initiated at the skin surface may induce an inflammatory response in lower skin layers (123,175).

Antioxidant Combinations

The cutaneous antioxidant system is complex and far from being completely understood. As pointed out above, the system is interlinked and operates as an antioxidant network (Fig. 2). Thus, an enhanced photoprotective effect may be obtained by applying appropriate combinations of antioxidants (see Table 10). As was shown in a human study, application of vitamin C or vitamin E alone resulted in modestly decreased erythema reaction (127). However, a much more pronounced effect was obtained by combining these two vitamins. Notably, the most dramatic improvement resulted from the coformulation of melatonin to-

Table 10 Photoprotective Effects of Topically Applied Antioxidant Combinations In Vivo

Compounds	Species	Endpoint(s)	Efficacy	Remarks	Reference
Vitamins E and C, BHT, and glutathione	Mouse	Erythema (MED)	Protective	BHT alone also protective	De Rios et al., 1978, (206)
Vitamins E and C	Yorkshire pig	Sunburn cell formation	Protective	Maximal protection in combination with sunscreens	Darr et al., 1996 (128)
Vitamin E acetate and α–glycosylrutin	Human	Chemiluminescence and reflection spectrometry of experimentally provoked polymorphous light eruption	Protective	Polymorphous light eruption induced by UVA radiation	Hadshiew et al., 1997 (178)
Vitamin E acetate, α–glycosylrutin and ferulic acid					
Vitamins E and C	Human	Erythema (skin color and skin blood flow)	Protective; maximal protection when vitamins E and C are combined with melatonin	No protection when administered after UVR-exposure; melatonin with sunscreening properties	Dreher et al., 1998 (127,180)
Vitamins E and C, and melatonin					

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gether with vitamin E and vitamin C. Studying the effect of distinct mixtures of topically applied antioxidants in photodermatoses, Hadshiew and associates demonstrated that the development and severity of polymorphous light eruption were significantly reduced by administration of a combination consisting of α-glycosylrutin, ferulic acid, and tocopheryl acetate (178). The authors hypothesized that a sunscreening effect of the substances employed was negligible, and that the photoprotection observed was due to reduction of UVA-induced oxidative stress.

Whereas the photoprotective effect of topical antioxidants applied before UVR exposure has been recognized, the effect of these compounds administered after irradiation is less obvious (see Tables 7 to 10). Diminished erythema formation was reported when antioxidants, such as α–tocopherol or α–tocopherol acetate, were topically administered after UVR exposure (125,141,142,179). However, these findings are in contrast to other studies that found no diminished UVR-related skin damage when antioxidants were applied after the irradiation (105,138,170). As was shown in a recent human study by Dreher and coworkers, neither vitamin E, nor vitamin C, nor melatonin, nor combinations thereof, led to a significantly lowered erythema formation when administered after UVB exposure (180). The authors concluded that ROS-induced skin damage is a rapid event, and antioxidants possibly prevent such damage only when present in relevant concentration at the site of action (e.g., superficial skin layers) at the beginning and during occurrence of oxidative stress.

Topical Application of Substances Other Than Conventional Antioxidants

Apart from increasing the skin's antioxidant capacity by topical application of antioxidants, other substances may serve to enhance the antioxidative capacity by preventing the formation of ROS or by increasing the formation, stability, or activity of constitutive skin antioxidants. Skin contains substantial amounts of iron, and chronic exposure to UVR was shown to increase the skin levels of nonheme iron (181). Iron participates as a catalyst in the formation of the highly damaging hydroxyl radical (15). Hence, topical application of certain iron chelators such as 2-furildioxime were demonstrated to be efficient in providing photoprotection alone (182) or in combination with sunscreens (183). Furthermore, a possible role of 1,25-dihydroxy-vitamin D_3-induced formation of metallothionein in cutaneous photoprotection was reported; Hanada and coworkers found a significantly lowered level of sunburn cell formation in mouse skin after UVB exposure by topical application of the active form of vitamin D_3 (184). The authors postulated that the cysteine-rich metallothionein may act as a radical scavenger. Supplementation with selenium is a further interesting approach in reducing UVR-induced skin damage. Selenium is an essential trace element in humans

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and animals and is the required constituent for GSH peroxidase. Applying topical selenium in the form of L-selenomethionine proved to reduce acute and/or chronic skin damage in mice (185) as well as in humans (186). Topical application of L-selenomethionine led to increased skin selenium levels, whereas free selenium was apparently not absorbed (187,188).

SUMMARY AND CONCLUSION

Animal and human studies have convincingly demonstrated significant photoprotective effects of ''natural'' and synthetic antioxidants when applied topically before UVA and UVB exposure. However, particularly with respect to UVB-induced skin damage, the photoprotective effects of most antioxidants were modest as compared to sunscreens. More successful in preventing such damage were appropriate combinations of antioxidants resulting in a sustained antioxidant capacity of the skin, possibly due to antioxidant synergisms. On the other hand, regarding photoprotective effects against UVA-induced skin alterations, which are largely determined by oxidative processes (75,189–192), topical administration of antioxidants might be particularly promising (193–195). In fact, topical application of antioxidants resulted in a remarkable reduction of UVA-induced ROS generation in mice (134), and diminished UVA-induced polymorphous light eruption in humans (178). Furthermore, topical application of antioxidants, particularly of vitamin C, was reported to diminish PUVA-induced erythema and sunburn cell formation (128,138,150,155,156).

Since UVA- and UVB-induced skin damage is not solely dependent on ROS formation and their reaction with numerous skin biomolecules, topical (as well as systemic) antioxidant supplementation cannot be presumed to give complete photoprotection (196). Other ROS-independent processes, such as DNA dimer formation, will persist in causing skin damage, regardless of the effectiveness of the antioxidant(s) administered. Therefore, efficient sunscreens are indispensable in the effective prevention of skin photodamage. However, antioxidants, in combination with sunscreens (128) or anti-inflammatory agents (135), seem to be highly effective adjuncts increasing the safety and the efficacy of photoprotective products.

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12
Protective Creams

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INTRODUCTION

Contact dermatitis is the most frequent manifestation of occupational skin disease. Since the course may be chronic leading to disability, and since treatment is frequently of limited efficacy, prevention should be emphasized to reduce the incidence and prevalence of irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). Apart from total elimination of cutaneous exposure to hazardous substances and the use of gloves or protective clothing, protective creams (PC) are additional tools in an integrated concept of preventive measurements. Skin protection in the workplace consists of preexposure PCs, mild skin cleansers, and postexposure skin-care products. PCs are designed to prevent skin damage due to irritant contact; skin cleaning should remove aggressive substances from the skin; and skin care is intended to enhance epidermal barrier regeneration (1).

CHEMISTRY AND MODE OF ACTION

Protective Creams with a Physical/Chemical Mode of Action

Even in recent years the prevailing opinion has been that PCs are effective in a purely physical way. Due to their composition, a barrier is built up that cannot

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be penetrated easily. In addition, lipophilic ointments should have benefit against hydrophilic irritants and lipophobic ointments against hydrophobic irritants. Water-in-oil emulsions are recommended against water-soluble irritants such as detergents, acids, alkalics, metal-working fluids, and even plain water. On the other hand, oil-in-water emulsions are offered against lipophilic irritants such as oils, varnishes, and organic solvents.

Special investigations have been undertaken to develop preparations with a dual mode of action, combining the different effects of hydrophilic and hydrophobic ingredients or developing foamy skin protectors containing stearic acid, propylene glycol, glycerol, sorbitol, and dimethylpolysiloxane. However, these so-called ''invisable gloves'' failed in a repetitive irritation test against the anionic detergent sodium lauryl sulfate (SLS) and against the solvent toluene (TOL) (2). Other preparations include a fatty amine amide acetate that binds to negatively charged carboxyl groups of keratin and the positive fatty ammonium ion of these substances binds firmly to the negative charge of the epidermis. This is supposed to build up a firm second layer on the skin, which prevents penetration of various agents in a steric manner (3).

Protective Creams with Special Ingredients

Some ingredients purportedly have special protective properties such as natural or synthetic tannery substances, zinc oxide, talcum, chelating agents, or other substances that can bind metal ions or reduce penetration through the skin. Zinc oxide has a covering effect. Tannin is used as a skin astringent in order to increase the mechanical resistance of the skin surface against microtraumas. Additionally, tannery agents cause a local decrease of perspiration, which seems to be helpful while wearing gloves (4). The decrease of swelling is caused by direct binding of the tanning substance to keratin. Chelating agents are used in order to protect against sensitizing substances. Tartaric acid and glycine chelate chromate and reduce chrome VI to chrome III, which is less allergenic (5).

EFFICACY OF PROTECTIVE CREAMS

Though PCs are one of the most common measures to prevent CD, their actual benefit at the workplace is still regarded with skepticism (6) and debated in recent reviews (7,8). Due to the fact that PCs are not considered to be drugs, but rather cosmetics, valid methods to show their efficacy have not been legally necessary. Because of new European Union (EU) laws for cosmetic standards, producers are now forced to provide better claim support. In addition, European Community (EC) regulations require the employer to provide PCs to workers at exposed workplaces for prevention of ICD. It is in the employers' interest that this invest-

ment is not based on unfounded claims, but on scientific data. Double-blinded, placebo-controlled clinical tests of PCs are still lacking because of methodological difficulties, ethical doubts, and the enormous expenditure for tests regarding the preventive benefit of PCs in practice. Therefore, in vivo and in vitro tests are used for the evaluation of PC efficacy, even though they are not considered to be close to real workplace situations.

Since Suskind introduced the slide test to evaluate PCs in the 1950s (9) various in vitro techniques and in vivo tests on animals or human skin were developed to investigate the efficacy of PCs as preexposure skin protectors (10–12). In recent years, noninvasive biophysical measurements have achieved great importance especially for clinically weak reactions. Mahmoud and Lachapelle (13,14) showed PCs to have some effect against the acute irritative and locally toxic action of solvents using skin biopsies and Doppler flowmetry. Also using a guinea pig model, Frosch et al. (15,16) carried out cumulative irritation by SLS, sodium hydroxide (NaOH), and TOL. Irritation was measured by a visual score and biophysiological techniques (evaporimetry and Doppler velocimetry).

Considering human models for PC evaluation, Frosch et al. (17) proposed the model of a repetitive irritation test (RIT) to examine efficacy of barrier creams in a human test model. After 30 min of treatment with two different products, SLS was applied daily to the ventral forearm of healthy volunteers for 2 weeks. Cutaneous irritation was evaluated by a visual score, evaporimetry, laser-Doppler velocimetry, and colorimetry. The authors observed a significant suppression of irritancy with one of the tested creams. In a subsequent paper, Frosch and Kurte (3) reported on the RIT with a set of four standard irritants (10% SLS, 1% NaOH, 30% lactic acid, and undiluted TOL) using the midback as a larger area than the forearm. Thus, three products could be compared simultaneously to a nonpretreated control site. The irritant cutaneous reactions were quantified by erythema score, transepidermal water loss, blood flow volume, and stratum corneum hydration. The tested products demonstrated a specific profile of efficacy against the four irritants used. Using the RIT, our group showed that four products tested were very effective against 10% SLS and three products showed a partial protective effect against all ionic irritants (18). However, the necessity of a 2-week period of cumulative irritation is still discussed and a model with repeated irritation of the forearms has been evaluated for further testing (19,20). Grunewald et al. (21) developed a repetitive washing procedure with SLS on the forearms for 7 days, demonstrating protection of skin function for the creams tested. Zhai and Maibach (22) presented an in vivo method using cyanoacrylate strips of protected skin samples to measure the effectiveness of PCs against two dye indicator solutions: methylene blue in water and oil red O in ethanol, representative of model hydrophilic and lipophilic compounds. One formulation was protective against the permeation of methylene blue and oil red O while the other was protective against oil red O only.

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Recently, perfluoropolyethers were shown to have some benefit in the prevention of irritation due to hydrophilic and lipophilic substances (23). As petrolatum is effective against water-soluble and water-insoluble irritants, it was recommended as a standard substance against which PCs may be compared (24).

Although PCs have been shown to reduce ACD in sensitized individuals under experimental conditions (25,26), their use in the prevention of ACD has been disappointing under practical conditions. However, recent publications indicate a benefit for some PCs used as ''active'' creams in the prevention of ACD like nickel dermatitis or poison ivy/oak ACD (5,27–31).

APPLICATION

PCs should be applied before contact with irritants, including an application after every break; repeated application is suggested. It is clear that for PCs to be effective, they must be applied frequently and in adequate amounts to all skin areas that need protection. In particular, application should be made with attention to the interdigital spaces. In a recent study, a simple method of determining and quantifying how exactly self-application of a PC was performed at the workplace is described. Using a fluorescence technique, it was shown that application was often incomplete, especially in the dorsal aspects of the hands and wrists (32). These findings indicate that people miss certain areas. Individuals should apply the cream systematically by anatomical regions, ensuring that each region is adequately covered.

To improve daily application, instructive brochures may be given to workers but they are usually not very successful. It was shown that the fluorescence technique is also a useful tool in demonstrating the most common mistakes in conjunction with an instructive videotape (33).

ADVERSE EFFECTS AND CONTRAINDICATIONS

While some authors reported a satisfactory protective action of PCs, others found no protection from or even aggravation of ICD. A foamy skin protector was not convincing in a guinea pig model and impressed by its aggravating effect of the irritation due to NaOH (2). Also using a guinea pig model, Goh showed that treatment with PC increases skin irritation by cutting oil fluids (34). Bomann and Mellström showed that absorption of butanol through stripped skin treated with PC was higher than absorption through skin not so treated (35). Recently, a PC was shown to cause an amplification of inflammation by TOL (18) and the protective properties against systemic absorption of solvents are less than adequate (35–37).

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Besides less efficacy against irritants or even amplification of barrier damage the creams themselves can induce ICD or ACD (38,39). Preservatives, cream bases such as wool alcohols, emulsifiers, and fragrances are potential allergens. Preparations marketed as invisible glove may feign a seeming protection that causes workers at risk to be careless about contact to irritants. Additionally, it is of utmost importance to apply PCs on intact skin only. They are not intended to be used on diseased skin, due to the irritant properties of some formulations (7,40,41).

CONCLUSION

Current PCs are still not perfect. Much effort is necessary to develop products that will give more protection and less side effects. Efficacy and cosmetic acceptance are both important qualities of PCs to be used for protective success at the workplace but the knowledge how they are used correctly is a basic condition. It goes without saying that their benefit in the prevention of ICD and ACD has to be evaluated in reliable studies. Results of animal experiments may not be valid for humans, particularly when dealing with irritants, in view of their complex action mechanisms and the high interindividual variability in susceptibility of human skin (22). Regarding the various models of investigation, the validation of a sensitive, standardized, and widely accepted model proved by interlaboratory standardization or controlled clinical studies at the workplace seems to be necessary. Clearly, studies both under experimental conditions and in the workplace are needed before a rational recommendation can be made as to whether a product is safe and effective for skin protection. PCs cannot be of benefit in all cases—only against individual irritants. The data of in vitro and in vivo tests underline the importance of careful selection of PCs for specific workplaces. Choosing the wrong preparation may worsen the effect of an irritant. Based on the data presented, PCs should be used more critically due to the noxious substances used at the workplace and complete labeling of the ingredients should be given on the packages.

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13
Seborrheic Dermatitis (Dandruff)

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Dandruff and seborrheic dermatitis are often mentioned together. Dandruff is the mildest manifestation of seborrheic dermatitis and it cannot be separated from seborrheic dermatitis. Therefore, what is mentioned in the literature for seborrheic dermatitis is also true for dandruff and vice versa. Seborrheic dermatitis is characterized by inflammation and desquamation in areas with a rich supply of sebaceous glands, namely, the scalp, face, and upper trunk (1). It is a common disease and the prevalence ranges from 2 to 5% in different studies. It is more common in males than in females. The disease usually starts during puberty and is more common around 40 years of age. Seborrheic dermatitis is characterized by red scaly lesions predominantly located on the scalp, face, and upper trunk. The skin lesions are distributed on the scalp, eyebrows, nasolabial folds, cheeks, ears, presternal and interscapular regions, axillae, and groin. Around 90 to 95% of all patients have scalp lesions and lesions on glabrous skin are found in approximately 60% of the patients. The lesions are red and covered with greasy scales. Itching is common in the scalp. Complications include lichenification, secondary bacterial infection, and otitis externa. The course of seborrheic dermatitis tends to be chronic with recurrent flare-up. A seasonal variation is observed with the majority of patients being better during the summertime. Mental stress and dry air are factors that may aggravate the disease. A genetic predisposition is an important factor. Seborrheic dermatitis is seen more frequently than expected in

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patients with pityriasis versicolor, *Pityrosporum folliculitis*, Parkinson's disease, major truncal paralysis, mood depression, and acquired immunodeficiency syndrome (1).

ETIOLOGY AND PATHOGENESIS

There are now many studies indicating that *Pityrosporum ovale* (*Malassezia*) plays an important role in seborrheic dermatitis (2). Many treatment studies describe the effectiveness of antimycotics, which reduces the number of *P. ovale*; recolonization leads to a recurrence of seborrheic dermatitis. The increased incidence of seborrheic dermatitis in patients with immunosuppressive disorders suggests that the relationship between *P. ovale* and the immune system is of importance.

P. ovale can activate complement by both the classic and alternative pathway (3). The humoral immune response to *P. ovale* in patients with seborrheic dermatitis and pityriasis versicolor has been studied using different antigen preparations and different techniques (3,4). Elevated titers in patients compared to controls as well as no difference in titers have been reported (3,4). In patients with seborrheic dermatitis, a reduced lymphocyte transformation response compared to healthy controls has been reported in two studies (5,6). However, in another study an enhanced lymphocyte stimulation response compared to healthy controls was found (7). In two recently published studies, no difference in lymphocyte stimulation response was found between patients with seborrheic dermatitis and healthy controls (8,9). In an immunological screening of patients with seborrheic dermatitis, we have found low (<0.7) responses in lymphocyte transformation tests to PHA and ConA in 13 of 30 patients (10). However, a recent study was not able to confirm that (8). Ashbee et al. found a normal PHA stimulation response in patients with seborrheic dermatitis compared to controls (7). In an earlier study we found a normal, but in the lower range (<1) CD4:CD8 ratio in 26 out of 30 patients with seborrheic dermatitis (10). Ashbee et al. found a normal CD4:CD8 ratio in patients compared to controls (7). Kieffer et al. found a low CD4:CD8 ratio in 13 of 19 patients with seborrheic dermatitis (11).

In a study by Neuber et al., IL-2 and IFN-γ production by lymphocytes from patients with seborrheic dermatitis was markedly depressed and IL-10 synthesis was increased after stimulation with *P. ovale* extract (5). In another paper by Kesevan et al., the *Pityrosporum* yeast suppressed the production of the proinflammatory cytokines IL-1β, IL-6, and TNF-α (12).

In an immunohistochemical study in patients with seborrheic dermatitis deposits of complement C3c and IgG were found in the stratum corneum below

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clusters of *P. ovale* (13). The local immune response in the skin may be different from the results obtained from in vitro studies on peripheral blood mononuclear cells and may better explain the inflammatory skin reaction seen in seborrheic dermatitis. In a recently fulfilled immunohistochemical study (data are still unpublished), we found an increase in all cellular markers in both lesional and nonlesional skin from patients with seborrheic dermatitis. We found an increase in markers for NK1 and CD16 positive cells (markers for natural killer cell function) as well as an increase in complement staining indicating that an irritant or nonimmunological stimulation of the immune system is important in seborrheic dermatitis. The reaction that we saw with the interleukins was complex, showing both an increase in the production of the inflammatory interleukins IL-1α, IL-1β, IL-6, and TNF-α, as well as interleukins responsible both for a Th1 and a Th2 reaction. It is important that no major differences were seen in the number of interleukin-associated cells between lesional and nonlesional skin in seborrheic dermatitis. However, the intercellular staining was more intense in lesional skin. The staining was also much higher in patients compared to healthy controls. The immune response in the skin of patients with seborrheic dermatitis is complex, but showed some similarities with the results obtained with *Candida* infections (14,15). *P. ovale* is a member of the normal skin flora and all individuals have both a humoral and a cellular immune response to this yeast (3,8). This is probably one of the important explanations why the immune response in the skin is more complex with diseases where this organism is involved. A strong stimulation of cells with natural killer function and complement activity may partly be explained by various enzymes (e.g., lipases) produced by *P. ovale* but further studies are needed to clarify this.

TREATMENT

Seborrheic dermatitis is a chronic disease and patients should be informed about the risk for relapse and predisposing factors. Stress and winter climate have a negative effect on the majority of patients and summer and sunshine have a positive effect. In patients with neurological diseases and especially in patients with immunosuppressive disorders, seborrheic dermatitis is more resistant to therapy. In a young individual with resistant lesions always think of HIV infection. Mild corticosteroids are effective in the treatment of seborrheic dermatitis. However, the disease recurs quickly often within a few days. Antifungal therapy is effective in the treatment of seborrheic dermatitis and, because it reduces the number of *P. ovale*, the time to recurrence is increased compared to corticosteroids. Antifungal therapy should be the primary treatment for this disease.

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Antifungal therapy for *P. ovale* is effective in treating most cases of seborrheic dermatitis and prophylactic treatment with antifungal drugs reduces the recurrence rate much more than corticosteroids (2,16–25). In one study, the combination of hydrocortisone and miconazole in an alcoholic solution was significantly more effective than hydrocortisone alone in reducing the number of *P. ovale* and the recurrence rate was also significantly lower with the combination therapy; 16% with the combination compared to 82% for hydrocortisone alone (2).

Ketoconazole is very effective in vitro against *P. ovale* with minimum inhibitory concentrations (MICs) in the range of 0.02 to 0.5 μg/mL. Oral ketoconazole has been effective in a double-blind, placebo-controlled trial in patients with seborrheic dermatitis of the scalp and other areas (18). However, oral ketoconazole should be reserved for patients not responding to topical therapy. In another double-blind, placebo-controlled study, ketoconazole 2% cream has been effective in the treatment of seborrheic dermatitis of the scalp and face (17), and in a comparative study between ketoconazole and hydrocortisone cream no difference was seen in effectiveness (20).

Ketoconazole shampoo used twice weekly is very effective in treating seborrheic dermatitis of the scalp (18). In a double-blind placebo-controlled study of ketoconazole shampoo used twice weekly for 4 weeks, 89% in the ketoconazole group was cured, compared with only 14% in the placebo group (18). Ketoconazole used once weekly has also been effective in preventing recurrence of dandruff in previously treated patients. Ketoconazole shampoo has been compared to ciclopirox olamine shampoo in the treatment of seborrheic dermatitis/dandruff (24). Both shampoos were equally effective and significantly more effective than placebo. However, at a follow-up visit 2 weeks after cessation of treatment, the recurrence rate was significantly lower in the ketoconazole group compared to the ciclopirox olamine group (24).

Other topical antimycotics are effective in the treatment of seborrheic dermatitis (2,16,21–25). Shampoos containing zinc pyrithione (21), selenium sulfide (16), or bifonazole (25) are also effective and widely used. Propylene glycol solution and shampoo has also been used successfully (22).

In severe inflammatory seborrheic dermatitis, topical treatment with antifungal therapy alone may not be effective. Some of these patients respond well to oral ketoconazole or itraconazole. Another therapy that can be effective is to combine potent topical corticosteroids with topical antifungal therapy. After clearance, many of these patients will remain free of lesions on prophylactic topical antifungal treatment. When lesions are covered with thick adherent scales, keratolytic therapy, especially in the scalp, is necessary. Seborrheic dermatitis especially in the scalp and external ear canal may be secondarily infected with bacteria. Often, in these patients, topical or oral antibacterial therapy in combination with regular treatment is indicated.

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Dermatotoxicology Overview

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INTRODUCTION

Cosmeceuticals are presumably relatively ''safe.'' Adverse skin responses associated with repetitive, low-dose exposure to consumer products are all too often not accurately predicted by the required assays. The need to market products with low risk of producing dermal and systemic injury to increase consumer satisfaction has led to the development of numerous assays to rank chemicals for their ability to injure the skin. Although these assays are not routinely mandated by regulatory agencies for cosmetics and skin care, the frequency with which they are conducted and their utility warrant attention.

The field of dermatotoxicology includes measurement of absorption of materials as well as assays that evaluate the ability of topically applied chemicals to induce or promote the development of neoplasia, trigger an immune response in the skin, directly destroy the skin (corrosion), irritate the skin, produce urticaria (hives), and produce noninflammatory painful sensations. The inflammatory responses of skin are the most common chemically induced dermatoses in humans.

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DERMATOPHARMACOKINETICS: RELATION TO PREDICTIVE ASSAYS

Although the skin's barrier properties are impressive, it has been shown to be a major route of entry under some exposure situations. Interest in dermatopharmacokinetics has increased as the skin has been reconsidered to be a route for systemic administration of drugs and chemicals, as well as a route of entry for toxins. A variety of assays, both in vivo and in vitro, for measuring absorption through the skin, have been developed (1,2) and many factors that govern absorption through the skin have been determined.

A major diffusion barrier of the skin is considered to be the stratum corneum. Absorption of chemicals through shunts, openings of skin appendages, and gaps in the stratum corneum associated with these structures have been considered (3). Absorption can be described as passive diffusion across this membrane by the equation, $J = (K_m C_v D_m) \div \delta$ [rate of absorption = (vehicle/stratum corneum partition coefficient $\times$ skin surface concentration $\times$ diffusion constant of penetrant in stratum corneum) divided by thickness of stratum corneum (4)]. Other factors that affect thermodynamic activity of the solution at the skin surface (e.g., pH and temperature) may vary flux (5,6). Vehicle influence cannot be overstated; for a specific concentration of chemical, thermodynamic activity may vary by 1000-fold from one vehicle to another (6). Other factors that affect percutaneous absorption include condition of the skin (7), age, surface area to which the material is applied (8), penetrant volatility, temperature and humidity (9), substantivity, and wash-and-rub resistance to removal from the skin and binding to the skin (10).

Once a chemical has gained access to the viable epidermis, it may initiate a local effect, be absorbed into the circulation and produce an effect, or produce no local or systemic effects. The viable epidermis contains enzymes capable of metabolizing exogenous chemicals (11), including a substantial cytochrome P450 system, esterases, mixed-function oxidases, and glucuronyltransferases. Early studies conducted in vitro using whole skin indicated that enzymatic activity in skin was only a fraction of the activity of the liver. However, when the surface area of the epidermis is taken into account, then enzymatic activities of the epidermis can range from 80% to 240% of those in liver (12).

IN VIVO PERCUTANEOUS ABSORPTION ASSAYS

Percutaneous absorption can be determined by applying a known amount of chemical to a specified surface area and then measuring levels of the chemical in the urine and/or feces. Because the analytical techniques to measure the chemical are not always available and because some chemicals may be metabolized, radiolabeled chemicals, ^{14}C or ^{3}H, are often used.

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In vivo studies have been conducted in humans and other species (12). Comparison of absorption rates of a number of compounds showed that absorption rates in the rat and rabbit tend to be higher than humans and that the skin permeability of monkeys and swine more closely resembles that of humans. No significant mouse–human skin comparisons exist. Guinea pig–human comparisons offer some promise for refinement of guinea pig–human irritation and sensitization extrapolations (13). Although these differences are not predicted by any single factor, they are not unexpected in light of differences in metabolism and in routes of excretion. Therefore, the metabolic capabilities of the species should be considered when selecting an animal model and designing the experiment. Although there is no question that pharmacokinetic studies of this type in humans or animals provide the best estimate of percutaneous absorption, the cost and difficulty in conducting well-controlled studies have led to the use of other in vivo assays that are poorer predictive tools and to the development of in vitro models.

IN VITRO PERCUTANEOUS PENETRATION ASSAYS

The excised skin of humans or animals can be used to measure penetration of chemicals. In vitro assays using excised skin utilize specially designed diffusion cells (1,14,15). The skin is stretched over the opening of a collecting receptacle, epidermal side up. The chemical is applied to the epidermis and fluid from the receptacle is assayed to measure the penetration of the chemical. This type of in vitro assay offers some advantages over in vivo assays: highly toxic compounds can be studied in human skin, large numbers of cells can be run simultaneously, diffusion through the membrane (eliminating other pharmacokinetic factors) can be studied, and these assays may be easier to conduct.

Comparison of penetration rates obtained from in vitro and in vivo assays have been made (1), often with a good correlation; however, with some, correlation was poor. Differences in the methods for some compounds could be explained on the basis of solubilities in the receptacle fluid and blood; others could not be explained. Skin of the weanling pig and miniature swine appear to be good in vitro models for most compounds (2). Although a limited number of studies have been reported, the skin of monkeys also appears to be a good model (8). Rat skin appears to be a good model for some compounds; however, when differences have been noted, they have been large.

ALLERGIC CONTACT DERMATITIS

Jadassohn (16) demonstrated that in some patients dermatitis was due to increased sensitivity following repeated contact with a substance and not the irritant proper-

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ties of the material. By 1930, a procedure for producing this hypersensitivity to chemicals in guinea pigs had been developed (17). Landsteiner and associates demonstrated that low-molecular-weight chemicals conjugate with proteins to form an antigen that stimulates the immune system to form a hyperreactive state (18); immunogenicity is related to chemical structure (19); and two types of immunological response exist, one transferable by serum and another transferred by suspensions of white blood cells (20). These mechanisms are succinctly provided by von Blomberg (21).

Appropriate planning and execution of predictive sensitization assays is critical. The first priority is to choose an appropriate experimental design. A common error in choosing an animal assay is using Freund's complete adjuvant (FCA) when setting dose–response relationships. The adjuvant provides such sensitivity that dose–effect relationships are muted. Choice of dose and vehicle appropriate to the assay and the study question is the second priority. Although dose must be high enough to ensure penetration, it must be below the threshold at challenge to avoid misinterpretation of irritant inflammation as allergic. Knowing the irritation potential of compounds will allow the investigator to design and execute these studies appropriately. Vehicle choice determines in part the absorption of the test material and can influence sensitization rate, ability to elicit response at challenge, and the irritation threshold.

QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIPS

Quantitative structure activity relationships (QSAR) describe a relationship of chemical structure to biological activity—in this case allergic contact dermatitis. A computer-assisted database describing the chemical structure and physicochemical parameters of an array of chemicals provides a facile approach to designing appropriate in vitro, animal, and human sensitization studies (22). In essence, searching the prior experimental data permits not only determination of relationship between structures and allergenicity, but provides insight into planning a given experiment. For example, if a closely related structure to the chemical of interest has been shown to be a potent allergen, the new chemical may be examined with a more quantitative assay.

GUINEA PIG SENSITIZATION TESTS

Predictive animal tests to determine the potential of substances to induce delayed hypersensitivity in humans are conducted most often in guinea pigs. Several tests

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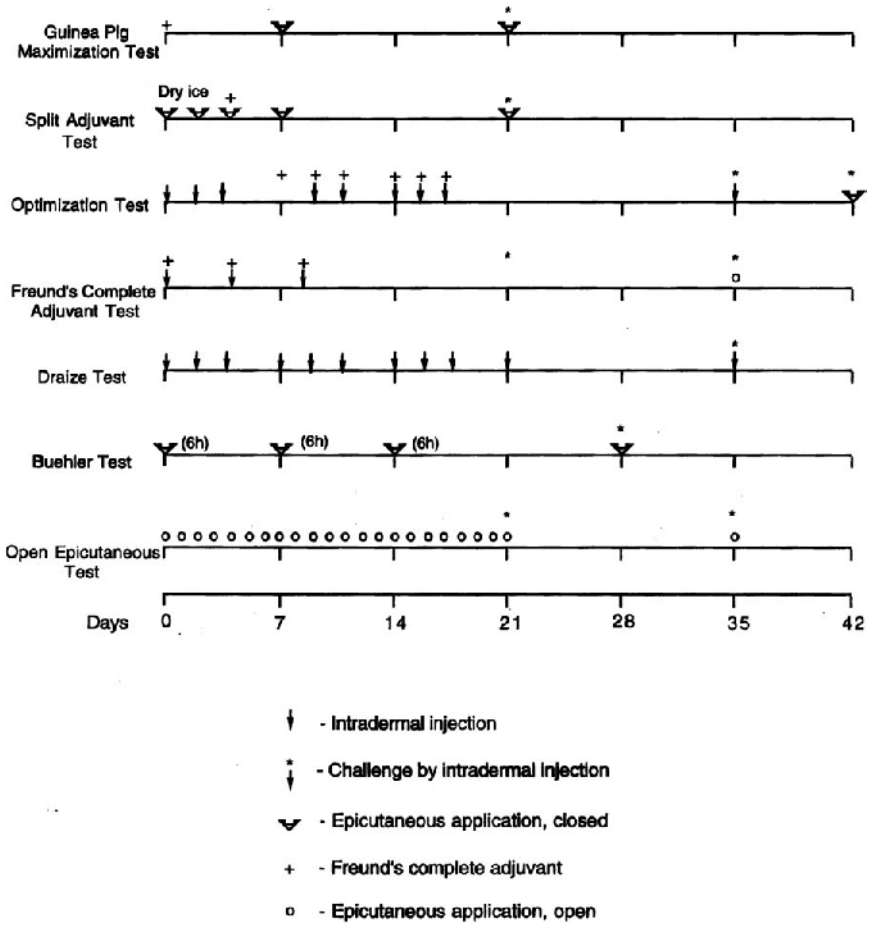
Table 1 Features of Most Commonly Used Assays to Predict Sensitization



have been described. All utilize young (1–3 months), randomly bred, albino guinea pigs. Most visually evaluate the responses using descriptive scales for erythema and edema. The tests differ significantly in route of exposure, use of adjuvants, induction interval, and number of exposures. The principal features of the most commonly used assays and assays acceptable to regulatory agencies to predict sensitization are summarized in Table 1 (23–25).

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DRAIZE TEST

The Draize sensitization test (DT) (26,27) was the first predictive sensitization test accepted by regulatory agencies. One flank of 20 guinea pigs is shaved and 0.05 ml of a 0.1% solution of test material in saline, paraffin oil, or polyethylene glycol is injected into the anterior flank on day 0. Every other day through day 20, 0.1 mL of the test solution is injected into a new site on the same flank. After a 2-week rest period, the opposite untreated flank is shaved and 0.05 mL of test solution is injected into each animal (challenge). Twenty previously untreated controls are injected at the same time. The test site is visually evaluated 24 h and 48 h after injection. A larger or more intensely erythematous response than that of controls is considered a positive response.

OPEN EPICUTANEOUS TEST

The open epicutaneous test (OET) (28) simulates the conditions of human use by utilizing topical application of the test material. The procedure determines the doses required to induce sensitization and to elicit a response in sensitized animals. The irritancy profile is determined by applying 0.025 mL of varying concentrations to a 2 cm^2 area of the shaved flanks of six to eight guinea pigs. Test sites are visually evaluated 24 h after application of test solutions to erythema. The dose not causing a reaction in any animal (maximal nonirritant concentration) and the dose causing a reaction in 25% of the animals (minimal irritant concentration) are determined. During induction, test solution is applied to flank skin of six to eight guinea pigs for 3 weeks, or 5 times a week for 4 weeks. A control group is treated with vehicle only. The highest dose tested is usually the minimal irritant concentration and lower doses are based on usage concentration or a stepwise reduction. Twenty-four to 72 h after the last induction treatment, each animal is challenged on the untreated flank. The minimal irritant concentration, the maximum nonirritant concentration and five solutions of lower concentrations are applied. Skin reactions are read on an all-or-none basis at 24, 48, and 72 h after application. The maximum nonirritating concentration in the vehicle-treated group is calculated. Animals in test groups that develop inflammatory responses to lower concentrations are considered sensitized.

BUEHLER TEST

The Buehler test (occlusion only) (29) also employs topical application. An absorbent patch, or vehicle alone, is placed on the shaved flanks of 10 to 20 guinea pigs. Test concentration varies from undiluted to usage levels. A concentration

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that produces slight erythema is optimum and is selected based on an irritancy screen conducted in other animals. This procedure is repeated 7 and 14 days after the initial exposure. Two weeks after the last induction patch, animals are challenged with patches saturated with a nonirritating concentration of test material and with the vehicle. After 6 h, the patch is removed and the area depilated. Test sites are visually evaluated 24 and 48 h after patch removal. Animals developing erythematous responses are considered sensitized (if irritant control animals do not respond).

FREUND'S COMPLETE ADJUVANT TEST

Freund's complete adjuvant test (FCAT) is an intradermal technique incorporating test material in a 50/50 mixture of FCA and distilled water. The description is summarized by Klecak (30).

OPTIMIZATION TEST

The optimization test resembles the DT, but incorporates the use of adjuvant for some induction injections and both intradermal and topical challenges (27). On day 1, one injection into the shaved flank and one into a shaved area of dorsal skin are given. Two and 4 days later, one injection into a new dorsal site is given. The test material is administered in saline during the first week. During the second and third weeks, test material is administered in FCA/saline every other day to a shaved area over the shoulders. Twenty test animals are treated and 20 controls are injected with vehicle alone. Thickness of a skinfold over the injection site is measured with a caliper. Any animal developing a reaction volume at challenge greater than the mean plus 1 standard deviation during induction is considered sensitized. A second challenge is conducted 45 days after the first injection. A nonirritating concentration of the test material in a suitable vehicle is applied to the flank skin, away from injection sites. Reactions are visually evaluated after 24 h using the 4-point erythema scale of the Draize primary irritancy scale. To classify materials as strong/moderate/weak/nonsensitizer, a classification scheme has been devised using results of exact Fisher test and number of positives detected.

SPLIT ADJUVANT TEST

The split adjuvant test (30) utilizes skin damage and FCA as adjuvants. An area of back skin of 10 to 20 guinea pigs is shaved to glistening, then treated with

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dry ice for 5 to 10 s. A layer of loose mesh gauze and stretch adhesive with a 2×2 cm^2 opening over the shaved area is placed around the animal. Approximately 0.2 mL of creams or solid test material, 0.1 mL if liquid, is spread over the test site and occluded. The concentration tested varies by irritancy potential, use conditions, etc. Two days later, the occlusive filter paper is removed, the test material reapplied, and the covering replaced. On day 4, the filter paper cover is removed, two injections of 0.075 mL FCA are given into the edges of the test site, the test material reapplied, and the site resealed. On day 7, the test material is reapplied and on day 9 the dressing is removed. Twenty-two days after the initial treatment, animals are challenged by topical application of 0.5 mL of test material to a 2×2 cm^2 area of the shaved midback. A group of naive controls, 10 to 20 animals, is treated by the same procedure at challenge. Twenty-four, 48, and 72 h after application, the dressing is removed and the test site is visually evaluated using a descriptive visual scale. Sensitization of individual animals is indicated by significantly stronger reactions than those of controls.

GUINEA PIG MAXIMIZATION TEST

The guinea pig maximization test (GPMT) (27,30) combines FCA, irritancy, intradermal injection, and occlusive topical application during the induction period. Two identical sets of 0.1-mL intradermal injections of 50/50 FCA/water, test material in water, paraffin oil, or propylene glycol and the same dose of test material in FCA/vehicle are placed on a filter paper, placed over the shaved injection site, covered with approximately 4×8 cm occlusive surgical tape, and secured in place. If the test material is nonirritating, the test site is pretreated with 10% sodium lauryl sulfate (SLS) in petrolatum on day 6 to provoke an irritant reaction. After 48 h, test and control (vehicle alone) animals are challenged on the shaved flank with the highest nonirritating concentration and with the vehicle. Solutions are applied to filter paper secured in place and patches removed 24 h later. Reactions are visually evaluated 24 and 48 h after patch removal. Reactions are considered positive when they are more intense than the response to vehicle and the responses to the test materials in controls. The test material is rated as a weak-to-extreme sensitizer, based on the incidence of positives in the test group (Table 2).

HUMAN SENSITIZATION ASSAYS

Chemicals can be tested for their ability to induce contact hypersensitivity in panels of human volunteers from whom informed consent is obtained. Allergic contact dermatitis to materials already in commercial use is sometimes detected

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Table 2 Guinea Pig Maximization Test (GPMT) Rating of Weak-to-Extreme Sensitizers

Sensitization rate (%)	Grade	Class
0–8	I	Weak
9–28	II	Mild
29–64	III	Moderate
65–80	IV	Strong
81–100	V	Extreme

by early induction patches. This does not reflect the particular test material's ability to induce sensitization. It merely indicates that under patch conditions, the material may elicit a response in presensitized individuals.

There are four basic predictive human sensitization tests in current use: (1) a single induction/single challenge patch test; (2) repeated insult patch test (RIPT); (3) RIPT with continuous exposure (modified Draize); and (4) the maximization test, all of which use similar customized patches (31,32). Principal features of human sensitization assays are summarized in Table 3. For assays other than maximization, 150 to 200 subjects are usually tested. Henderson and Riley (33) statistically showed that if no positive reactions are observed in 200 randomly selected subjects, as many as 15/1000 of the general population may react (95% confidence). As sample size is reduced, the likelihood of unpredicted adverse reactions in the general population increases.

REPEAT INSULT PATCH TESTS

In the Draize human sensitization test (34), an occlusive patch containing the test material is applied to the upper arm or upper back of 200 volunteers for 48 h. The test site is evaluated at patch removal for erythema and edema. This process is repeated until a total of 9 to 10 patches have been applied. Ten to 14 days after application of the last induction patches, subjects are challenged via a patch applied to a new site for 48 h. Sites are visually evaluated at removal of the patch and the response at challenge is compared to the response to patches applied early in induction.

MODIFIED DRAIZE HUMAN SENSITIZATION TEST

The RIPT procedure was modified to provide continuous patch exposure to the test material during a 3-week induction period (35,36). Patches are applied to

Table 3 Principal Features of Human Sensitization Assays

Test	No. subjects	Concentration/ amount of test material	Vehicle	Skin site	Patch type	*Induction* No. patches	Duration	Rest	Challenge
Schwartz	200	Fabric			Fabric	1	5 days	10 days	48-h patch; observe 10 days
Schwartz	200	1-in. fabric, liquid or powder		Arm, thigh or back	Cellophane covered with 2 × 2 in. Elastoplast	1	72 h	7–10 days	72 h; same site; observe 3 days
''Prophetic'' Schwartz-Peck	200	1/4-in.^{2} 4-ply gauze, liquid saturated[a]	Petrolatum or corn oil	Arm or back	1-in.^{2} nonwaterproof cellophane covered with 2-in.^{2} adhesive plaster	1	24, 72, or 96 h	10–14 days	48-h; observe 3 days: compare new and old formulas
''Repeated insult'' Shelanski	200	Proportional to area of ultimate use	Mineral oil		Occlusion: follows Schwartz test	10–15	24 h every other day: same site	2–3 weeks	48-h patch
''Repeated insult'' Draize	100 males 100 females	0.5 mL or 0.5 g		Arm or back	1 in.^{2}	10	24 h alternate days	10–14 days	Repeat patch on new site
Modified Draize	200	0.5 mL or 0.5 g high concentration	Petrolatum	Arm	Square BandAid, no perforations	10	48 h	2 weeks	Patch on new site 72 h with nonirritant concentration
''Maximization'' (Kligman)	25	1 mL 5% SLS[b], followed by 1 mL 25% test material	Petrolatum	Forearm or calf	1.5-in.^{2} Webril occluded with Blenderm. held with perforated plastic tape	5 (same site)	24 h SLS followed by 48-h test material for each of 5 inducing applications	10 days	1-in.^{2} patch on lower back or forearm: 0.4 mL of 10% SLS for 1 h followed by 0.4 mL of 10% test material for 48 h
Modified ''maximization''	25	Same as maximization	Petrolatum	Forearm or calf	Same as maximization	7	24 h SLS followed by 48-h test material for each of 7 inducing applications	10 days	2% SLS for 0.5 h followed by 48-h patch with test material

[a] Modified for solids, powders, ointment and cosmetics. Concentration, amount, area and site of application are considered important in evaluating results. Authors recommended that cosmetics be tested uncovered.

[b] Sodium lauryl sulfate (SLS) pretreatment is used to produce moderate inflammation. SLS is mixed with test material when compatible. SLS is eliminated when the test material is a strong irritant. Table modified from Patrick E, Maibach HI. Predictive skin irritation tests in animals and humans. In: Marzulli FN, Maibach HI, eds. Dermatotoxicology, 3rd ed. New York: Hemisphere Publishing 1991:201–222.

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the outer upper arm each Monday, Wednesday, and Friday, until a total of 9 to 10 patches have been applied. Fresh patches are applied to the same site unless moderate inflammation has developed when the patches should be placed on adjacent noninflamed skin. This produces a continuous exposure of 504 to 552 h compared to a total exposure period of 216 to 240 h for RIPT of comparable induction periods. In addition, induction concentrations are increased to levels above usage exposure. Two weeks after induction, subjects are challenged by exposure of a new site to a patch for 48 to 72 h at a nonirritating concentration. Test sites are evaluated at 0 and 24 h after removal.

IRRITANT DERMATITIS

Historically, skin irritation has been described by exclusion as localized inflammation not mediated by either sensitized lymphocytes or by antibodies (i.e., nonimmunogenic). Application of some chemicals directly destroys tissue, producing skin necrosis at the site of application (i.e., corrosive chemicals). Chemicals may disrupt cell functions and/or trigger the release, formation, or activation of autocoids that produce local increases in blood flow, increase vascular permeability, attract white blood cells in the area, or directly damage cells. The additive effects of these mediators result in local skin inflammation (i.e., acute irritants). A number of as-yet poorly defined pathways involving different processes of mediator generation appear to exist. Although no agent has yet met all the criteria to establish it as a mediator of skin irritation, histamine, 5–hydroxytryptamine, prostaglandins, leukotrienes, kinins, complement, reactive oxygen species and products of white blood cells have been strongly implicated as mediators of some irritant reactions (37).

Some chemicals do not produce acute irritation from a single exposure but may produce inflammation following repeated application to the same area of skin [cumulative irritation (38)]. Studies on skin corrosion are conducted in animals, using standardized protocols as it is not appropriate to conduct screening studies in humans. But acute irritation is sometimes evaluated in humans after animal studies have been completed. Tests for cumulative irritation in both animals and humans have been reported.

IN VITRO ASSAYS

Numerous in vitro assays for irritation exist. Rougier et al. summarize these assays and offer guidelines as to their potential validation (39).

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IRRITATION TESTS IN ANIMALS

Draize-Type Tests

Primary irritation and corrosion are most often evaluated by modifications of the method described by Draize (24). The Federal Hazardous Substance Act (FHSA) adopted one modification as a standard procedure (22). The backs of six albino rabbits are clipped free of hair. Each undiluted material is tested on two 1-in.^{2} sites on the same animal (one site is intact and one is abraded in such a way that the stratum corneum is opened but no bleeding produced). Each test site is covered with two layers of 1-in.^{2} surgical gauze and secured in place. The entire trunk of the animal is then wrapped with rubberized cloth or other occlusive impervious material to retard evaporation of the substances and hold the patches in position. Twenty-four and 48 h after application the wrappings are removed and the test sites evaluated for erythema and edema, using a prescribed scale. Modifications of the Draize procedure that have been proposed include changing the species tested (40), reduction of exposure period, use of fewer animals and testing on intact skin only (41). Several governmental bodies utilized their own modification of the Draize procedure for regulatory decisions. The FHSA, DOT, Environmental Protection Agency (EPA), Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), and OECD guidelines are contrasted to the original Draize methods. All Draize-type tests are used to evaluate corrosion as well as irritation. When severe reactions that may not be reversible are noted, test sites are observed for a longer period. Delayed evaluations are usually made on days 7 and 14, but maybe as late as 35 days.

Non-Draize Animal Studies

Animal assays to evaluate the ability of chemicals to produce cumulative irritation have been developed (42). Those assays used often are not as well standardized as Draize-type tests and many variables have been introduced by multiple investigators.

Repeat application patch tests in which diluted materials are applied to the same site each day for 15 to 21 days have been reported using several species (the guinea pig or rabbit being most commonly used) (42). Because the degree of occlusion is an important determinant of percutaneous penetration, the choice of covering materials may determine the sensitivity of a given test (43). A reference material of similar use or one that produces a known effect in humans is included in almost all repeat application procedures. Test sites are evaluated for erythema and edema, either using the scales of the Draize-type tests or more descriptive scales developed by the investigator.

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Human Irritation Tests

Because only a small area of skin need be tested, it is possible to conduct predictive irritation assays in humans, provided systemic toxicity (from absorption) is low. Human tests are preferred to animal tests in some cases because of the uncertainties of interspecies extrapolation. Many forms of a single application patch test have been published. Custom-made apparatus to hold the test material have been designed (29,43). Duration of patch exposure has varied between 1 and 72 h. The single application patch procedure outlined by the National Academy of Sciences (NAS) Publication 1138 (44) incorporates important aspects of assays. For new materials or volatiles, a relatively nonocclusive tape (e.g., Micropore, Dermical, or Scanpore) should be used. Increasing the degree of occlusion with occlusive tapes (e.g., Blenderm) or chamber devices generally increases the severity of responses. A 4-h exposure period was suggested by the NAS panel. However, it is desirable to test new materials and volatiles for shorter periods (30 min to 1 h) and many investigators apply materials intended for skin contact between 24- and 48-h periods. After the period of exposure, the patches should be removed and the area cleaned with water to remove any residue. Responses are evaluated 30 min to 1 h and 24 h (to allow hydration and pressure effects to subside) after patch removal. Persistent reactions may be evaluated for 3 to 4 days. The Draize scales for erythema and edema have no provision for scoring papular, vesicular, or bullous responses. Therefore, integrated scales ranging from 4 to 16 points have been published and are generally preferred to the Draize scales.

Most multiple application patch tests were patterned after human sensitization studies with 24-h exposures, with or without a rest period between patches. The early work of Kligman and Wooding (45) forms the basis for the irritant dose 50 (ID_{50}) comparative system.

The cumulative irritation assay (46) was used to compare antiperspirants, deodorants, and bath oils to provide guidance for product development. A 1-in.^{2} patch of Webril was saturated with test compound and applied to the skin of the upper back. After 24 h, the patch was removed, the area evaluated, and a fresh patch applied. The procedure was repeated daily for up to 21 days. The IT_{50} [as described by Kligman and Wooding (45)] was used to evaluate and compare test materials. Modifications of the cumulative irritation assay have been reported (44,47) and newer chamber devices have replaced Webril with occlusive tape by some. Many variables of the chosen test procedure (e.g., vehicle, type of patch, concentration tested) may modify the intensity of the response (48,49). Differences in intensity of responses have also been linked to differences in age (50), sex (50), and race (51).

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CONTACT URTICARIA SYNDROME

Contact urticaria syndrome (CUS) has been defined as a wheal-and-flare response that develops within 30 to 60 min after exposure of the skin to certain agents (52,53). Symptoms of immediate contact reactions can be classified according to their morphology and severity:

Itching, tingling, and burning with erythema is the weakest type of immediate contact reaction.
Local wheal and flare with tingling and itching represents the prototype reaction of contact urticaria.
Generalized urticaria after local contact is rare, but can occur from strong urticaria.
Symptoms in other organs can appear with the skin symptoms in cases of immunological contact urticaria syndrome.

The strength of the reactions may vary greatly and often the whole range of local symptoms can be seen from the same substance if different concentrations are used (54). In addition, a certain concentration of contact urticant may produce strong edema and erythema reactions on the skin of the upper back and face but only erythema on the volar surfaces of the lower arms or legs. In some cases, contact urticaria can be demonstrated only on damaged or previously eczematous skin and it can be part of the mechanism responsible for maintenance of chronic eczemas (25). Because of the risk of systemic reactions (e.g., anaphylaxis), human diagnostic tests should only be performed by experienced personnel with facilities for resuscitation on hand. Contact urticaria has been divided into two main types on the basis of proposed pathophysiological mechanisms, namely, nonimmunological and immunological (55).

NONIMMUNOLOGICAL CONTACT URTICARIA

Nonimmunological contact urticaria (NICU) is the most common form and occurs without previous exposure in most individuals. The reaction remains localized and does not cause systemic symptoms to spread to become generalized urticaria. Typically, the strength of this type of contact urticaria reaction varies from erythema to a generalized urticarial response, depending on the concentration, skin site, and substance. The mechanism of nonimmunological contact urticaria has not been delineated, but a direct influence on dermal vessel walls or a nonantibody-mediated release of histamine, prostaglandins, leukotrienes, substance P, other inflammatory mediators, or different combinations of these mediators represents possible mechanisms (56). The most potent and best studied substances producing nonimmunological contact urticaria are benzoic acid, cinnamic

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acid, cinnamic aldehyde, and nicotinic esters. Under optimal conditions, more than half of a random sample of individuals show local edema and erythema reactions within 45 min of application of these substances if the concentration is high enough.

IMMUNOLOGICAL CONTACT URTICARIA

Immunological contact urticaria (ICU) is an immediate type 1 allergic reaction (52). The molecules of a contact urticant react with specific IgE molecules attached to mast-cell membranes. The cutaneous symptoms are elicited by vasoactive substances, mainly histamine, released from mast cells. Other mediators of inflammation may influence the degree of response. Immunological contact urticaria reaction can extend beyond the contact site and generalized urticaria may be accompanied by other symptoms, such as rhinitis, conjunctivitis, asthma, and even anaphylactic shock. The term ''contact urticaria syndrome'' was therefore suggested by Maibach and Johnson (55). Fortunately, the appearance of systemic symptoms is rare, but it may be seen in cases of strong hypersensitivity or in a widespread exposure and abundant percutaneous absorption of an allergen.

GUINEA PIG EAR SWELLING TEST

Predictive assays for evaluating the ability of materials to produce nonimmunological contact urticaria have been developed. Lahti and Maibach (57) developed an assay in guinea pigs using materials known to produce urticaria in humans. One-tenth of a milliliter of the material (or control solvent) is applied to one ear of the animal. Ear thickness is measured before application and then every 15 min for 1 or 2 h after application. The maximum response is a 100% increase in ear thickness (within 50 min after application).

Materials can also be screened for nonimmunological contact urticaria in humans. A small amount of the test material is applied to a marked site on the forehead and the vehicle is applied to a parallel site. The areas are evaluated at about 20 to 39 min after application for erythema and/or edema (52).

Differentiation between nonspecific irritant reactions and contact urticaria may be difficult. Strong irritants (e.g., hydrochloric acid, lactic acid, and phenol), can cause clear-cut immediate whealing if the concentration is high enough, but the reactions do not usually fade away quickly. Instead, they are followed by signs of irritation (erythema, scaling, or crusting) 24 h later. Some substances have only irritant properties (e.g., benzoic acid and nicotinic acid esters), some are pure irritants (e.g., SLS), and some have both these features [e.g., dimethyl sulfoxide (DMSO) and formaldehyde].

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TRIMELLITIC ANHYDRIDE-SENSITIVE MOUSE ASSAY

The respiratory allergen, trimellitic anhydride (TMA), has been shown to induce IgE production and immediate ear swelling in mice sensitized to it (58). These authors showed that TMA-sensitized mice have a biphasic ear-swelling response with early (30 min–2 h) and late (24–48 h) phases after topical application of TMA. It was concluded that the first swelling was due to either immediate-type immunological processes or NICU, and the second swelling was due to contact hypersensitivity (i.e., allergic contact dermatitis). This relatively simple method could possibly be a useful tool to study the pharmacology of CU. However, further validation of this model is still required.

SUBJECTIVE IRRITATION AND PARESTHESIA

Cutaneous application of some chemicals elicits sensory discomfort—tingling and burning without visible inflammation. This noninflammatory painful response has been termed subjective irritation (59). Materials reported to produce subjective irritation include DMSO, salicylic acid, amyl–dimethyl-p-amino benzoic acid and 2-ethoxy ethyl-p-methoxy cinnamate, which are ingredients of cosmetics and over-the-counter drugs. Pyrethroids, a group of broad-spectrum insecticides, produce a similar condition that may lead to temporary numbness, which has been called paresthesia (60). Only a portion of the human population seems to develop nonpyrethroid subjective irritation. For example, only 20% of subjects exposed to 5% aqueous lactic acid in a hot, humid environment developed stinging response (59). Prior skin damage (e.g., sunburn, pretreatment with surfactants, and tape stripping) increases the intensity of responses in stingers. Recent data show that stingers develop stronger reactions to materials causing nonimmunological contact urticaria. The mechanisms by which materials produce subjective irritation have not been extensively investigated. Pyrethroids directly act on the axon, interfering with the channel-gating mechanism and impulse firing (61). It has been suggested that agents causing subjective irritation act via a similar mechanism because no visible inflammation is present.

An animal model was developed to rate paresthesia to pyrethroids and may be useful for other agents (60). Both flanks of 300 to 450 g guinea pigs are shaved and 100 μL of the test material (or vehicle) is spread over approximately 30 mm^2 on separate flanks. The animal's behavior is monitored by an unmanned video camera for 5 min at 0.5, 1, 2, 4, and 6 h after application. Subsequently, the film is analyzed for the number of full turns of the head made, usually accompanied by attempted licking and biting of the application sites. Using this technique, it was possible to rank pyrethroids for their ability to produce paresthesia and corresponded to the ranking available from human exposure.

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HUMAN ASSAY

As originally published, the human subjective irritation assay required the use of a 110°F environmental chamber with 80% relative humidity (59). Sweat was removed from the nasolabial fold and cheek, then a 5% aqueous solution of lactic acid was briskly rubbed over the area. Those who reported stinging for 3 to 5 min within the first 15 min were designated as stingers and were used for subsequent tests. Subjects were asked to evaluate the degree of stinging as 0 = no stinging; 1 = slight stinging; 2 = moderate stinging; 3 = severe stinging.

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The Legal Distinction in the United States Between a Cosmetic and a Drug

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The Federal Food, Drug, and Cosmetic Act (FD&C Act) establishes substantially different regulatory requirements in the United States for cosmetics and drugs. This chapter traces the history of U.S. regulatory policy for these two categories of products, discusses the application of U.S. law to products that fall within both categories at the same time (i.e., cosmetic drugs†), and considers potential strategies for resolving the long-standing concern that the drug provisions of the Act impose overly stringent requirements on cosmetic drugs.

HISTORICAL OVERVIEW

Cosmetic products have been used by humans since before recorded history. Archeologists date the earliest discovered cosmetics to about 10,000 B.C. (1). By the height of the ancient Roman civilization, virtually all types of cosmetics that

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† The term ''cosmeceutical'' has no legal or regulatory meaning and no other accepted definition, and therefore is not used in this chapter.

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are available today were in widespread use. In his landmark *Natural History*, Pliny the Elder (23–79 A.D.) described such cosmetic products as hair dye, eyelash dye, eyebrow dye, freckle removers, rouge, deodorants and antiperspirants, depilatories, wrinkle removers, hair preservatives and restorers, bust firmers, sunburn products, complexion aids, moisturizers, mouthwashes and breath fresheners, toothpaste, face powder, and perfume (2). Cosmetics have continued to be widely used from these ancient times to the present.

During the 19th century, virtually all government regulation of private enterprise in the United States was conducted at the city, county, and state levels. Because of the Supreme Court's narrow interpretation of the power of the federal government to regulate interstate commerce, federal laws regulating consumer products did not emerge until the first decade of the 20th century. Thus, the first laws explicitly regulating cosmetics were enacted by the states. The earliest known state regulatory law explicitly mentioning cosmetics was enacted by Massachusetts in 1886. That law included all cosmetics within the statutory definition of a drug, thus imposing the same regulatory requirements on both cosmetics and drugs (3).

From 1879 through 1906, Congress held hearings and debated the enactment of a federal food and drug law (4). Although bills introduced in Congress during 1898–1900 explicitly defined the term ''drug'' to include all cosmetics (5), the inclusion of cosmetics was deleted from the drug definition in 1900 as part of a legislative compromise (6). As a result, cosmetics were not included when the legislation was finally enacted as the Federal Food and Drugs Act of 1906 (7).

Implementation of the 1906 Act was delegated by Congress to the U.S. Department of Agriculture (USDA). Subsequently, it was redelegated to the Federal Security Agency (FSA), then the Department of Health, Education, and Welfare (DHEW), and now the Department of Health and Human Services (DHHS). Since 1930, the specific agency responsible for the 1906 Act and its successor statute, the Federal Food, Drug, and Cosmetic Act of 1938 (8) has been the Food and Drug Administration (FDA) (9). For editorial purposes, throughout this chapter all references to the agencies and departments responsible for implementing federal food and drug laws shall be to the FDA.

Not long after enactment of the 1906 Act, the FDA concluded that its jurisdiction should be expanded to include both cosmetics and medical devices (10). When the Roosevelt Administration introduced a bill to replace the 1906 Act (11), cosmetics were included (12) through a separate definition and separate regulatory requirements. Although the provisions relating to cosmetics were revised periodically during the 5 years of congressional consideration, the separate definition and separate regulatory requirements were retained in the final FD&C Act when it was enacted in 1938 (13). In the intervening 62 years, these provisions have not been amended.

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LEGISLATIVE HISTORY OF THE COSMETIC AND DRUG PROVISIONS OF THE 1938 ACT

The 1906 Act had defined a drug to include:

> . . . all medicine and preparations recognized in United States Pharmacopoeia or National Formulary for internal or external use, and any substance or mixture of substances intended to be used for the cure, mitigation, or prevention of disease of either man or other animals (14).

From the time that the legislation that ultimately became the FD&C Act was initially introduced until it was finally enacted, substantial attention was focused on the specific definitions of food, drug, and cosmetics, and the interaction among these three definitions. Out of these deliberations, the following important principles and policies emerged.

First, the 1938 Act, like the 1906 Act, classified products according to their intended use. In a paragraph from the 1935 Senate Report on the legislation, Congress established the policy that the representations of the sellers with respect to a product would determine its classification:

> The use to which the product is to be put will determine the category into which it will fall. If it is to be used only as food it will come within the definition of food and none other. If it contains nutritive ingredients but is sold for drug use only, as clearly shown by the labeling and advertising, it will come within the definition of drug, but not that of food. If it is sold to be used both as a food and for the prevention or treatment of disease it would satisfy both definitions and be subject to the substantive requirements for both. The manufacturer of the article, through his representations in connection with its sale, can determine the use to which the article is to be put. For example, the manufacturer of a laxative which is a medicated candy or chewing gum can bring his product within the definition of drug and escape that of food by representing the article fairly and unequivocally as a drug product (15).

This principle remains the touchstone for product classification under the 1938 Act.

Second, from the outset, the FDA sought to expand the definition of a drug from the narrow definition included in the 1906 Act. The 1906 Act limited the drug definition to products intended to prevent or treat disease. The FDA was concerned that, although it was able to regulate food products represented for use in weight reduction, it could not exert jurisdiction over nonfood chemicals represented for the same purpose because obesity was not regarded as a disease. Accordingly, from the initial bill to the final law, the drug definition was expanded to include articles ''intended to affect the structure or any function of the body of man or other animals'' (16).

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Third, Congress determined that the definitions of food, drugs, and cosmetics should not be mutually exclusive. Because the representations made for the product would determine the proper classification of the product, and thus classification was within the sole control of the seller, Congress concluded that the product should be subject to whatever statutory requirements are established for whatever product classifications applied, based upon those representations:

> It has not been considered necessary to specify that the definitions of food, drug, and cosmetic shall not be construed, other than to the extent expressly provided, as mutually exclusive. The present law does not have such a clause relating to the definitions of food and drug and there has never been a court decision to the effect that these definitions are mutually exclusive, despite the fact that repeated actions have been brought, for example, against filthy foods bearing unwarranted therapeutic claims, alleging these products to be adulterated as food because of their filth, and misbranded as drugs because of their false and fraudulent therapeutic claims (17).

Thus, dual and even triple classification of a product as a food, drug, and cosmetic was contemplated by Congress under the 1938 Act.

Fourth, Congress realized that there must be one exception to the general rule of nonexclusive definitions. All food is intended to affect the structure or function of the human body. Accordingly, Congress explicitly excluded food from the structure/function prong of the drug definition, but not from the disease prong.

In the Senate debate on the legislation in April 1935, the exclusion of food from the structure/function prong of the drug definition was expanded, without discussion, to include cosmetics (18). That bill was not passed by the House of Representatives, however, and no subsequent legislation retained the cosmetic exclusion. Accordingly, any cosmetic represented to affect the structure or function of the human body is classified as a drug as well as a cosmetic and must meet the statutory requirements for both categories of products.

Fifth, Congress also included in the 1938 Act, as it had in the 1906 Act, a third prong of the drug definition to include articles recognized in specified pharmacopeias. This was intended, however, to include pharmacopeial articles only when they are in fact represented for disease or structure/function purposes (19). Accordingly, this prong of the definition may be excluded from further consideration in this chapter.

With these principles and policies established, Congress enacted the FD&C Act in 1938 with the following two pertinent definitions. A drug was defined in section 201(g) to mean:

> . . . (1) articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them; and (2) articles intended for use in

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> the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; and (3) articles (other than food) intended to affect the structure or any function of the body of man or other animals . . . .

A cosmetic was defined in section 201(i) to mean:

> . . . articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance . . . .

Parts of the drug definition not pertinent here have been revised since 1938, but the central core of the definition has not been altered. No part of the cosmetic definition has been changed. Thus, the controlling definitions have remained in place for the entire 62-year history of the FD&C Act.

IMPLEMENTATION OF THE FD&C ACT

The regulatory consequences of classifying a product as a drug rather than as a cosmetic are substantial. A drug will almost invariably be determined by the FDA to be a ''new drug'' that required substantial preclinical toxicological testing, clinical testing under an investigational new drug (IND) application, submission of a new drug application (NDA) requesting FDA approval, and ultimately marketing under substantial FDA postapproval requirements, including drug good manufacturing practices (GMP) regulations (20). New drugs typically require a decade or more for research and development prior to FDA approval and require the investment of hundreds of millions of dollars. In short, it is only the very rare cosmetic product that could justify this level of investment. It is therefore essential that cosmetic products be formulated and labeled in such a way as to avoid the drug definition.

Initial FDA Action Under the FD&C Act

FDA scientists recognized very early that all cosmetics penetrate the skin and thus inherently affect the structure or function of the body:

> . . . there are few if any substances which are not absorbed through the intact skin, even though the idea is prevalent that the skin is a relatively effective barrier to its environment (21).

Nonetheless, the FDA recognized that Congress fully intended a separate category of cosmetic products regardless of their inherent effect on the structure or function of the body, as long as no structure/function or disease claims were made for them.

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The FDA sought to establish policy on the distinction between a cosmetic and a drug in three ways. First, FDA issued formal trade correspondence that set forth advisory opinions on the classification of products. Second, the agency published pamphlets and other educational materials with examples of product classification. Third, it brought court action to contest the legality of cosmetic products with labeling that contained what the agency concluded to be drug claims. From this body of literature and precedent has emerged, over six decades, a number of well-developed examples:

A suntan product is a cosmetic but a sunscreen product is a drug.
A deodorant is a cosmetic but an antiperspirant is a drug.
A shampoo is a cosmetic but an antidandruff shampoo is a drug.
A toothpaste is a cosmetic but an anticaries toothpaste is a drug.
A skin exfoliant is a cosmetic but a skin peel is a drug.
A mouthwash is a cosmetic but an antigingivitis mouthwash is a drug.
A hair bulking product is a cosmetic but a hair growth product is a drug.
A skin product to hide acne is a cosmetic but an antiacne product is a drug.
An antibacterial deodorant soap is a cosmetic but an antibacterial anti-infective soap is a drug.
A skin moisturizer is a cosmetic but a wrinkle remover is a drug.
A lip softener is a cosmetic but a product for chapped lips is a drug.

This list is illustrative, not exhaustive.

Products that are represented only to change the structure or function of the hair or nails are regarded as cosmetics and not drugs. For example, permanent waves and cuticle removers are cosmetics, not drugs (22). Products that are represented to affect the hair or nails systemically, on the other hand, are regarded as drugs.

Cosmetic products represented as ''hypoallergenic,'' and thus with reduced allergic potential, remain classified as cosmetics and not as drugs (23). Only if these products are represented to treat specific reactions or diseases would they be classified as drugs.

Inclusion of an active ingredient in a cosmetic does not automatically classify it as a drug, unless the active ingredient is so closely identified with therapeutic properties that the mere use of the term would connote a drug claim. For example, use of the term ''penicillin'' or ''AZT'' would preclude classification of the product solely as a cosmetic because of their well-recognized therapeutic purposes (24). In many instances, however, ingredients can be used in both cosmetic and drug products. When the FDA banned all topical nonprescription drug products containing hormones, the agency stated that cosmetics could continue to contain hormones without becoming drugs if the chemical name of the specific hormone was included in the ingredient statement and the word ''hormone'' was not used in the labeling or advertising (25).

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In many instances, the context of a word or phrase must be considered before a determination can be made about proper classification of the product as a drug or cosmetic. A product represented as a treatment for disease is a drug, but a product represented as a beauty treatment is a cosmetic. A product represented to kill germs that cause infection is a drug but a product tht is represented to kill germs that cause odor is a cosmetic.

These examples illustrate the difficulty in drawing a clear and definitive distinction between these two categories of products. Nonetheless, these distinctions have come to be understood both by FDA and by industry and serve the extremely useful purpose of guiding decisions in this area.

The Wrinkle Remover Cases of the 1960s

In the early 1960s, the cosmetic industry developed a line of products, broadly characterized as ''wrinkle remover'' products, containing ingredients intended to smooth, firm, and tighten the skin temporarily and thus to make wrinkles less obvious. In 1964, the FDA seized several of these products, alleging that they were drugs under the FD&C Act. The resulting litigation produced three decisions by U.S. District Courts and two decisions by U.S. Courts of Appeals involving three products: Line Away, Sudden Change, and Magic Secret.

The District Court in the Line Away case took the position that, by intending to smooth and tighten the skin, Line Away had as its objective affecting the structure of the skin and thus was a drug (26). The Court of Appeals agreed, citing the ''strong therapeutic implications'' of the promotional material (27).

The District Court in the Sudden Change case concluded that the product was represented merely to alter the appearance of the skin and thus was a cosmetic (28). The Court of Appeals, however, reversed the District Court in a split decision. The majority held that the claims that that product would give a ''face lift without surgery'' and would ''lift out puffs'' had ''physiological connotations'' (29). The majority went out of its way, however, to state that all of the traditional cosmetic claims (e.g., that a product will soften or moisturize the skin) remain within the cosmetic category. One judge dissented on the ground that the two claims cited by the majority as drug claims were indistinguishable from such cosmetic claims as smooths, firms, tones, and moisturizes the skin.

Finally, the District Court in the Magic Secret case determined that the product was a cosmetic, not a drug, based on the conclusion that the claims were less exaggerated than in the other two cases. The court held that the claim that the product caused an ''astringent sensation'' would not be regarded by consumers as doing anything other than altering their appearance (30).

By this time, it was apparent both to the FDA and to the regulated industry that further litigation would be unproductive. Industry sought to modify its claims in order to bring them within the cosmetic boundaries established by the FDA

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administrative precedent and the judicial decisions. The FDA concluded to provide any further guidance with respect to the distinction between a drug and a cosmetic through the OTC Drug Review, which was initiated in the early 1970s.

The OTC Drug Review

Under the Drug Amendments of 1962 (31), which were enacted following the thalidomide disaster in order to strengthen drug regulation in the United States, the FDA was required to review every new drug application (NDA) that had become effective on the basis of an agency safety review between 1938 and 1962 in order to determine whether the drug was effective as well as safe. For prescription drugs, FDA submitted the pre-1962 NDAs for review by the National Academy of Sciences, under the Drug Efficacy Study Implementation (DESI) program. For nonprescription drugs (also called over-the-counter or OTC drugs), the FDA chose a different approach. Under procedures promulgated in 1972 (32), the FDA established advisory committees to review all of the pharmacological categories of OTC drugs and to prepare reports on the safety, effectiveness, and labeling for all existing OTC drugs. The advisory committee reports, together with a proposed monograph, were published in the Federal Register for public comment. After reviewing the public comment, the FDA published its own conclusions together with a tentative final monograph for further public comment. Following its consideration of the second round of public comments, the FDA promulgated a final monograph establishing the conditions for safe and effective use including required and permitted labeling of the OTC drugs that fell within that drug category. An OTC drug ingredient that was not included in a final monograph could no longer be used as an active ingredient in an OTC drug following the effective date of the final monograph, but could be used as an inactive ingredient or as a cosmetic ingredient.

The OTC Drug Review inherently raised issues relating to the distinction between a cosmetic and a drug. All of the traditional cosmetic drug products—sunscreens, antiperspirants, antidandruff shampoos, anticaries toothpaste, skin protectants, hormone creams, acne products, and so forth—were reviewed under the OTC Drug Review. The FDA made clear that only the drug and not the cosmetic aspects of cosmetic drugs were subject to review and evaluation, and ultimately a final monograph, under this program. Thus, in many of the advisory committee meetings and subsequent reports (33), as well as in the preambles to the tentative final (34) and final (35) monographs, there has been substantial discussion about the dividing line between a drug claim and a cosmetic claim for a cosmetic drug. In several instances, the FDA has explicitly stated that a final monograph covered only products making drug claims and did not cover cosmetic claims for the product or products making only cosmetic claims.

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The distinction between a cosmetic and a drug became important early in the OTC Drug Review process. Based on an advisory committee recommendation, FDA published regulations banning three substances as unsafe for use: hexachlorophene (36), TBS (37), and zirconium (38). Recognizing that these substances could properly be used both in drugs and in cosmetics, the FDH published parallel regulations to assure that both types of uses would be banned.

For the most part, the OTC Drug Review has proceeded without major controversy with respect to the classification of cosmetic and drug claims. In general, the FDA has followed the traditional cosmetic/drug distinctions described earlier in this chapter. In a few remaining monographs, however, the FDA has proposed to change its policy with respect to important products. It has proposed to reclassify ''kills germs that cause odor'' from the cosmetic category to drug status (39). It has proposed to set a limit on cosmetic use of hormone ingredients, above which they would automatically become drugs (40). The resolution of these matters remains uncertain. Although the FDA had previously stated that suntan products are cosmetics (41), it proposed to reclassify them as drugs, but then retained them as cosmetics (with a required sunburn warning) in the final regulations (42). Industry, in turn, has asked the FDA to classify sunscreen ingredients when used in nonbeach traditional cosmetic formulations as cosmetic ingredients rather than as drugs, in order to encourage the cosmetic industry to include sunscreen ingredients in skin-care products for public health protection wherever feasible, but the FDA rejected this approach.

The Warning Letters of the Late 1980s

For a period of 15 years following the conclusion of the wrinkle remover cases, the FDA pursued cosmetic/drug issues largely through the OTC Drug Review and seldom, if ever, through Regulatory Letters or direct court action. Based upon new product technology and the conclusion that the consuming public was becoming increasingly sophisticated about skin-care products and their claims, the cosmetic industry gradually became more aggressive with cell rejuvenation and other antiaging promotional claims. As a result of research and development in the intervening years, new and more effective products were now on the market.

Two defining events served to initiate a new round of FDA enforcement activities against skin-care claims in the late 1980s (43). First, in 1986 the well-known South African heart surgeon, Christiaan Barnard, made a tour of the United States on behalf of a cosmetic company to promote its skin care product, Glycel. Barnard made extravagant claims for Glycel on the television program, Nightline, with FDA Commissioner Frank Young participating on the same program. Second, an attorney for a major cosmetic company wrote Dr. Young to protest the claims being made for Glycel. As a result, the FDA began to issue Regulatory Letters not only to the manufacturer of Glycel but also to other leading

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members of the industry (44). More than 20 Regulatory Letters were sent in the first wave, and when the FDA concluded that the response was unsatisfactory the agency sent another 20. Complex negotiations ensued among the FDA, individual companies, and a consortium of companies. The FDA established the agency position on the matter with a letter from the FDA Associate Commissioner for Regulatory Affairs, John Taylor:

> We consider a claim that a product will affect the body in some physiological way to be a drug claim, even if the claim is that the effect is only temporary. Such a claim constitutes a representation that the product is intended to affect the structure or function of the body and thus makes the product a drug under 21 U.S.C. 321(g)(1)(C). Therefore, we consider most of the anti-aging and skin physiology claims that you outline in your letter to be drug claims. For example, claims that a product ''counteracts,'' ''retards,'' or ''controls'' aging or the aging process, as well as claims that a product will ''rejuvenate,'' ''repair,'' or ''renew'' the skin, are drug claims because they can be fairly understood as claims that a function of the body, or that the structure of the body, will be affected by the product. For this reason also, all of the examples that you use to allege an effect within the epidermis as the basis for a temporary beneficial effect on wrinkles, lines, or fine lines are unacceptable. A claim such as ''molecules absorb'' . . . and expand, exerting upward pressure to ''lift'' wrinkles ''upward'' is a claim for an inner, structural change (45).

The Associate Commissioner did offer some guidelines for cosmetic claims:

> While we agree with your statements that wrinkles will not be reversed or removed by these products . . . we would not object to claims that products will temporarily improve the appearance of such outward signs of aging. The label of such products should state that the product is intended to cover up the signs of aging, to improve the appearance by adding color or a luster to skin, or otherwise to affect the appearance through physical means . . . .
>
> However, we would consider a product that claims to improve or to maintain temporarily the appearance or the feel of the skin to be a cosmetic. For example, a product that claims to moisturize or soften the skin is a cosmetic.

Following the FDA letter, one company brought court action to obtain a declaratory judgment that its product was a cosmetic rather than a drug, but the court ruled that a Regulatory Letter could not be contested in this way, and thus the issue remained unresolved (46). Individual companies eventually worked out their issues with the FDA and thus the agency was not required to bring formal court action against even one product.

The Alpha-Hydroxy Acid (AHA) Products of the 1990s

In the early 1990s, the cosmetic industry developed and marketed a line of products containing alpha-hydroxy acids such as glycolic, lactic, and citric acid that

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occurred in natural food products, to cleanse dead cells from the surface of the skin and assist moisturization. The AHAs have been used in consumer products at relatively modest levels, usually at 10% or lower, in contrast with very high levels used in professional skin peeling products (47). It is universally accepted that the AHA products are the most effective skin-care beauty products that the industry has ever developed. As a result, they have become extremely popular with consumers and gained substantial media and regulatory attention.

The FDA has raised two questions about the AHA products. First, the agency has questioned the claims being made. The FDA has sought to adhere to the guidelines established in the November 1987 letter on the antiaging and cell rejuvenation products. Second, the FDA has also questioned the safety of these products, not on the ground that there are known toxicological concerns but rather on the ground that their safety is unproven. In contrast with the cell rejuvenation claims of the 1980s, however, the FDA has not launched another wave of Warning Letters. A company that had obtained FDA approval of NDAs for antiaging drugs, frustrated by this lack of FDA action, brought a private false advertising case under section 43(a) of the Lanham Act (48) against a competitor making aggressive claims for a cosmetic product, but lost in both the District Court (49) and the Court of Appeals (50).

Use of Foreign Marketing Experience

As noted above, the cosmetic industry has been forced to stay within the confines of traditional cosmetic claims for skin-care products that could potentially justify stronger promotion, because the only other alternative is the bottomless pit of the IND/NDA process for drugs. To create a more realistic alternative, the FDA has sought to modify its position on OTC drugs.

When the OTC Drug Review was initiated in 1972, the FDA announced two policies that were designed to confine the scope of the Review. First, the Review included only those products on the market prior to the final procedural regulations, published in June 1972. This date was later extended to December 1975. Second, the Review included only products marketed in the United States, and excluded those marketed abroad. As a result, it was impossible to market in the United States any nonprescription drug that had been sold abroad before the cutoff date or that was developed at any time, anywhere in the world, after the cutoff date.

These two policies were adopted for management, not legal, reasons. The OTC Drug Review was an enormous undertaking, and the FDA concluded that it was essential to establish limitations in order to avoid a perpetual process. Nonetheless, these two policies had a major adverse impact. Some products marketed abroad have important public health benefits. For example, sunscreen products providing protection against both ultraviolet A (UVA) radiation and ultravio-

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let B (UVB) radiation were available in Europe for at least 15 years before they became available in the United States. The FDA refused to bring these products within the OTC Drug Review until it finally relented in 1997 (51). In the interim, U.S. residents were denied important public health protection solely because of this policy.

Recognizing the adverse public health consequence of its policy, and in light of a court decision invalidating a parallel policy for food ingredients (52), the FDA has now opened up the issue of expanding the OTC Drug Review to include new conditions under the OTC drug monograph system based upon foreign marketing experience (53). The FDA has thus far published an advance notice of proposed rulemaking on this issue, and an actual proposed change in the current regulations, but it may be some years before final action is taken on it.

In the interim, additional pressure is being placed on the FDA to change its policy in order to achieve international harmonization in the regulation of cosmetics and nonprescription drugs. It is difficult, if not impossible, to reconcile the FDA policy that excludes foreign marketing experience with the requirements of the General Agreement on Tariffs and Trade (GATT) (54). The recently enacted Food and Drug Administration Modernization Act of 1997 also requires the FDA to work toward international harmonization and mutual recognition agreements relating to drugs between the European Union and the United States (55). The combination of all of these efforts may well produce a more flexible approach toward FDA approval of nonprescription cosmetic drugs.

If the FDA were to recognize foreign marketing experience and engage in international harmonization, the distinction between a cosmetic and a drug in the United States could become less crucial. A number of products that are marketed as cosmetic drugs in the United States are classified solely as cosmetics in Europe. Cosmetic drugs can also be marketed in Europe with less restrictions than apply in the United States. Once a cosmetic drug is on the market in Europe, entry into the United States could become easier based upon international harmonization and mutual recognition principles.

The Rationale of the Tobacco Initiative

In August 1995, the FDA published two notices in the Federal Register relating to the proposed regulation of tobacco (56). The first notice set forth the proposed regulation governing cigarettes. The second notice consisted of an analysis supporting the agency's decision on the matter. Normally, regulation of cigarettes would have little or nothing to do with regulation of cosmetics. The rationale provided by the FDA for asserting its jurisdiction over cigarettes, however, as

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well as some of the specific discussion in the Federal Register preambles, are of substantial importance to the cosmetic/drug distinction.

As discussed above, the FD&C Act provides that a drug includes articles ''intended to affect the structure or any function of the body.'' In its analysis relating to cigarettes, the FDA took the position that the ''intent'' required under this definition means the ''objective'' intent of the manufacturer, not the ''subjective'' intent (i.e., the manufacturer's representation for the product). The FDA contended that ''objective'' intent requires a ''reasonable person'' test, and that a manufacturer is charged with the reasonable foreseeability—the natural and foreseeable consequences—of its action. Thus, the FDA asserted that it has authority under the FD&C Act to classify products as drugs where they inherently result in nontherapeutic but pharmacological effects even though no pharmacological or therapeutic claims are made for the products. The following examples were given by the FDA: topical hormones and sunscreens. The FDA analysis stated, however, that courts have distinguished between ''remote physical effects'' that would not make a product inherently a drug and ''significant effects on structure or function'' which the agency concluded clearly fall within the drug definition (57).

In its final regulation published in August 1996 (58), the FDA adhered to this position. The FDA categorically rejected the contention that the intended use of a product must be derived solely from the manufacturer's subjective intent (i.e., promotional claims for the product). The FDA did, however, reiterate that the structure/function provision would not extend to products that have a ''remote physical effect on the body'' (59).

The U.S. District Court that reviewed this matter upheld the FDA position on ''intended use'' (60). On appeal, however, the U.S. Court of Appeals overturned the District Court and declared the FDA regulations unlawful (61). In a divided decision, the majority of the Court of Appeals agreed with the District Court that ''no court has ever found that a product is 'intended for use' or 'intended to affect' within the meaning of the [Act] absent manufacturer claims as to that product's use,'' but then went on to decide the case on completely different grounds. The majority concluded, as a matter of statutory construction, that the FDA has no jurisdiction over tobacco products under the FD&C Act, and thus it was unnecessary to determine the scope of the ''intended use'' provision in the structure/function prong of the drug definition. The dissenting judge agreed with the FDA interpretation of intended use.

As a result, we are left with an FDA interpretation, a District Court agreement with that interpretation, two judges on the Court of Appeals who questioned the FDA interpretation but determined it was irrelevant, and one judge on the Court of Appeals who also agreed with the FDA interpretation. In short, the state of the law remains quite uncertain in this area. Even if the FDA interpretation

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were upheld, however, it would still exclude all cosmetics with structure/function effects that are remote or insignificnat.

Labeling and Manufacturing Difficulties for Cosmetic Drugs

Compliance with the combined cosmetic and drug provisions of the FD&C Act can be difficult and aggravating. FDA regulations have in the past sought to accommodate cosmetic drug labeling requirements (62), however, and the FDA Modernization Act specifically reconciled the two different approaches to ingredient labeling (63). To the extent that FDA continues to ignore the labeling complexities of cosmetic drugs—as it did, for example, in promulgating the final regulations for nonprescription sunscreen drugs (64) and for the new labeling requirements for all nonprescription drugs (65)—concerns about the dividing line between a cosmetic and a drug will be greatly aggravated. Although the FDA has declined formally to acknowledge different good manufacturing practice standards for cosmetic drugs (66), in practice cosmetic drugs are usually not held to the identical requirements.

Budgetary Impact on the FDA

The ability of the FDA to monitor and bring regulatory action with respect to claims for cosmetic products must take into account the resources available to the agency for this purpose. During the past several years, the FDA has experienced a flat budget. Because of the inexorable impact of inflation, this has been tantamount to a substantial reduction in available resources. At the same time, the FDA has been pursuing its tobacco initiative and a presidential initiative on food safety. As a result of all of these budgetary factors, the FDA announced in 1998 that it was reducing the staff of the Office of Cosmetics and Colors by 50% and cutting back or eliminating many cosmetic regulatory programs (67). This reduction is so substantial that it propelled the cosmetic industry to request and obtain restoration by Congress of adequate funds to assure that the FDA has a credible cosmetic regulatory program. FDA cosmetic officials are also reaching out to FDA drug officials for cooperation and assistance in discharging their duties.

POTENTIAL FUTURE APPROACHES

For more than 30 years, there has been widespread debate about whether, and how, the current statutory definitions of cosmetic and drug should be changed. Virtually every option has been considered, from making no change at all to modest or even substantial legislative changes.

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Advocates of leaving the statute unchanged contend that, in general, there is already sufficient flexibility in the law to permit valid cosmetic claims and that any attempt to change the legislation might well result in a worse situation rather than a better one. Even the November 1987 FDA guidelines provide industry with a great deal of flexibility. Creative marketing has found a way to convey the benefit of innovative new cosmetic products to consumers, as shown by experience with the AHA products. Thus, there is little to be gained, and potentially a great deal to be lost, by Congress considering changes in the cosmetic provisions of the FD&C Act that have stood the test of 60 years of experience without a single amendment.

Advocates of moderate change contend that all that would be needed is to insert the two words ''and cosmetics'' in the parenthetical exclusion that currently exists in the structure/function prong of the drug definition—the approach taken by the Senate in April 1935 (68)—with the result that both food and cosmetics would be excluded from this portion of the definition. This would allow cosmetics to make structure/function claims comparable to the structure/function claims available to dietary supplements and conventional food (69). It would be necessary to obtain clear legislative history that a structure/function claim is not an implied disease claim, as the FDA once contended for food products (70). Advocates of this minimalist legislative approach acknowledge, however, that they can offer no assurance that Congress would not reexamine other portions of the cosmetic provisions of the FD&C Act and perhaps make additional changes.

Advocates for a more extensive legislative approach offer a wide variety of potential statutory changes. Some advocate creating an entire new category of cosmetic drugs that would have its own separate regulatory requirements and prohibitions, halfway between those for drugs and those for cosmetics. Others argue for imposing the same premarket safety requirements for cosmetic drugs as for other drugs, but excluding claims from premarket review or approval. Once again, these advocates acknowledge that Congress could, in the process of establishing any such new statutory scheme, also review and change the existing cosmetic provisions of the FD&C Act.

In the more than 30 years that this subject has been debated, no new legislation has been proposed to address the matter. Over the same period of time, industry has found ways to accommodate the existing FDA requirements and to reconcile advances in technology with current regulatory policy.

CONCLUSION

The history set forth in this chapter reflects the inherent uncertainty in attempting to formulate any bright line between a cosmetic and a drug. Even with legislation, whatever new statutory definitions or standards that might be enacted would inev-

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itably raise close questions of judgment that would continue to evolve over time. Accordingly, legislation will not eliminate the uncertainty inherent in the cosmetic/drug distinction and thus is not the only or even the preferred solution to this matter.

The FDA has substantial administrative discretion to determine the line between a cosmetic and a drug. By assuring the safety of cosmetic ingredients through the Cosmetic Ingredient Review program (71), the cosmetic industry has substantially reduced concern about the safety of marketed cosmetic products. International harmonization activities have already led the FDA to explore opening U.S. requirements to include foreign marketing experience, and the FDA Modernization Act requirements with respect to international harmonization and mutual recognition will accelerate this approach. It is thus more likely that a reasonable approach to the cosmetic/drug distinction will be found through administrative and international action rather than through legislation.

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16
Drugs Versus Cosmetics: Cosmeceuticals?

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REGULATORY: JAPAN

Regulatory Environment

The legal classification of topical products in Japan is different from that in the United States and Europe, where they are classified into only two categories—drugs and cosmetics. In Japan, there are also regulations covering cosmetic products with pharmacological action, called quasidrugs, which are ranked between cosmetics and drugs (1). Each definition of drugs, cosmetics, and quasidrugs in the regulations of The Pharmaceutical Affairs Law (2) reads as follows:

Drugs are articles as defined below.

1. Articles recognized in the official Japanese pharmacopoeia.
2. Articles (other than quasidrugs) that are intended for use in the diagnosis, cure, or prevention of disease in humans or animals, and that are not equipment or instruments (including dental materials, medical supplies, and sanitary materials).
3. Articles (other than quasidrugs and cosmetics) that are intended to affect the structure or any function of the body of humans or animals, and that are not equipment or instruments (paragraph 1, article 2 of the law).

Quasidrugs are articles that have the purposes given below and exert mild actions on the human body, or similar articles designated by the Minister of Health and Welfare. They exclude not only equipment and instruments but also any article intended, in addition to the following purposes, for the use of drugs described in 2 and 3 above.

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1. Prevention of nausea or other discomfort, or prevention of foul breath or body odor.
2. Prevention of prickly heat, sores, and the like.
3. Prevention of falling hair, or hair restoration or depilation.
4. Killing or prevention of rats, flies, mosquitoes, fleas, etc., for maintaining the health of humans or animals (paragraph 2, article 2 of the law).

Examples of quasidrugs, as designated by the Minister of Health and Welfare (MHW Notification No. 14, 1961) include: (1) cotton products intended for sanitary purpose (including paper cotton); (2) products with a mild action on the human body [i.e., hair dyes; agents for permanent waving; products that combine the purposes of use as stipulated in paragraph 3, article 2 of the law (on cosmetics), with the purpose of prevention of acne, chapping, itchy skin rash, chilblain, etc., as well as disinfection of the skin and mouth (so-called medicated cosmetics) and bath preparations].

The term ''cosmetic'' means any article intended to be used by means of rubbing, sprinkling, or by similar application to the human body for cleaning, beautifying, promoting attractiveness, altering the appearance of the human body, and for keeping the skin and hair healthy, provided that the action of the article on the human body is mild. Such articles exclude the articles intended, besides the above purposes, for the use of drugs described in 2 or 3 above, and quasidrugs (paragraph 3, article 2 of the law).

Cosmeceuticals in Japan

A current definition of cosmeceuticals would cover those products ''that will achieve cosmetic results by means of some degree of physiological action'' (3). This product category is ranked between cosmetics and drugs. It is a well-known fact that Japan is ahead of most other countries in coping with the legal issues. A category of pseudodrugs that are what we now refer to as cosmeceuticals has already been established in the Pharmaceutical Affairs Law (4). The phrase pseudodrugs corresponds to the legal category of quasidrugs. Actually, many of the topical products corresponding to the cosmeceuticals fall into the category of quasidrugs. In the Pharmaceutical Affairs Law, quasidrugs are defined as articles having ''a fixed purpose of use'' and ''a mild action on the body'' or similar articles designated by the Minister of Health and Welfare. Their types, purpose of use, principal product form, indications, and effects are described in Tables 1 and 2 (2,5).

The manufacturers of quasidrugs are required to obtain government approval before marketing. Approval of a product under application for manufacturing (import) is contingent upon a judgment by the Ministry of Health and Welfare

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regarding its adequacy as a quasidrug in view of its effectiveness, safety, etc. It should be noted, therefore, that the examination procedures for approval, as well as the data and documentation required to be submitted upon filing differ according to indications and effects of the products (2). The following data must be submitted, depending on the kind of ingredients, etc.: (1) data on origin, background of discovery, use in foreign countries; (2) data on physicochemical properties, specifications, testing methods; (3) data on stability; (4) data on safety; and (5) data on indications or effects.

The scope of data actually to be attached to the application depends on the type of quasidrug: (1) new quasidrugs that obviously differ from any one of previously approved products with regard to active ingredient, usage, and dosage and/or indications or effects; (2) quasidrugs identical with previously approved quasidrug(s); or (3) other quasidrugs that are other than those specified in (1) and (2) above (2).

For a product under application to be approved as a quasidrug, it is prerequisite that the purpose of its use is within the scope stipulated by the Pharmaceutical Affairs Law. Thus, approval of a product as a quasidrug is determined by an integrated judgment of various factors such as its ingredients, quantity (composition), indications and effects, usage and dosage, and dosage form. For example, those products whose effects are not mild and thus come under the category of poisons or deleterious drugs are *not* approved, even if their indications, effects, and dosage forms are within the scope of quasidrug legislation. Likewise, products for which the purpose of use deviates from the scope of quasidrug are not approved either, even if their effects are mild (2).

When viewed taking into account the essential characteristics of quasidrugs for example, it is inappropriate for products such as mouth refreshers to include any medicinal indications and effects relevant to ''treatment,'' such as morning sickness and sterilization and disinfection of the mouth (2).

Since a quasidrug under the law may be sold and used by any person without specific restriction, it should be a product that, in principle, can be easily and directly used by any person without involving any complex process (2). An active ingredient used in a chemically pure (bulk drug) form is usually considered a drug and not suitable for over-the-counter use. Therefore, such a product is not acceptable as a quasidrug. Generally, simplicity in handling and usage constitutes another potent factor (2).

Cosmeceuticals in the Future

With respect to cosmetic requirements, the demand for fashion has strengthened, but, at the same time, a tendency to place importance on efficacy has also emerged (6). This trend has become increasingly strong with the transition toward a gerontocracy, who wish to delay the biological process of aging and remain young as

Table 1 Types, Purposes of Use, Indications, and Effects of Quasidrugs

Type of quasidrugs	Scope of purpose of use and principal product forms		Scope of indications and effects
	Purpose of use	Principal product form	Indications and effects
1. Mouth refreshers	Oral preps for prevention of nausea or other indisposition	Pill, plate, troche, liquid	Heartburn, nausea and vomiting, motion sickness, hangover, dizziness, foul breath, choking, indisposition, sunstroke
2. Body deodorants	External agents to prevent body odor	Liquid, ointment, aerosol, powder, stick	Body odor, perspiration odor, suppression of perspiration
3. Talcum powders	Agents to prevent prickly heat, sores, etc.	Powder for external application	Prickly heat, diaper rash, sores, razor burn
4. Hair growers (hair nutrients)	External agents to prevent loss of hair and to grow hair	Liquid, aerosol	Hair growth, prevention of thinning hair, itching and falling hair, promotion of hair growth, dandruff, loss of hair after illness or childbirth, hair nutrition
5. Depilatories	External agents for hair removal	Ointment, aerosol	Hair removal
6. Hair dyes (including color and dye removers)	External agents for dying hair, removing hair or dye colors. Excluding agents for physical hair dying	Powder, tablet, liquid, cream, aerosol	Hair dying, hair decoloring, removal of hair color dye
7. Permanent waving agents	External agents for waves in the hair etc.	Liquid, cream, powder, paste, aerosol, tablet	Creation and retention of waves in the hair, straightening frizzy, curly or wavy hair, and retaining that condition
8. Sanitary cotton products	Cotton (including paper cotton) used for sanitary purposes	Cotton products, gauze	Sanitary napkins for absorbing and managing menses; cotton for cleaning, for wiping clean the skin and cavities of babies, for wiping clean the breasts and nipples when nursing, for wiping clean the eyes, genitals, and anus

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9. Bath preparations	External agents to be dissolved, as a rule, in the bath (excluding bath soaps)	Powder, granule, tablet, soft capsule, liquid, etc.	Prickly heat, roughness, ringworm, bruises, stiff shoulder, sprains, neuralgia, eczema, frostbite, hemorrhoids, tinea, chills, athlete's foot, scabies, itch, lumbago, rheumatism, fatigue recovery, chaps, cracks, chills before and after childbirth, acne
10. Medicated cosmetics (including medicated soaps)	External agents combining cosmetic purposes and resembling cosmetics in forms	Liquid, cream, jelly, solid, aerosol	See Table 2
11. Medicated dentifrices	External agents combining cosmetic purposes and resembling ordinary dentifrices in forms	Paste, liquid, powder, solid, tooth wet-powder	Making the teeth white, cleaning and refreshing the mouth, prevention of pyorrhea, prevention of gingivitis, prevention of tartar, prevention of dental caries, prevention of occurrence and progress of dental caries, prevention of foul breath, removal of tobacco stains
12. Repellents	Agents for repelling insects such as flies, mosquitoes, fleas, etc.	Liquid, stick, cream, aerosol	Repelling mosquitoes, gnats, stinging flies, fleas, house ticks, bedbugs, etc.
13. Insecticides	Agents for killing and eliminating insects such as flies, mosquitoes, fleas, etc.	Mat, stick-incense, powder, liquid, aerosol, paste	Killing of insects; exterminating and preventing sanitary insect pests such as flies, mosquitoes, fleas, etc.
14. Rodenticides	Agents for killing and eliminating rats and mice		Killing of rats and mice; expelling, exterminating, or preventing rats and mice
15. Soft contact lens disinfectants	Agents to disinfect soft contact lens		Disinfectant for soft contact lenses

Source: Modified from Refs. 2 and 5.

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Table 2 Types of Medicated Cosmetics

Type	Indications and effects
1. Shampoos	Prevention of dandruff and itching Prevention of perspiration odors in the hair and on the scalp Cleaning of the hair and scalp a. Keeping the hair and scalp healthy b. Making the hair supple (Choose either a or b)
2. Rinses	Prevention of dandruff and itching Prevention of perspiration odors in the hair and on the scalp Supplementing and maintaining moisture and fat of the hair Prevention of split, broken, or branched hairs a. Keeping the hair and scalp healthy b. Making the hair supple (Choose either a or b)
3. Skin lotions	Chapping and roughness of the skin Prevention of prickly heat, frostbite, chaps, cracks, acne Oily skin Prevention of razor burn Prevention of spots and freckles due to sunburn Burning sensation after sunburn or snow burn Bracing, cleaning, and conditioning the skin Keeping the skin healthy; supplying the skin with moisture
4. Creams, milky lotions, hand creams, cosmetic oils	Chapping and roughness of the skin Prevention of prickly heat, frostbite, chaps, cracks, acne Oily skin Prevention of razor burn Prevention of spots and freckles due to sunburn Burning sensation after sunburn or snow burn Bracing, cleaning and conditioning the skin Keeping the skin healthy; supplying the skin with moisture Protection of the skin; prevention of dry skin
5. Shaving agents	Prevention of razor burn Protection of the skin for smoother shave
6. Sunburn prevention agents	Prevention of chapping due to sunburn and snow burn Prevention of sunburn and snow burn Prevention of spots and freckles due to sunburn Protection of the skin
7. Packs	Chapping and roughness of the skin Prevention of acne Oily skin Prevention of spots and freckles due to sunburn Burning sensation after sunburn or snow burn Making the skin smooth Cleaning the skin
8. Medicated soaps (including face cleaning agents)	Soaps which are mainly bactericides Cleaning, sterilizing and disinfecting the skin Prevention of body odor, perspiration odor, and acne Soaps mainly containing anti-inflammatory agents Cleaning of the skin; prevention of acne, razor burn, and chapping

Source: Refs. 2 and 5.

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long as possible. However, the desire to look young and beautiful is shifting to a desire to protect the health of the skin (6).

In addition, with the increasingly sophisticated research into the skin, technology has been generating new active ingredients for antiaging skin-care products. However, some of them, such as antiwrinkle products, fall into neither of the three categories—drugs, quasidrugs, or cosmetics. No existing specifications (Tables 1,2) (2,5) of quasidrug are suitable for such products. How, then, should these products be categorized?

In the United States, a drug is defined as ''an article intended for the use in the diagnosis, mitigation, treatment or prevention of disease or intended to affect the structure or any function of the body.'' According to the current federal Food, Drug and Cosmetic Act, written in 1938, cosmetics are defined as ''articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance'' without affecting structure or function (7–9). If, for example, a nonmedicated shampoo is designated ''dandruff'' shampoo, simply by virtue of the fact that it removes loose dandruff flakes as part of the cleansing process, then it would be classified as a cosmetic shampoo (8). However, a shampoo that controls dandruff flaking would be categorized as a drug, and known as an ''antidandruff'' shampoo (8). On the other hand, an antidandruff shampoo would be regarded as a quasidrug in Japan if its action on the human body was mild.

Generally, topically applied quasidrugs are intended to mollify flaws of the skin and have a mild action on the human body, while drugs are intended to treat diseases (10). Therefore, hair-growing products having mild action on male pattern baldness, which is not a disease (1), and are considered quasidrugs; on the other hand, products intended for alopecia areata, which is a kind of disease, are regarded as drugs. Aging of skin, as in wrinkling, for example, is not a disease. We should also keep in mind that ''high efficacy'' would not always involve ''strong action.'' There will probably be many cosmeceutical products with mild action showing good efficacy. Accordingly, those new cosmeceutical products intended for antiaging of the skin could be categorized as quasidrugs. Legally, the Minister of Health and Welfare can add new or novel types of product to the current list of quasidrugs (10).

Regarding this matter, a review ''. . . of the scope of efficacy by adding new effects, will increase incentives toward research and developments in the technological standards and quality of cosmetics'' was included in the policy for promoting the Japanese cosmetic industry (6,11) published in May, 1984 by the Pharmaceutical Industry Policy Council, a consulting body of the Director of Pharmaceutical Affairs Bureau, the Ministry of Health and Welfare. The Japan Cosmetic Industry Association has set up an ad hoc subcommittee within its

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technical committee to review the scope of indications and effects of cosmetics and quasidrugs (12). We hope this effort will be successful.

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17
Efficacy of Barrier Creams

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INTRODUCTION

The concept of barrier creams (BC) has been around since the early twentieth century. In practice, their utilization remains the subject of a lively debate; some suggest that inappropriate BC application may induce additional irritation rather than benefit (1–5).

To evaluate BC efficacy, in vivo and in vitro methods have been developed. In particular, recent bioengineering techniques provide more accurate quantitative data than has traditionally been supplied by clinical studies dependent on visual scoring.

We review the investigative details of the pertinent scientific literature and summarize methodology and efficacy of BC.

BARRIER CREAMS

BC are designed to prevent or reduce the penetration and absorption of various hazardous materials into skin, preventing skin lesions and/or other toxic effects from dermal exposure (1,2,4–7). Alternate terms for BC include ''skin protective creams (SPCs),'' ''protective creams (PCs),'' ''protective ointments,'' ''invisible glove,'' and ''barrier,'' ''protective,'' ''prework'' or ''after-work'' creams, lotions, emollients, and/or gels (7–9). Frosch et al. (4) suggest that the term ''skin protective creams'' seems more appropriate because most do not provide a barrier comparable to the stratum corneum.

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Table 1 Influence of Indulona and Ivosin on the Percutaneous Absorption of 0.017 M Chromate Solution $k \times 10^5/min^{-1}$[a]

Series no.	Barrier cream	Volume applied mL/cm^2	Interval[b] min	<3.4 *n*	3.4–6.6 *n*	6.7–10.1 *n*	10.2–13.5 *n*	13.6– *n*	Mean relative absorption $k \times 10^5/min^{-1}$ 10 exp.	χ^2 (*c*), analysis of variance (*v*) compared with series 1
1	No barrier cream			—	4	4	—	2	8.8 ± 1.4[c]	—
2	Indulona	0.05	1	3	2	4	—	1	(5.7–6.7)[d]	$0.05 < p < 0.10$ (*c*)
3	Indulona	0.10	1	—	3	4	1	2	9.0 ± 1.2[c]	$p > 0.2$ (*v*)
4	Indulona	0.10	15	4	1	3	2	—	(5.5–6.8)[d]	$0.02 < p < 0.05$ (*c*)
5	Indulona	0.15	1	—	5	—	5	—	8.2 ± 0.1[c]	$p < 0.2$ (*v*)
6	Ivosin	0.025	1	2	2	3	3	—	(6.7–7.3)[d]	$0.10 < p < 0.20$ (*c*)
7	Ivosin	0.05	1	7	1	2	—	—	(2.1–4.5)[d]	$0.001 < p < 0.01$ (*c*)
8	Ivosin	0.10	1	8	—	2	—	—	(1.8–4.5)[d]	$p < 0.001$ (*c*)
9	Ivosin	0.10	15	5	5	—	—	—	(2.5–4.2)[d]	$0.001 < p < 0.01$ (*c*)
10	Ivosin	0.15	1	5	3	2	—	—	(3.2–4.7)[d]	$0.001 < p < 0.01$ (*c*)

[a] Modified from Ref. 13.
[b] Interval between application of barrier cream and test substance.
[c] Standard error.
[d] See text.

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METHODOLOGY AND EFFICACY OF BARRIER CREAMS

In 1940, Schwartz et al. (10) introduced an in vivo method to evaluate the efficacy of a vanishing cream against poison ivy extract utilizing visual erythema on human skin. The test cream was an effective prophylaxis against poison ivy dermatitis as compared to unprotected skin.

Sadler et al. (11) performed qualitative tests to evaluate the efficacy of barrier creams. One method used the fluorescence of a dyestuff and eosin as a measure of penetration; another measured the rates of penetration of water through barrier creams. These methods are rapid and simple, but provide only qualitative estimates. They introduced an apparatus for measuring the permeability of films of barrier creams.

Wahlberg (12,13) employed an isotope technique disappearance measurement for documenting the inhibiting effect of barrier creams on chromate (^{51}Cr) percutaneous absorption in guinea pigs (Table 1 and Fig. 1) (13). In this series 2,4 Indulona® (which contains Na_2H_2EDTA, $CaNa_2EDTA$ and acidum ascorbicum as active ingredients) and 6, 10 Ivosin® (which contains the hydrochloride of a copolymerizate of *p*-divinyl benzol and *p*-m-dimethylaminomethyl-

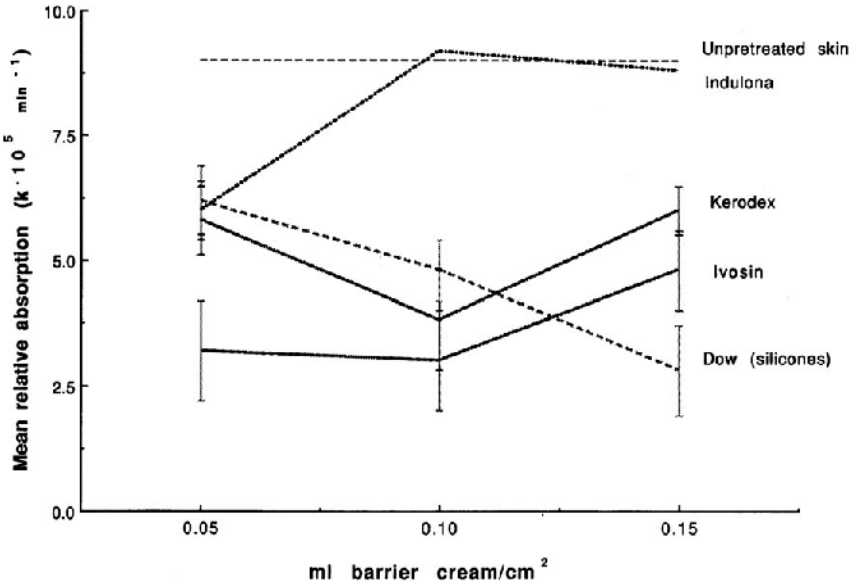

Figure 1 The effect of barrier creams on the percutaneous absorption of a 0.017 *M* aqueous solution of chromate. Three different volumes per unit area (mL/cm^2) were studied. (Modified from Ref. 13.)

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styrol) absorption decreased as a result of pretreatment. In some cases, the disappearance technique was not sufficiently sensitive to permit quantitative determination. The disappearance measurements distinguished between different barrier creams, volumes per unit area, and intervals between application of cream and chromate (13).

Langford (14) introduced in vitro studies to determine the efficacy of the formulated FC resin complex to prevent solvent penetration through treated filter paper, solvent repellency on treated pigskin, and penetration of radio-tagged sodium lauryl sulfate through treated hairless mouse skin. The penetration rate of

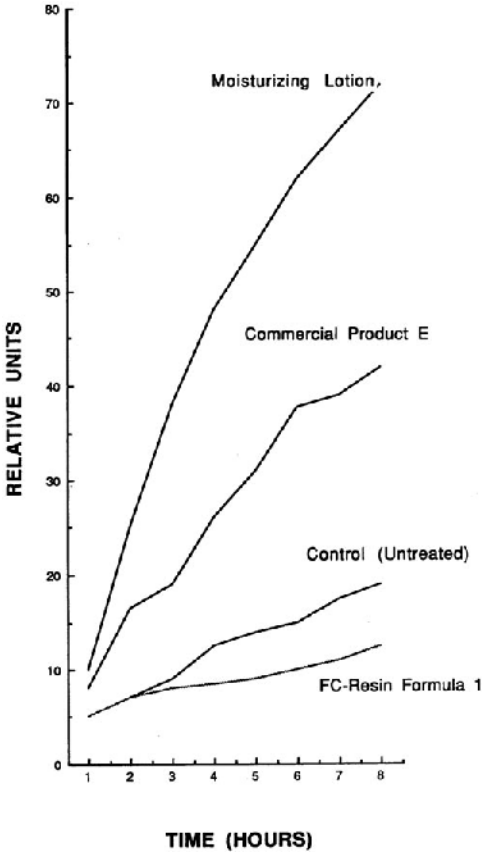

Figure 2 Penetration rate of radio-tagged sodium lauryl sulfate/ETOH through hairless mouse skin. (Modified from Ref. 14.)

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radio-tagged sodium lauryl sulfate/ETOH through hairless mouse skin is shown in Figure 2.

Reiner et al. (15) examined the protective effect of ointments both on guinea pig skin in vitro and on guinea pigs in vivo. The permeation of ''toxic agent 4''® through unprotected and protected skin within 10 h is plotted in Figure 3 as a function of time. The permeation values were determined radiologically and enzymatically. Permeation of ''toxic agent 4''® was markedly reduced by polyethylene glycol ointment base and ointments containing active substance. In in vivo experiments on guinea pigs, mortality was greater after applying the toxic

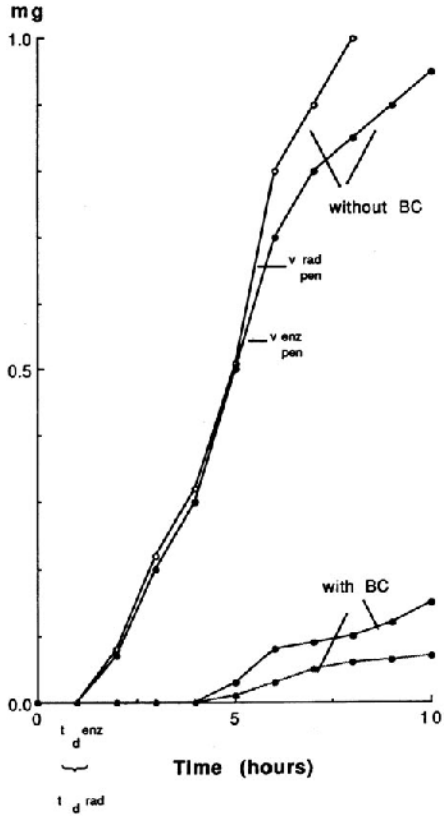

Figure 3 Permeation of the ''toxic agent 4''® through unprotected and protected skin as a function of time. ❍ = Rad data; ● = enzyme data; BC = reactive PEG ointment such as S4–S8. (Modified Ref. 15.)

agent to unprotected skin. All formulations with nucleophilic substances markedly reduced the mortality rate.

Lachapelle et al. (16–19) utilized a guinea pig model to evaluate the protective value of barrier creams and/or gels by laser Doppler flowmetry and histological assessment. In addition, the blood concentration of *n*-hexane of the control group and the gel-pretreated group was determined. Figure 4 shows partial results (19), which correlated invasive (blood levels) and noninvasive techniques.

Loden (7) evaluated the effect of barrier creams on the absorption of (^{3}H)-water (^{14}C)-benzene, and (^{14}C)-formaldehyde into excised human skin. The control and the barrier-cream-treated skin was exposed to the test substance for 0.5 h,

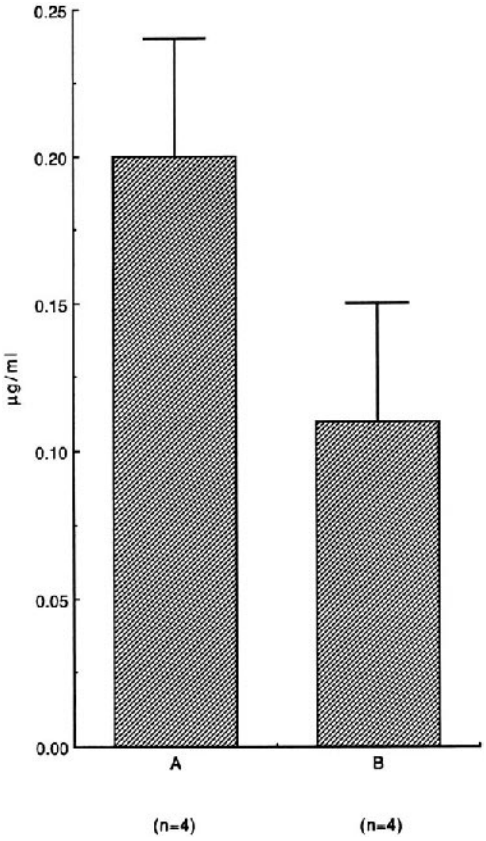

Figure 4 Mean ± SD (µg/mL) of blood levels of *n*-hexane in two groups of guinea pigs exposed for 30 min. (A) control group; (B) gel-pretreated group ($p < 0.05$). (Modified from Ref. 19.)

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whereupon absorption was determined. The experimental cream ''water barrier'' reduced the absorption of water and benzene but not formaldehyde. Kerodex 71® cream slightly reduced benzene and formaldehyde absorption. Petrogard® and ''Solvent Barrier''® did not affect the absorption of any of the substances studied (Fig. 5). One advantage of the method is the use of human skin. The effects of the barrier cream on the skin and the test substance mimic the in vivo situation. Another advantage is that the method is quantitative.

Frosch et al. (2–4,20,21) developed the repetitive irritation test (RIT) in the guinea pig and in humans to evaluate the efficacy of BC using a series of bioengineering techniques. The pretreated and untreated test skin (guinea pig or humans) was exposed daily to the irritants for 2 weeks. The resulting irritation was scored on a clinical scale and assessed by biophysical technique parameters. Some test creams suppressed irritation with all test parameters; some failed to show any effect; and some exacerbated the condition (Fig. 6) (4).

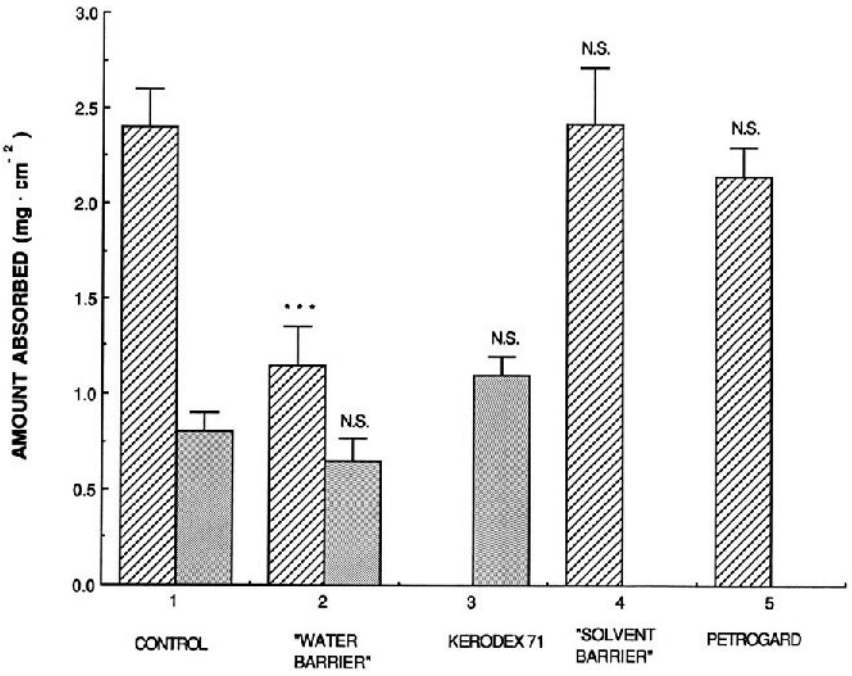

Figure 5 The amount of water absorbed into control skin and skin treated with barrier creams during 0.5 h of exposure. Skin from two donors was used (hatched and dotted columns). Values are means ± SE of the number of experiments within parenthesis. ***$p < 0.001$; n.s., not significantly different from control. (Modified from Ref. 7.)

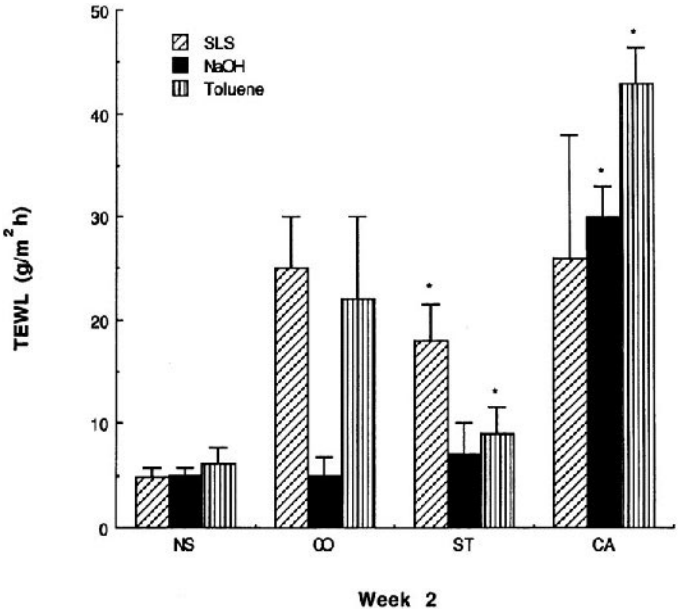

Figure 6 The effect of barrier creams: Stokoderm Salbe (ST) and Contra Alkali Creme (CA) in the guinea pig model after 2 weeks of treatment with three irritants (sodium lauryl sulfate, SLS; sodium hydroxide, NaOH; toluene). CO, control animals; NS, normal skin (untreated). Shown are the data of the transepidermal water loss (TEWL). Significant differences from control animals and barrier-cream-treated animals are indicated by an asterisk (*) ($p < 0.05$). (Modified from Ref. 4.)

Treffel et al. (22) measured the effectiveness of barrier creams on human skin in vitro against three dyes (eosin, methylviolet, and oil red O) with varying *n*-octanol/water partition coefficients (0.19, 29.8, and 165, respectively). Barrier cream efficacy was assayed by measurements of the dyes in the epidermis of protected skin samples 30 min after application. Penetration depths of the dyes into the stratum corneum are shown in Figure 7. The ΔTTC (%) related to the number of the cellophane tape strips made from the skin sample controls. The dyes were present in high amounts in the superficial layers of the stratum corneum; ΔTTC due to eosin in the first strip was lower than that obtained with both the other dyes (not statistically significant). Oil red O penetrated in greater amounts into the deeper stratum corneum. The amount of the three dyes at the bottom of the stratum corneum remains, however, low. The efficacy of barrier creams against the three dyes in several cases showed data contrary to manufac-

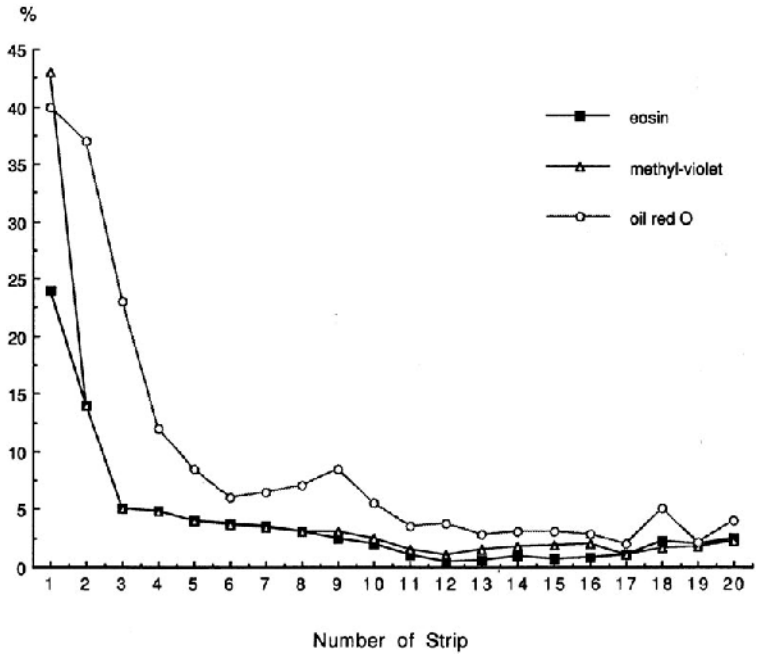

Figure 7 Δ total color change measurement in the stratum corneum expressed in percentage. (Modified from Ref. 22.)

turers' information. There was no correlation between the galenic parameters of the assayed products and the protection level, indicating that neither the water content nor the consistency of the formulations influenced the protection effectiveness.

Fullerton and Menne (23) evaluated that the protective effect of ethylenediaminetetraacetate (EDTA) barrier gels against nickel contact allergy using in vitro and in vivo methods. Thirty milligrams of barrier gel were applied on the epidermal side of the skin in vitro and a nickel disk was applied above the gel. Twenty-four hours after application, the nickel disk was removed and the epidermis separated from the dermis. Nickel content in epidermis and dermis was quantified by adsorption differential pulse voltammetry (ADPV). The distributions of nickel in the epidermis and the dermis after 24 h of occluded application of the two nickel disks made from alloy A and alloy B are in Figure 8. The amount of nickel in the epidermal skin layer after application of the barrier gels was significantly reduced compared to the untreated control.

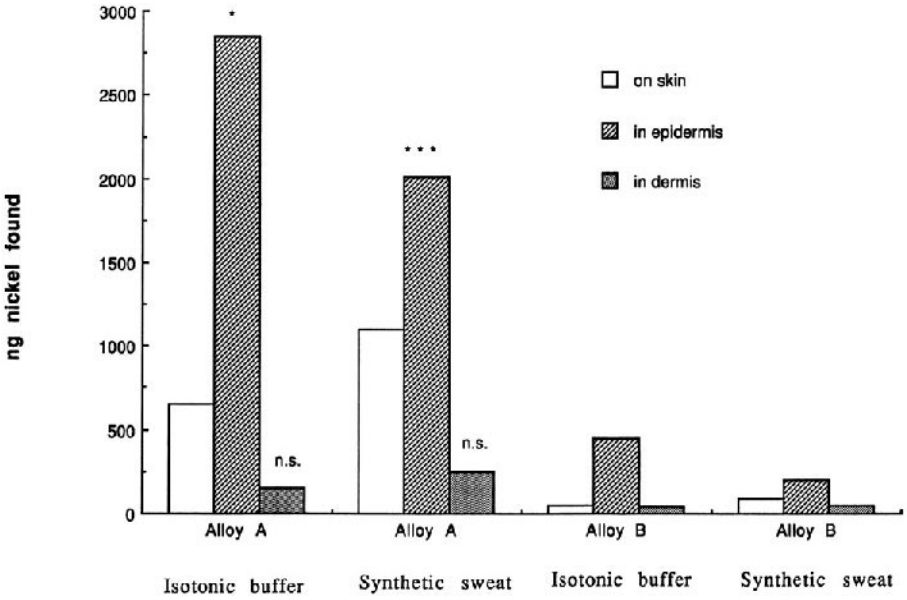

Figure 8 Distribution of nickel in epidermis and dermis after 24 h occluded application of two nickel disks made from alloy A and alloy B. Recipient mediums were isotonic phosphate buffer and synthetic sweat (skin from donor A). Statistics: 2-sample *t* test comparing mean skin compartment distribution of nickel after application of alloy B and alloy A. Comparison for isotonic phosphate buffer and synthetic sweat as recipient medium, respectively. n.s., no significance; $*p < 0.05$; $***p < 0.001$. (Modified from Fullerton Ref. 23.)

In vivo patch testing of nickel-sensitive patients was performed using nickel disks made of metal alloy A and Carbopol® barrier gel systems with and without added EDTA (gel type A and B). Test preparations and nickel disks were removed 1 day postapplication and the sites evaluated. Reduction in positive test reactions was highly significant.

Zhai (5) developed an in vivo method in human skin to measure the effectiveness of skin protective creams against dye indicator solutions: methylene blue in water and oil red O in ethanol, which are representative of model hydrophilic and lipophilic compounds. Solutions of 5% methylene blue and 5% oil red O were applied to untreated and protective cream pretreated skin with the aid of aluminum occlusive chambers, for 0 h and 4 h. At the end of the application time, the materials were removed, and consecutive skin surface biopsies (SSB) were obtained. The amount of dye penetrating into each strip was determined

by colorimetry. Two creams exhibited effectiveness, but one cream enhanced a cumulative amount of dye (Fig. 9).

Zhai et al. (24) introduced a facile approach to screening protectants in vivo in human subjects. Two acute irritants and one allergen were selected: sodium lauryl sulfate (SLS) represented a household irritant that can produce contact dermatitis; the combination of ammonium hydroxide (NH^4OH) and urea was used to simulate diaper dermatitis; and Rhus was used to evaluate the effect of model protective materials. Test materials were spread over onto test area, massaged, allowed to dry for 30 min, and reapplied with another 30-min drying period. The model irritants and allergen were applied with an occlusive patch for 24 h. Inflammation was scored with an expanded 10-point scale at 72 h postapplication. Most test materials statistically suppressed the SLS irritation and Rhus allergic reaction rather than NH^4OH and urea-induced irritation (Fig. 10).

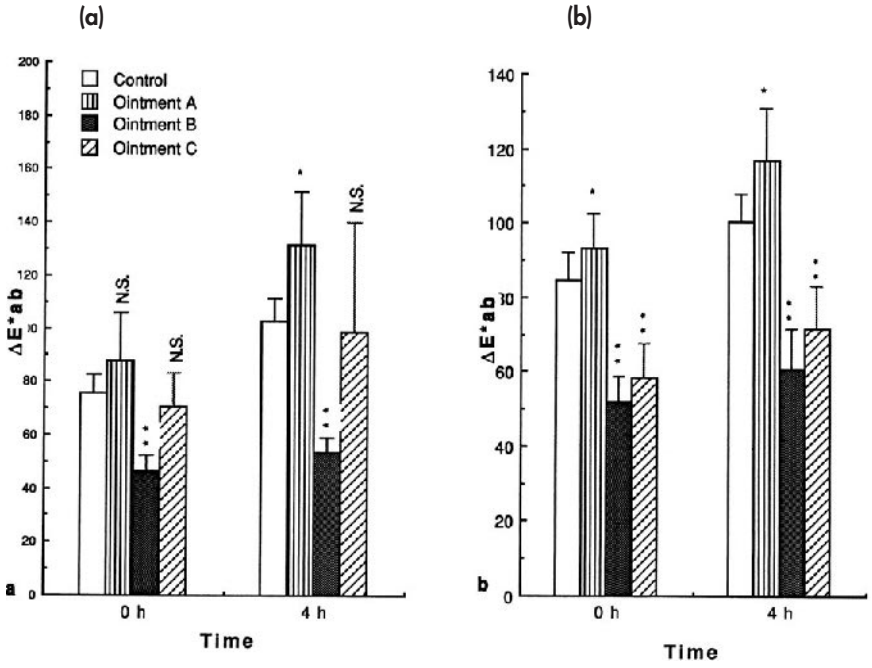

Figure 9 The amount of methylene blue and oil red O absorbed into control skin and skin treated with barrier creams: (a) methylene blue; (b) oil red O. Results expressed as the means ± s.d. of ΔE^*ab. Statistical differences in comparison to the control indicated by an asterisk (*) ($p < 0.05$) to **($p < 0.01$); n.s., not significant. (Modified from Ref. 5.)

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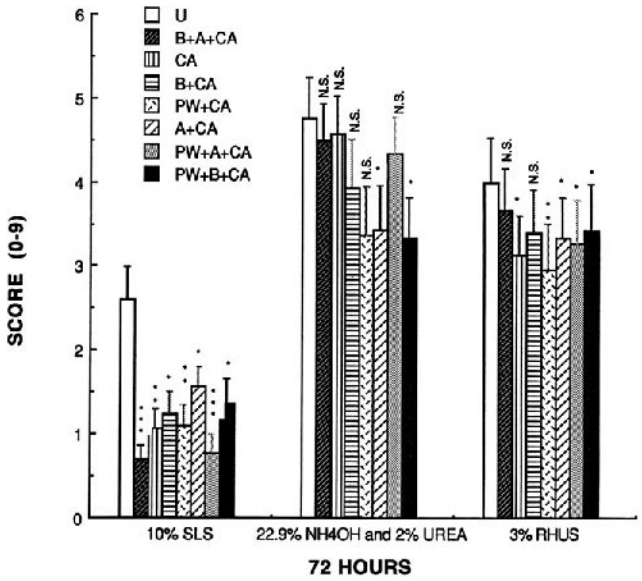

Figure 10 Efficacy of protective materials against skin irritation following treatment by two acute irritants (10% SLS, 22.9% NH^4OH and 2% urea) and 1 allergen (3% Rhus). Results expressed as the means ± SE. Statistical differences in comparison with untreated skin site. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; n.s., not significant. (Modified from Ref. 24.)

Zhai et al. (25) utilized an in vitro diffusion system to measure the protective effectiveness of quaternium-18 bentonite (Q18B) gels to prevent 1% concentration of [^{35}S]-sodium lauryl sulfate penetration by human cadaver skin. The accumulated amount of [^{35}S]-SLS in receptor-cell fluid was counted to evaluate the efficacy of the Q-18B gels over a 24-h period. These test gels significantly decreased SLS absorption when compared to unprotected skin control samples (Table 2). The percent protection effect of three test gels against SLS percutaneous absorption was 88%, 81%, and 65%, respectively (Fig. 11).

CONCLUSIONS

1. Some BCs reduce the local damage due to various irritants and allergens.
2. Using inappropriate BC may enhance irritation rather than provide benefit.
3. In vitro methods are recommended in screening procedures for barrier cream candidates because of their simplicity, speed, and safety.

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Table 2 Cumulative Amount of [^{35}S]-SLS and Protection Effect Percent

	Control	A	B	C
Receptor fluid	0.43 ± 0.4	0.05 ± 0.05**	0.08 ± 0.05**	0.15 ± 0.2*
Skin content	14.19 ± 11.1	5.3 ± 7.3	3.95 ± 4.1	4.7 ± 3.5
Skin wash	78.6 ± 12.7	83.81 ± 12.0	83.19 ± 14.9	83.76 ± 10.6
% Protection effect		88%	81%	65%

Statistical differences in comparison with the control. *$p < 0.05$; **$p < 0.01$. Values are mean ± s.d. of percent of applied dose.
Source: Modified from Ref. 25.

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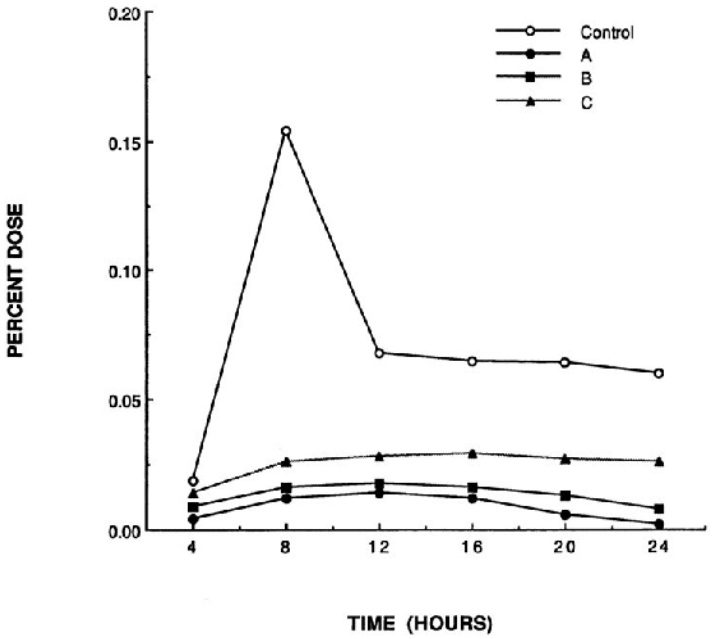

Figure 11 The cumulative amount absorbed into control skin and skin treated with three gels during 24-h exposure. Values are means of percent of applied dose. (Modified from Ref. 25.)

4. Animals may be used to generate kinetic data. Percutaneous absorption in pigs and monkeys shows a closer similarity to that in humans. But no animal, with its complex anatomy and biology, can simulate human absorption for all compounds. Therefore, the best estimate of human percutaneous absorption is determined by in vivo studies in humans.

5. Noninvasive bioengineering techniques are valuable in quantifying the inflammation response to various irritants and allergens when BCs are to be evaluated.

6. The accuracy of measurements of the efficacy of BCs depends on the use of proper methodology.

7. Above all, the clinical efficacy of BCs should be assessed in real rather than in experimental circumstance.

In the end, despite the power of these models, well-controlled field trials are required to define the relationship of the model to the occupational setting.

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Nevertheless, appropriate use of models should lead to formulation refinement and greater insight.

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18
Contact Urticaria Syndrome and Claims Support

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INTRODUCTION

Contact urticaria syndrome (CUS) was first defined by Maibach and Johnson (1) and, since then, numerous reports of contact urticaria to a variety of compounds such as foods, preservatives, fragrances, plant and animal products, metals and other things, continue to be reported. Therefore, it is important to determine, in a scientific manner, whether and in what dose a particular substance causes contact urticaria. Accurate experimental models are required to document urticaria-inducing properties of a substance; protocols to quantify efficacy of formulations that putatively inhibit CUS are also proposed. This chapter outlines current scientific knowledge and approaches to experimental methodology.

SYMPTOMS AND SIGNS

Immediate contact reactions, such as contact urticaria, appear within minutes to about 1 h after exposure of the urticariant to the skin. The patient may complain of local burning, tingling or itch, and swelling and redness may be seen (wheal and flare). Symptoms may extend extracutaneously, inducing, for example, bronchial asthma. In the most severe cases, anaphylactoid reactions may occur. A staging system of CUS has been described (see Table 1).

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Table 1 Staging of Contact Urticaria

	Cutaneous reactions only
Stage 1:	Localized urticaria (redness and swelling)
	Dermatitis (eczema)
	Nonspecific symptoms (itching, tingling, burning)
Stage 2:	Generalized urticaria
	Extracutaneous reactions
Stage 3:	Bronchial asthma (wheezing)
	Rhinitis, conjunctivitis (runny nose, watery eyes)
	Orolaryngeal symptoms (lip swelling, hoarseness, difficulty swallowing)
	Gastrointestinal symptoms (nausea, vomiting, diarrhea, cramps)
Stage 4:	Anaphylactiod reactions (shock)

Source: Ref. 11.

EPIDEMIOLOGY

Kanerva et al. (2,3) gathered statistical data on occupational contact urticaria in Finland. The incidence more than doubled from 89 reported cases in 1989 to 194 cases in 1994. From 1990 to 1994, a total of 815 cases was reported. The most common causes were, in decreasing order, cow dander, natural rubber latex (NRL), and flour/grains/feed. These three groups comprised 79% of all cases. Reflecting this, the most affected occupations (per 100,000 workers) were bakers, processed food preparers, and dental assistants, in decreasing order. Contact urticaria, therefore, is a common problem that may affect many people in the course of their daily lives.

MECHANISMS OF CONTACT URTICARIA

CUS can be described in two broad categories: nonimmunological contact urticaria (NICU) and immunological contact urticaria (ICU). The former does not require presensitization of the patient's immune system to an allergen, whereas the latter does. There are, however, contact urticaria reactions of unknown mechanism, and these are unclassified.

Nonimmunological Contact Urticaria

NICU is the most frequent immediate contact reaction (4) and occurs, without prior sensitization, in most exposed individuals. The symptoms may vary according to the site of exposure, the concentration, the vehicle, the mode of exposure, and the substance itself (5).

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The mechanism of NICU is not well understood. It was previously assumed that histamine was released from mast cells in response to exposure to an eliciting substance. However, the H_1 antihistamines—hydroxyzine and terfenadine—do not inhibit NICU to benzoic acid, cinnamic acid, cinnamic aldehyde, or methyl nicotinate in prick tests, but they do inhibit reactions to histamine itself (5,6). Therefore, mechanisms that do not involve histamine may mediate NICU for these substances.

Evidence suggests that prostaglandins may mediate NICU. Oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit nonimmunological reactions (7). Lahti et al. (6), used laser Doppler flowmetry to demonstrate a reduction in NICU-induced erythema in subjects pretreated with NSAIDs. This group believed that inhibition of prostaglandin metabolism may explain this effect.

Supporting this, Morrow et al. (8) demonstrated an increase in plasma PGD_2 following the topical application of 1% sorbic acid to the human forearm. The time course of PGD_2 peaks correlated temporally with the observed intensity of cutaneous vasodilatation. Notably, histamine and PGE_2 levels at peak erythema were not significantly higher than pretreatment levels. This suggests that the release of vasodilatory prostaglandins induced by sorbic acid was selective for PGD_2, and that histamine is not involved in sorbic acid contact urticarial reactions. The release of PGD_2 was a dose-dependent effect, increasing with greater concentrations of sorbic acid, until reaching a plateau between 1 to 3%. Pretreating the subjects with oral aspirin (325 mg b.i.d. for 3 days) attenuated the observed cutaneous vasodilatation and inhibit release of PGD_2. In later studies, based on the same model, this group demonstrated similar results with benzoic acid–and nicotinic acid–induced contact urticaria (9,10).

These studies add evidence to the argument that prostaglandin metabolism is significant in the pathophysiology of CUS. Also, they not only suggest that NSAIDs are useful as a treatment but also that experimental subjects should avoid these drugs when participating in a contact urticaria study.

Ultraviolet A and ultraviolet B light also inhibits immediate nonimmunological contact reactions. Notably this effect can last for 2 weeks after irradiation and inhibits skin sites that were not directly irradiated (7). The authors suggest that there may be a systemic effect rather than simply a local one; however, the mechanism by which ultraviolet light inhibits NICU is not known.

Immunological Contact Urticaria

ICU is less frequent in clinical practice than the NICU form. It is a type 1 hypersensitivity reaction mediated by IgE antibodies, specific to the eliciting substance (11). Therefore, prior immune (IgE) sensitization is required for this type of contact urticaria.

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This sensitization can be at the cutaneous level, but also via mucous membranes, for example, in the respiratory or gastrointestinal tracts. Notably, ICU reactions may spread beyond the site of contact and progress to generalized urticaria and, in the most severe case, to anaphylactic shock.

People with an atopic background (personal or family background of eczema, hayfever, or asthma) are predisposed toward the immunological form of contact urticaria.

A well-studied example of ICU is allergy to natural rubber latex (NRL), which is found in a wide variety of products, such as balloons, condoms, and, importantly, surgical or protective gloves. ICU to NRL is a major occupational hazard in occupations that utilize such gloves (e.g., the health care profession).

Typically, latex gloves cause a wheal-and-flare reaction at the site of contact. This can affect either the person wearing the gloves or the person being touched by the wearer. In a study of 70 German patients with contact urticaria, 51% suffered rhinitis, 44% conjunctivitis, 31% dyspnea, 24% systemic symptoms, and 6% severe systemic reactions during surgery (12). In addition to direct skin contact, allergy may be caused by airborne NRL (13). Clearly, sensitized, yet undiagnosed, individuals are at risk when contacting ICU allergens.

Cross allergy can also induce ICU reactions: the patient may be sensitized to one protein but reacts to other proteins that contain the same (or similar) allergenic molecule. In the example of latex allergy, patients may also experience symptoms from banana, chestnut, and avocado (14). This phenomenon places ICU patients at further risk.

SITE SPECIFICITY OF CONTACT URTICARIA REACTIONS

Characteristics of the skin and also of its sensitivity to urticariants vary from site to site. This is an important consideration in experimental design, discussed below, and in diagnosis. Shriner and Maibach (15) used laser Doppler flow to map the regions of the human face most sensitive to NICU induced by benzoic acid: the neck was the most sensitive area, followed by the perioral and nasolabial folds. The least sensitive area was the volar forearm. The authors conclude that the neck, nasolabial, and perioral areas are the most sensitive to test for potential NICU to this agent. Lahti (5) found that the back was more sensitive than the hands, ventral forearms, or the soles of the feet, in his study of benzoic acid sensitivity at various body sites.

HUMAN EXPERIMENTAL PROTOCOLS

Human subjects may be used to determine the potential for a product to cause CUS in the human population. The protocols for ICU and NICU are the same,

although ICU requires volunteers who are presensitized to the product. Subject selection, dosing, test site, application methods, and analysis are discussed in this section.

Subject Selection

To test a product for use in the general population, it is desirable to recruit a random pool of volunteers. However, this may introduce several confounding factors, such as age, skin disease, atopic tendency, and medication (such as NSAIDs) that may alter the results. Therefore, subjects must be chosen with particular regard to the aim of the study and screened carefully for inclusion and exclusion criteria, and for possible confounding factors.

Spriet et al. (16) suggest that subjects can be considered in three categories: serious sufferers, symptomatic volunteers, and healthy volunteers. It is likely that the latter are most suitable for testing new products, whereas the former two groups may be better suited to ICU studies or investigating claims that a product already in use causes CUS. Ideally, subjects should be representative of the population at which the product is aimed.

Site Selection

In the diagnostic investigation of a patient, the site affected in the patient's history may be tested. However, in a new product test trial, it is preferable to test the site at which the product is to be used. However, this may not be convenient for volunteers, and so concealed sites such as the volar aspect of the forearm or the upper back, may be chosen. Importantly, the site selected should be consistent in patients and controls, as different areas of the skin may demonstrate different sensitivities to the urticariant, thereby distorting comparability of the data. As noted above, different areas of the skin have varying capacity to induce urticaria, which should be considered when a site is chosen. Even in ICU, different skin sites may vary in their ability to elicit contact urticaria (17).

A history of skin disease may also affect the result. A test that is negative in nondiseased skin may in fact be positive in previously diseased or currently affected skin (18). If the initial studies are negative, it may be desirable to select subjects who are symptomatic and use the affected sites to test the substance.

Paired Comparison Studies

Paired comparison studies allow rapid comparison between treated and untreated groups. Randomized matched pairs can be grouped for treatment and control, or the subjects can be used as their own controls by applying the test substance and controls on separate sites. The latter is preferred, because each subject may have

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several doses applied to their skin, providing more data from a smaller pool of subjects. Furthermore, this decreases intersubject variation and confounding, thus providing better control.

Serial Doses

Performing studies at different doses of the product will allow the investigator to build a dose–response profile. This may indicate a minimum dose that causes a threshold response in the study group and also the dose at which a maximum response is seen. Extrapolating these data to the general population may give manufacturers an indication of a safe concentration for an ingredient to be included in a product. Dose–response analysis may also demonstrate that there is no safe concentration for that ingredient, or, indeed, that there is relatively little risk.

Examples of concentrations that have been used in dilution series in alcohol vehicles are 250, 125, 62, 31 mM for benzoic acid and 50, 10, 2, 0.5 mM for methyl nicotinate (7).

Application Techniques

Commonly used topical application techniques in both immunological and nonimmunological contact urticaria are the *open test* and the *chamber test.* A *use test* can be employed in known sufferers. A positive reaction comprises a wheal-and-flare reaction and sometimes an eruption of vesicles.

1. In the *open test*, 0.1 mL of the test substance is spread over a 3×3-cm area on the desired site. Lahti (7) suggests that using alcohol vehicles, with the addition of propylene glycol, enhances the sensitivity of this test compared with previously used petrolatum and water vehicles. The test is usually read at 20, 40, and 60 min, in order to see the maximal response. Immunological contact urticaria reactions appear within 15 to 20 min, and nonimmunological ones appear within 45 to 60 min after application (11).
2. The *chamber test* is an occlusive method of applying the substance to be tested. These are applied in small aluminum containers (Finn Chamber, Epitest Ltd., Hyrylä, Finland) and attached to the skin via porous tape. The chambers are applied for 15 min, and the results are read at 20, 40, and 60 min. The advantages of this method are that occlusion enhances percutaneous penetration, and therefore possibly the sensitivity of the test; also, a smaller area of skin is required than in an open test. For unexplained reasons, this occlusion may provide less responsivity than in the open test.

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3. The *use test* is a method in which a subject known to be affected uses the substance in the same way as when the symptoms appeared (e.g., putting surgical gloves on wet hands provokes latex ICU).

Other techniques, used in the assessment of ICU are the prick test, the scratch test, and the chamber prick test. RAST can be used to determine cross-reactivity (11,13).

CUS Inhibition

The above models can be employed to test the capability of a substance to inhibit CUS. This may be by topical application or by systemic means. Topical putative inhibitors can be studied by the paired comparison method, using multiple test sites and a control on the same subject. This allows serial dosing, with either the urticariant or the inhibitor, to identify its protective potential against a known urticariant. In systemic studies of an oral putative CUS inhibitor, for example, subjects can be randomized into matched pairs for treatment and control. Following systemic administration, a known urticariant can be applied topically in various doses, as outlined above, and the response assessed.

CLINICAL ASSESSMENT AND QUANTITATIVE METHODS

Previously, dermatological studies of the skin have scored the degree of urticaria by means of visual assessment by an experienced observer, usually a dermatologist. There are several advantages and disadvantages to this technique. Advantages are that it is inexpensive, visual scoring is rapid, subjects are regularly assessed so that the study can be curtailed if adverse reactions are severe, and unexpected findings can be handled by the investigator. However, simple observation may introduce error, inter- and intraobserver variation. This is especially important in larger studies, which may involve a team of investigators.

Visual observations are also often graded on an ordinal (nonlinear) scale (e.g., rating reactions as weak, moderate, or severe). As these data are not in linear numerical form, that statistical analysis is not as powerful as for quantitative data. In many studies, subjects report symptoms, also on an ordinal scale; this, again, is a subjective analysis prone to variation error.

In contrast, a quantitative analysis may provide linear numerical data that are easily reproducible and accurate in standardized conditions. Rather than providing a score, measured data allow for statistical comparison such as mean values and standard deviations. This adds to our understanding of the properties of the test substance. Thus, objective measurements can clearly benefit dermatology studies.

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Table 2 Scale to Score Erythema

Score	Description
1+	Slight erythema, either spotty or diffuse.
2+	Moderate uniform erythema.
3+	Intense redness.
4+	Fiery redness with edema.

Source: Ref. 18a.

Visual Scoring of Contact Urticaria

Contact urticaria can be graded visually by marking the degree of erythema and edema on an ordinal scale. Tables 2 and 3 provide examples.

Measurement of Erythema

Erythema, redness of the skin, is part of the skin inflammatory response that reflects localized increase in capillary blood flow elicited. Therefore, erythema can be measured by both the redness and the blood flow in the inflamed area.

Measuring Color

Two techniques have been used to measure color: remittance spectroscopy and tristimulus chromametry. Elsner gave detailed descriptions of the two techniques (19,20). Essentially, both methods detect light remitted from illuminated skin. Remittance spectroscopy employs multiple sensors to ''scan'' the light over the whole visible spectrum, producing a spectrogram. This differs from a tristimulus chromameter, in which the remitted light is transmitted to three photodiodes, each with a color filter with a specific spectral sensitivity: 450 nm (blue),

Table 3 Scale to Score Edema

Score	Description
1	Slight edema, barely visible or palpable.
2	Unmistakable wheal, easily palpable.
3	Solid, tense wheal.
4	Tense wheal, extending beyond test area.

Source: Ref. 18b.

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550 nm (green), 610 nm (red). The data from a colorimeter are expressed as a color value.

Remittance spectroscopy has been used to measure erythema in contact urticaria (21,22). This group evaluated remittance spectroscopy compared to visual scoring in the assessment of urticarial prick test reactions. They found that there was a significant difference between negative and positive reactions, and between positive and strong positive reactions (+/++). Baseline skin had an erythema index of 36, compared to 72 for a positive reaction. Negative skin sites had a slightly, but not significantly, raised erythema index, resulting from a dermographic reaction related to the procedure of the test itself. Notably, remittance spectroscopy was not as effective in discerning between the stronger reactions (++/+++), possibly because of the reduction of blood flow and hemoglobin content associated with the whitening of the center of the lesion and also because the blood flow may already have been maximized.

Laser–Doppler Blood Flowmetry

Several studies have identified a reliable correlation between skin blood flow measured by laser–Doppler flowmetry (LDF) and cutaneous inflammation (23–27). Bircher (28) reviews the use of LDF to study the role of various mediators in altering cutaneous blood flow.

The LDF technique measures the Doppler frequency shift in monochromatic laser light backscattered from moving red blood cells. This shift is proportional to the number of erythrocytes times their velocity in the cutaneous microcirculation. This noninvasive technique measures a surface area of 1 mm^2 and a depth of 1 to 1.5 mm. The 1-mm depth will therefore measure the upper horizontal plexus, consisting of arterioles, capillaries, and postcapillary venules. LDF does not measure the deep horizontal plexus that lies at the subcutaneous dermal junction. Detailed review of the principles, techniques, and methodology can be found in Berardesca et al. (22).

The changes in blood flow can be expressed in two ways. Either as the net change in cutaneous blood flow over the time of the experiment, which is given by the area under the curve (AUC), or as the maximal increase in flow over the baseline value (PEAK). Following a measurement of baseline blood flow, the product can be applied and posttreatment flow can be measured. The change in blood flow provides an indication of the degree of inflammation caused.

Measurement of Edema

Ultrasound has been used to quantify the edema component of urticaria. Agner and Serup (29) demonstrated a significant difference in skin thickness compared to controls in irritant reactions to sodium lauryl sulfate, nonanoic acid, and hydro-

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chloric acid. Serup et al. (30) used ultrasound to measure edema in patch tests, expressed in millimeters. Agner (31) suggests that A-mode ultrasound scanning is a simple, reproducible method of measuring skin thickness. One disadvantage, however, is that the technique is dependent on an experienced operator, which can potentially introduce observer error.

ANIMAL EXPERIMENTAL PROTOCOLS

Animal models are potentially useful to identify putative contact urticariants.

NICU

The guinea pig ear lobe resembles human skin in its reaction to contact urticariants (7,32), and is an established model for NICU. A positive reaction is seen as erythema and swelling of the ear, which can be quantified by measuring the thickness of the ear.

ICU

Laurema et al. (33) considered a possible animal model for ICU, topically presensitizing mice to trimellitic anhydride (TMA), which is known to cause IgE-mediated reactions. Topical TMA was applied to the dorsum of mice ears 6 days after they had been sensitized, eliciting a biphasic ear-swelling response. However, further studies are required to validate this model.

CONCLUSION

In conclusion, study of contact urticaria is possible with both human and animal subjects in whom a combination of subjective and objective analysis can identify potential immunological and nonimmunological contact urticariants.

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Decorative Products

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INTRODUCTION

Decorative cosmetics are principally concerned with beautifying and decoration, rather than functionality. No discussion of decorative products can be complete without a full understanding of the importance of color, a prime component of every decorative cosmetic. Conventional pigments create color by absorption of certain wavelengths of incident light. The color perceived corresponds to that of the wavelengths reflected. Formulation of decorative cosmetics has been an exciting challenge for cosmetic chemists. Before formulating any color cosmetic product, one must check the current regulations in the country where the proposed product will be sold to make sure all the colors conform to those regulations. The following is a practical guide for the formulator and covers a maximum of technical and regulatory issues in an easy-to-use format.

COLOR

Color Additive Regulation

In the past, colorants had been used in cosmetics without any consideration for their possible toxicity. Today, all countries have regulations that control the type and purity of colors that may be used in cosmetics.

USA: Food and Drug Administration (FDA)

21 CFR 73, 74: POSITIVE LIST (1): colors listed for general cosmetic use, including eye area only if stated specifically, or external only, meaning no contact with mucous membranes. Hair dyes and true soaps are exempt.

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Europe (EU): European Commission (EC)

Directive 76/786, ANNEX IV (2): POSITIVE LIST: colors listed for ingested use, general, including eye area, external, or rinse off.

Japan: Ministry of Health and Welfare (MHW)

MHW Ordinance No. 30 (3): POSITIVE LIST: coal-tar colors. Premarket approval by MHW for all other cosmetic ingredients, including inorganic and natural colorants.

Color Additives: Definitions

Primary/straight color: a color that is pure, containing no extenders or diluents.

Dye: a color that is soluble in the medium in which it is dispersed (i.e., FD&C Blue #1).

Pigment: a color that is insoluble in the medium in which it is dispersed (i.e., FD&C Blue #1 A1 lake, black iron oxide).

Lake: a water-insoluble pigment composed of a water-soluble straight color strongly absorbed onto an insoluble substratum through the use of a precipitant (i.e., FD&C Blue #1 A1 lake). Generally, 10 to 40% color.

Toner: a pigment that is produced by precipitating a water-soluble dye as an insoluble metal salt (i.e., D&C Red #6 barium salt; D&C Red #7 calcium salt).

True pigment: a pigment that, based on its chemistry, precipitates as it is formed (i.e., D&C Red #36).

Extender: a pigment, diluted on substrate (a) during manufacture by precipitation; or (b) postmanufacture by intimate milling or mixing.

Note: FDA has considered any certified colorant mixed with a diluent to be a lake: D&C Red 30 plus talc; D&C Red #7 CA lake on calcium carbonate.

UNITED STATES REGULATIONS

21 CFR Part 73 (4): Listing of color additives exempt from certification. Inorganic pigments, powdered metals, and naturally derived colorants approved for food, drug, and/or cosmetic use. Listed permitted uses: (1) food; (2) ingested/externally applied drugs; (3) general cosmetic; (4) eye area only if mentioned; (5) external (no mucous membrane) (i.e., ultramarines, ferric ammonium); and (6) ferrocyanide not permitted in lip or bath products.

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21 CFR Part 74 (5): Listing of color additives subject to certification. Synthetic organic dyes and pigments. Each batch must be submitted by the manufacturer to the FDA for certification that specifications are met. Listed permitted uses (as in Part 73) of four certified organic dyes and their lakes for eye area use: (1) FD&C Blue #1; (2) FD&C Red #40; (3) FD&C Yellow #5; and (4) D&C Green #5.

21 CFR Part 82 (6): Listing of certified provisionally listed colors. Lakes: FD&C: Aluminum or calcium salt on alumina. D&C: sodium, potassium, barium, calcium, strontium, or zirconium salt on alumina, blanc fixe, gloss white, clay, titanium dioxide, zinc oxide, talc, rosin, aluminum benzoate, calcium carbonate. A salt prepared from straight color (i.e., D&C Red #6) by combining the color with a basic radical.

Proposed permanent listing of color additive lakes (Ref. 7): (1) list substrate (i.e., D&C Red #27 aluminum lake on alumina); (2) extenders of insoluble straight colors will no longer be called lakes (i.e., D&C Red #30); (3) permit blends of previously certified straight colors in a lake (i.e., FD&C Blue #1 and Yellow #5 aluminum lake; (4) all lakes to be prepared from previously certified batches of straight color would necessitate process changes for D&C Reds #6, #7, and #34; and (5) abbreviations permitted for cosmetic ingredient labeling, omitting FD&C, precipitate, and substrate designation (i.e., Blue 1).

EUROPEAN COMMUNITY

Directive 76/786, as amended (8).

Annex IV. List of coloring agents allowed in cosmetic products. List by color index number. Part 1: permanently listed; Part 2: provisionally listed.

Four fields of application:

1. All cosmetic products.
2. All cosmetic products, except those intended to be applied in the vicinity of the eyes, in particular eye makeup and makeup remover.
3. Allowed exclusively in cosmetic products intended not to come into contact with mucous membranes (including the eye area).
4. Allowed exclusively in cosmetic products intended to come into contact only briefly with skin (not permitted in nail preparations).

Lakes and salts. If a color index number is listed in Annex IV, then the pure color plus its salts and lakes are allowed, unless prohibited under Annex II (the list substances that cosmetics may not contain). Exception: barium, strontium, and zirconium.

Prohibited under Annex II, but where a footnote ''3'' appears in Annex IV, ''the insoluble barium, strontium, and zirconium lakes, salts, and pigments

. . . shall also be permitted. They must pass the test for insolubility which will be determined by the procedure in Article 8 (insoluble in 0.1 N HC1).

Purity criteria. Only colors designated by an ''E,'' those also permitted for food use, must meet the general specification for food colors: <5 ppm As; <20 ppm Pb; <100 ppm Sb, Cu, Cr, Zn, $BaSO_4$ separately; <200 ppm of those together. None detectable: Cd, Hg, Se, Te, Th, U Cr^{+6} or soluble Ba. Sixth amendment to the directive is currently adopted. Update of purity criteria is being considered; test methods may be stipulated.

JAPAN

MHW ordinance No. 30 (1966) as amended by MHW ordinance No. 55 (1972) (9)

Positive list. 83 coal-tar colors: must be declared on cosmetic product label; fields of application: oral, lip, eye area, external, rinse-off.

Inorganic/natural colorants. Listing, specifications, test methods: Japan standards of cosmetic ingredients (JSCI); comprehensive licensing standards of cosmetics by category (CLS); and Japan cosmetic ingredient dictionary (CLS).

U.S. Colorants not Permitted/Restricted in Japan

Pigments. D&C Red #6 Ba Lake; D&C Red #21 A1 Lake; D&C Red #27 A1 Lake; D&C Red #33 Zr Lake; D&C Orange #5 A1 Lake.

Substrates: Aluminum benzoate: 0.5% maximum in lipstick; Rosin: 7.0% maximum in lipstick; Calcium carbonate: Not permitted.

Inorganic Pigments. In general, inorganic colors are more opaque, more light fast, more solvent-resistant but not as bright as organic colors. They may be affected by alkali and acid. Inorganic colorants are formed from compounds of the transition elements. Color is produced due to the ease with which the outer ''d'' electrons can absorb visible light and be promoted to the next higher energy level.

Iron oxides	Red	Fe_2O_3
Good stability, opacity	Brown	
	Burgundy	Fe_2O_3
	Black	Fe3O4
	Yellow	FeOOH
Chromium oxide	Green	Cr_2O_3
Good stability, opacity		

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Chromium hydroxide Good stability, lower tinting strength	Aqua	$Cr_2O_3\ _XH_2O$
Ultramarines Good light stability; lower tinting strength; unstable to acid	Blue Violet Pink	$Na_x(AlSi0_4)_yS_z$
Manganese violet Good light stability; lower tinting strength; unstable to water	Violet	$NH_4MnP_2O_7$
Ferric ammonium Ferrocyanide Lower light stability; high tinting strength; unstable to alkali, salts; difficult dispersion	Deep blue	FeNH4Fe(CN)6
Ferric ferrocyanide: Physical/chemical stability as above; precipitated on a substrate (i.e.; Mica)	Deep blue	Fe [Fe(CN)6]3 XH2O
Titanium dioxide Medium light stability, good chemical stability, high opacity	White	TiO2 Anatase Rutile

ORGANIC PIGMENTS

Organic pigments are characterized by: transparency; variable chemical and physical stability; and ''clean,'' bright colors.

Color is produced by chromophoric groups, generally electron donors:

-N=N- -C=O
-NO2 -C=S
-NO

Shade is modified or intensified by auxochromes, generally electron acceptors:

-NH2 -OH
-NHR -OCH3
-NR2

CATEGORIES OF ORGANIC COLORANTS

AZO Colorants: -N=N-

Insoluble (unsulfonated): D&C Red #36; light stable.
Soluble (sulfonated); D&C Red #33, FD&C Red #40, FD&C Yellow #5, FD&C Yellow #6; stable to acid, alkali, light, bleed in water.
Slightly soluble (sulfonated/insoluble salt): D&C Red #6; D&C Red #7, D&C Red #34; color shift in acid and alkali; light fast; resistant to oil bleed.
Oil-soluble (unsulfonated): D&C Red #17

Xanthenes

D&C Orange #5; D&C Red, D&C Red #21; D&C Red #27 ''staining dyes''; structure changes with pH; poor light stability; bleed in solvent.

Triarylmethane

FD&C Blue #1, FD&C Green #3; water-soluble; poor light stability.

Anthraquinone

D&C Green #5; good light stability.

Quinoline

D&C Yellow #10, D&C Yellow #11; oil-soluble.

Indigoid

D&C Red #30; good chemical, light, bleed resistance; exception: acetone-soluble.

STABILITY OF ORGANIC PIGMENTS

True pigments > toners > true lakes. Light: anthraquinone > quinone > indigoid > azo > triarylmethane > xanthene. Heat: True pigments stable to heat. Toners: D&C Red #7 Ca lake changes reversibly; lakes: D&C Red #27 A1 lake changes

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irreversibly. pH: 4–9; metal ions: unstable; solubility: True lakes tend to bleed in water; fluorescein lakes bleed in solvent.

Natural Dyes (10)

Generally used in foods, there is no restriction on their use in cosmetics. For the most part, the resistance of natural dyes to heat, light, and pH instability is much inferior to their synthetic counterparts. A further disadvantage is that they often tend to exhibit strong odors.

Color	Description	Source
Yellow	Curcumim	Turmeric
Yellow	Crocin	Saffron
Orange	Capsanthin	Paprika
Orange	Annato	Annatto
Orange	Cartenoids	Carrots
Red	Cochineal	Coccus cactii
Red	Betanine	Beetroot
Red	Anthocyanins	Red berries
Green	Chlorophylls	Lucerne grass
Brown	Caramel	Sugars

All of the above are of vegetable origin, with the exception of cochineal, which is extracted from the crushed insects *Coccus cactii.*

COLOR CHEMISTRY AND MANUFACTURE

The property of a colorant makes it absorb more in one part of the visible spectrum than another is its chemical constitution. Molecules like atoms exist in different electronic states. Since molecules contain two or more nuclei, they also possess energies of rotation and vibration. This theory applies to both organic and inorganic colorants. With the inorganic colorants, colored compounds are obtained with the ions of the transition elements which have atomic numbers 22 to 29.

INORGANIC PIGMENTS

Titanium Dioxide

A brilliant white pigment. Two crystal types occur: anatase and rutile. Two manufacturing processes are employed: (1) sulfate—either crystal may be produced;

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or (2) chloride—only rutile crystals are formed properties. Crystals of both rutile and anatase are tetragonal, rutile having greater hiding power due to the closer packing of the atoms in the crystal. Refractive indices are 2.55 for anatase and 2.71 for rutile. Opacity is the result of the light-scattering ability of titanium dioxide. Light, heat, and chemical stability are excellent. Additionally, in the United States, titanium dioxide is a category I sunscreen.

Zinc Oxide

Zinc ore is roasted and purified at 1000°C. Two methods of manufacture are utilized: (1) French (indirect); and (2) American (direct).

Properties: Zinc oxide forms transparent hexagonal crystals; whiteness is due to the light scattering of the extremely fine particles. Refractive index is 2.0. Hiding power is less than titanium dioxide. Primary use is for antibacterial and fungicidal properties. Heat and light stability are good. It is soluble in acid and alkali. Zinc oxide in the United States is a category I skin protectant and a category III sunscreen.

Iron Oxides

These are used in all types of cosmetic products. By blending black, red, and yellow in certain properties, brown, tans, umbers, and sienna may be produced. Yellow iron oxide is hydrated iron II (ferrous) oxide, $Fe_2O_3XH_2O$. It is produced by the controlled oxidation of ferrous sulfate. Red iron oxide (chemically Fe_2O_3) is obtained by the controlled heating (at about 1000°C) of yellow iron oxide. Black iron oxide is Fe_2O_4 and is a mixture of ferrous and ferric oxide and is prepared by controlled oxidation of ferrous sulfate under alkaline conditions.

Ultramarines

Theoretically these are polysulfide sodium/aluminum sulfosilicates. They range in color from blue to violet, pink, and even green. A mixture is calcined at 800°C to 900°C for 4 to 5 days. Shades are determined by reaction time, formula variations, and particle size; ultramarine violets and pinks are obtained by treating ultramarine blue with HCl at 275°C, and removing some sodium and sulfur from the molecule.

Manganese Violet

Chemically manganese violet is $MnNH_4P_2O_7$. It is manufactured by heating manganese dioxide with ammonium dihydrogen phosphate and water. Phos-

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phous acid is added and the mixture is heated until the violet color develops.

Iron Blue

Chemically iron blue is ferric ammonium ferrocyanide, $Fe[Fe(Cn)_6]_3$. Sodium ferrocyanide and ferrous sulfate are reacted in the presence of ammonium sulfate. Pigments prepared with sodium or potassium salts are called ferric ferrocyanide.

Chromium Oxide (Cr_2O_3)

A dull yellow green pigment may be prepared by blending an alkali dichromate with sulfur or a carbonaceous material. Reduction to chrome (III) oxide is achieved in a kiln at 1000°C.

Chromium Hydroxide [$Cr_2O(OH)_4$]

A bright bluish-green pigment prepared by the calcination of a bichromate with boric acid at 500°C. The mass during cooling is hydrolyzed with water, yielding a hydrate.

Hydrated Alumina

Chemically hydrated alumina (Al_2O_3 X H_2O) give little opacity and are almost transparent.

Barium Sulfate

Barium sulfate is relatively translucent and may be used as a pigment extender.

ORGANIC PIGMENTS

Organic pigments are chiefly conjugated cyclic compounds based on a benzene ring structure, although some heterocyclic ones exist. There are three main types: lakes, toners, and true pigments. Organic pigments are seldom used without a diluent or substrate in order to maintain color consistency from batch to batch. A true pigment is an insoluble compound that contains no metal ions (e.g., D&C Red #30 and D&C Red #36). They are the most stable. A lake is essentially an insoluble colorant, produced by precipitating a permitted soluble dye to a permitted substrate. In cosmetics, most lakes are based on aluminum, although zinconium lakes are also found. Stabilitywise, true aluminum lakes can be

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affected by extremes of pH, resulting in reforming of the soluble dye or ''bleeding.'' They are fairly transparent and not particularly light-fast. Toners are colorants made with other approved metals besides aluminum, such as barium and calcium. Generally, they are more resistant to heat, light, and pH, although extremes of pH can result in shade changes. Generally, many organic colorants are unsuitable for certain cosmetics because of their chemical nature. D&C Red #36 a typical nonsoluble azo color, is not recommended for lipstick because of its very slight solubility in oils and waxes, when it tends to crystallize upon continual reheating of the lipstick mass. Soluble azo dyes such as FD&C Yellow #5 and #6 and D&C Red #33 lakes are often used in lipstick and nail lacquer. Sparingly soluble types such as D&C Red #6 is not highly soluble but the barium lake of Red #6 and the calcium lake of Red #7 are the most popular colors for cosmetics. Colors in this group do not need a substrate to make them insoluble. The D&C Red #6 and #7 lakes are widely used in lipstick and nail lacquer because of high strength, bright hues, good light fastness, chemical, and heat stability. Non-azo-soluble dyes such as D&C Red #21, Orange #5, and Red #27 all are fluoresceins and act as a pH indicator and will change accordingly. They all stain the skin and D&C Red #27 gives the strongest blue stain.

QUALITY CONTROL OF COLORANTS

Establishment of Standards

1. Insure that product development is performed with material representatives of supplier's production.
2. Prior to purchase, evaluate at least three lots; establish standard in consultation with the supplier.
3. Supplier and end user should agree on specifications, standard, and test methods.

Test Methods

Shade evaluation: Methods should predict performance of the colorant under use conditions. Light source for visual evaluations to be specified.

Dyes: visual or spectrophotometric evaluation of solutions.

Pigments: cannot be evaluated as received due to variable degree of agglomeration. Visual or instrumental evaluation is made of wet and dry dispersions prepared under defined conditions to a defined degree of dispersion.

Vehicles	Dispersion equipment
Talc	Osterizer
Nitrocellulose lacquer	Hoover muller, three roll
Acrylic lacquer	mill, or ball mill
Castor oil	

Heavy metals: wet chemical; atomic absorption spectroscopy (AAS); inductive coupled plasma (ICP).

Particle size: wet/dry sieve analysis; optical microscopy; laser diffraction; sedimentation.

Bulk density: Fischer–Scott volumeter.

pH.

PEARLESCENT PIGMENTS AND OTHER SPECIALTY PIGMENTS

Pearlescent Pigments

The most important requirement for a substance to be pearlescent is that its crystals should be platelike and have a high refractive index. A thin, transparent, platy configuration allows light to be transmitted. A pearlescent material should have a smooth surface to allow specular reflection and be nontoxic. Generally, the most transparent formulation of pearlescent pigments should be used and grinding or milling the pearl pigments should be avoided, and pearls that complement one another should be blended.

Organic Pearls

These pearls produce a bright silver effect and can be obtained from fish scales as platelets or needles that are highly reflective. The materials responsible for the pearl effect are crystals of a purine called guanine. Guanine is chiefly used in nail enamel.

Inorganic Pearls

Bismuth Oxychloride. Bismuth oxychloride produces a silvery–gray pearlescent effect and is synthesized as tetragonal crystals. Crystal sizes vary from approximately 8 μm, which gives a soft, opaque, smooth luster and 20 μm, which give a more brilliant sparkling effect. Its major disadvantage in use is poor light stability that may cause darkening after prolonged exposure. UV absorbs in the finished products are used to overcome this defect. BioCl is chiefly used to pearl nail enamels, lipsticks, blushes, and eye shadows. BioCl may be modified by deposition on mica, titanium dioxide and mica, or talc. Inorganic pigments may

be bonded to BioCl and then deposited on mica. All these alter the final effect on the finished product.

Titanium Dioxide–Coated Micas. Titanium dioxide coated micas are extensively used in decorative cosmetics. They exist in several different forms: (1) Silver–titanium dioxide uniformly coats platelets of mica: rutile crystals give a brilliant pearl effect because of a higher refractive index than the anatase grade. (2) Interference pearlescent products can be made by altering the thickness of the film. At a certain thickness, interference of light can take place so that some wavelengths of the incident light are reflected and others transmitted. The colors created are complimentary to each other. As the layers become thicker, the reflection goes from silvery white, to yellow–gold, red, blue, and green. Additionally, colorants such as iron oxides can be laminated with this interference film providing a two-color effect.

Pigment Pearls

Colored pearls are produced by laminating a layer of iron oxides on titanium dioxides–coated mica producing a color and luster effect.

Specialty Pigments

In addition to BioCl and the titanium dioxide–coated mica systems, polyester foil cut into regular shapes which have been epoxy coated with light-fast pigments have been used for nail enamels and body makeup. Finally, aluminum powder and copper/bronze powder have been used as reflective pigments, especially in eye shadows. For cosmetic use, as in aluminum powder, 100% of the particles must pass through a 200 mesh screen; 95% must pass through a 325 mesh (44 millimicron) screen.

TREATED PIGMENTS

Surface-treated colors and substrates allowed chemists to enhance the aesthetic and functional qualities of their formulations. The benefits of using these treatments may be divided into two categories: those evident in the finished cosmetic product, and the benefits derived from process improvements. Consumer benefits include hydrophobicity yielding greater wear, improved skin adhesion, smoother product feel, improved optical appearance, moisturization, and ease of application. Processing benefits include ease of dispersion, pressability, less oil and moisture absorption, and uniformity.

The following surface treatments are commercially available:

Amino acids: N-Lauroyl Lysine, acyl amino acid (11): natural; good skin adhesion; pH balanced; heat-sensitive.

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Fluorochemical: perfluoropolymethylisopropyl ether perfluoroalkyl phosphate: hydrophobic and lipophobic greatly enhance wear; heat and shear resistance.
Lecithin (12): natural; exceptionally smooth, silky skin feel, particularly in pressed products; heat-sensitive, slightly soluble in water.
Metal soaps (Zn Mg Stearate): good skin adhesion; enhanced compressibility.
Natural wax: natural; moisturizing skin feel; good skin adhesion; heat-sensitive (low m.p.)
Nylon: pure mechanically coated; smooth skin feel.
Polyacrylate: enhanced wetting in aqueous systems; feel is not very good, but is usually used in dispersion.
Polyethylene: hydrophobic; waxy, smooth skin feel; enhanced compressibility; heat sensitive.
Silicone (polymethylhydrogensiloxane): methicone will be chemically bonded and cannot be removed later; hydrophobic; achieves full color development; main use is to improve wetting.
Other silicones: no potential for hydrogen evolution; dimethiconol; absorbed dimethicone; silicone/lecithin.
Silane: extremely hydrophobic, lipophilic; no hydrogen potential.
Titanate ester: isopropyl triisostearyl titanate (13): enhances wetting in oil; smooth skin feel; high pigment loading; lowers oil absorption of pigments.

MICROFINE PIGMENTS

Microfine/ultrafine/nanosized: Pigments have a primary particle size below 100 nm; larger agglomerates/aggregates can be present. Properties such as surface area, bulk density, vehicle absorption, and UV absorption differ significantly from those of conventional pigment. Microfine titanium dioxide, zinc oxide, and iron oxides can be utilized in a range of color cosmetics to provide unique visual effects as well as UV protection. In pressed powders, anhydrous, and emulsified formulations, significant SPF values can be achieved in formulations having a translucent, natural looking finish. With microfine pigments, formulations for darker skin tones can be formulated which avoid the ''ashy'' or ''made-up'' appearance caused by conventional opaque pigments.

LIGHT-DIFFUSING PIGMENTS

Some of the requirements for light-diffusing pigments include a high refractive index, reflection to be diffused, translucency and primarily diffuse transmission.

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Skin has a refractive index of 1.60. Examples of light diffusers include $BaSO_4$, silica, silica spheres coated on mica, $TiO_2/BaSO_4$-coated mica, Al_2OH_3/mica, ultrafine TiO_2/mica, ultrafine TiO_2/polyethylene, ethylene acyrates copolymer, polymethyl methacrylate, and many others. These products are chiefly used in powders to create illusions and hide wrinkles.

MAKEUP TECHNOLOGY

Types of color cosmetics: foundation; blushers; mascara; eyeliner; eye shadow; lip color; nail color.
Purpose: improve appearance; impart color; even out skin tones; hide imperfections; protection.
Types of formulations: suspensions; aqueous; anhydrous.
Emulsions: oil-in-water; water-in-oil.
Powder: pressed; loose.
Anhydrous: wax, solvent; stick; pan; tube.

Powder

The term powdered cosmetics are generally used to describe face powders, eyeshadows, and blushers. When the product is applied to the skin, the shade must not significantly change when worn, must feel smooth in use, making it easy to apply, and adhere well for a reasonable time, without reapplication.

Face Powders

Some of the attributes of a satisfactory face powder are the following: (1) gives smoothness to overall texture; (2) gives added skin translucency when excess is buffed; (3) makes the skin appear more refined and finer textured; (4) helps set the makeup base and adds longevity to the make-up overall; (5) suppresses surface oil and shine. Generally there is a wide range of raw materials used in powdered cosmetics and many of these carry over into the formulation of other decorative cosmetics.

Talc

Talc is the major component of most face powders, eye shadows, and blushers. Chemically it is a hydrated magnesium silicate. Cosmetic talcs are mined in Italy, France, Norway, India, Spain, China, Egypt, Japan, and the United States. Typically talcs are sterilized by gamma irradiation. Particle size should pass through a 200-mesh sieve. Cosmetic talc should be white, free of asbestos, have high

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spreadability or slip, with low covering power. Micronized talc is generally lighter and fluffier but less smooth on the skin than regular grades. Although talc is fairly hydrophobic, treated talcs have been used to enhance its texture. In some products talc is present in up to 70% of the formulation.

Kaolin

Kaolin or china clay is a naturally occurring, almost white, hydrated aluminum silicate. It does not exhibit a high degree of slip. Kaolin has good absorbency, is dense, and is sometimes used to reduce bulk densities in loose powder products. It provides a matte surface effect that can reduce slight sheen left by some talc products.

Calcium Carbonate

Calcium carbonate or precipitated chalk has excellent absorption properties. It provides a matte finish and has moderate covering powder. High levels should be avoided, or an undesirable, dry, powdery feel can result.

Magnesium Carbonate

Magnesium carbonate is available in a very light, fluffy grade that absorbs well and is often used to absorb perfume before mixing it into face powders.

Metallic Soap

Zinc and magnesium stearate are important materials for imparting adhesion to face powders, and usually incorporated at 3 to 10% of the formulation. Stearates add some water repellency to formulas while too high levels give a blotchy effect on the skin. Zinc stearate, besides imparting adhesions, gives a smoothing quality to face powders. Aluminum stearate and lithium stearates have also been used. High levels can make pressed formulation too hard.

Starch

Starch in used in face powders to give a ''peachlike'' bloom and provides a smooth surface on the skin. One problem attributed to rice starch is that when moistened it tends to cake. Also, the wet product may provide an environment for bacterial growth.

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Mica

Chemically mica is potassium aluminum silicate dihydrate. Cosmetic mica is refined and ground to particles of 150 μm or less. It imparts a natural translucence when used up to 20% in formulations of face powder blushes. Mica is available as wet ground that is creamy or dry ground that is matte. Sericite is a mineral, similar to white mica in shape and composition. It has a very fine grain size and a silky shine. It is soft and smooth and has a slippery feel on the skin. Sericite may be coated with silicone and other treatments for better water repellency and skin adhesion.

Polymers

Polymers are chiefly texture enhancers used at levels of 3 to 40% depending on whether they are to be included in a loose or pressed powder. Among these polymers, we find nylon-12 and nylon-6, lauroyl lysine, boron nitride (makes active ingredients spread more uniformly on inactive bases), polyethylene, polypropylene, ethylene acrylates copolymer (very sheer, will not affect binder in pressed powders, processing temperature less than 85–90°), polymethyl methacrylate (PMMA) and silica beads (can carry oily ingredients into a system; increase wear on oily skin), polyurethane powders, silicone powders, borosilicate, microcrystalline cellulose, acrylate copolymers, teflon® and teflon® composites (effective at low concentrations, 1–5%), polyvinylidene copolymers (very light–ultra low density), and composite powders that are coated on inexpensive beads to reduce costs and increase effectiveness, like nylon/mica, silica/mica, lauryl lysine/mica and boron nitride/mica. Many of these polymers are treated with silicones, titanates, lecithin, etc., for increased effectiveness.

Colorants

Titanium dioxide and zinc oxide, both pigmentary and ultrafine, organics, inorganics, carmine and pearlescent pigments either predispersed or treated are found in all face powders because the textures of these colorants are not very satisfactory.

Perfumes

The use of perfumes is important for face powder, which requires them because most of the raw materials used are earthy smelling and should be masked. Perfumes should show stability and low volatility.

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Preservatives

Preservation of face powders is usually not a problem since they are used dry, but small amounts of antibacterials are recommended. Powdered eye shadows should always contain antibacterials such as parabens, imidazolidinyl urea, and others.

Loose Face Powders

This type has declined in popularity in favor of pressed face powder products. The smoothness of loose face powder can be enhanced by use of the aforementioned texture enhancers. In the manufacturing process, all ingredients except the pearls, if required, are combined in a stainless steel ribbon blender. Mixing time can be as long as 1 or 2 h, depending on the size of the batch and evenness of the color. Perfume, if required, is slowly sprayed into the batch, and blended until homogeneous. The batch is then pulverized through a hammer mill and the color is checked. Color adjustments are made, if necessary, in the ribbon blender and the batch is repulverized. Any pearl or mica is then added for a final mix. The batch is then stored and made ready for filling into appropriate containers.

Pressed Face Powders

Pressed face powders are more popular than loose powders because of their ease of application and portability. The basic raw materials are the same as loose powder except that a binder must be used to press the cake into a tin-plate godet. If water-based binders are used, aluminum godets should be considered to prevent corrosion. The properties of a binder are is follows: provides creaminess to the powder, aids in compression and adhesion, develops colorants, enhances water-resistance and pick-up and deposit. If the binder level is too high, it may be difficult to remove the powder with a puff. Also, high levels may lead to glazing of the powder surface, making it waxy looking, with little or no pay-off. Fatty soaps, kaolin, polyethylene, teflon® synthetic wax and calcium silicate are some of the binder systems used. Use levels of binder are between 3 to 10%, depending on formulation variables. Silicone-treated pigments have given rise to pressed face powders that may be used wet or dry. When used dry, they are usually smoother than regular pressure powders. When a wet sponge is applied to the cake, no water penetrates the cake; the water is repelled. These ‘‘two-way’’ cakes can be used either as a foundation or face powder. When formulating pressed powders, care must be taken so that the raw materials used do not corrode the godets or attack the plastic packaging materials. The manufacture of pressed powders, including the mixing and color-matching process, is similar to loose powders. Sometimes the powder mix is pulverized without binder and then again

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after its addition. Pearls are usually added during the blending process and preferably without the milling operation, which can damage the pearl. If milling a batch containing pearl becomes necessary, it should be done with the mill screen removed. Powder pressing is often more successful if the powder is kept for a few days to allow the binder system to fully spread, especially when pearls are present. The most commonly used presses for face powder are the ALITE high-speed hydraulic press and the KEMWALL, CAVALLA, or VE. TRA. CO. presses. The pressures used and the speed of pressing depends on the characteristics of the individual formulation and the size of the godet.

Powder Blushers

The attributes of blushers are as follows: (1) add color to the face; (2) give more dimension to the cheekbones; (3) harmonize the face-balance between eye makeup and lipstick; (4) create subtle changes in the foundation look when lightly dusted over the face. Pressed powder blushers are similar to face powder formulations, except that a greater range of color pigments are used. The three basic iron oxides and one or more of the lakes are used to achieve various blusher shades. Blushers are usually applied with a brush. Manufacture and pressing is similar to face powders. Care should be taken than only nonbleeding pigments be used to avoid skin staining. Total pigment concentration ranges from 2 to 10%, excluding pearls. Pressed powder rouges were once popular and contained high levels of colorants (10–30%). Usually they are applied from the godet with the finger so that glazing may frequently occur if the rouge is improperly formulated.

Pressed Powder Eyeshadows

Eye shadows in general have the following functions: (1) Add color and personality to the face; (2) sharpen or soften the eyeball itself; (3) create the illusion of depth or bring out deep-set eyes; (4) create light and dark illusions for subtle character changes; and (5) can be used wet or dry for different illusions. The technology is similar to other pressed powder products but the permitted color range is limited. In the United States the only synthetic organic pigments that may be used in eye products are FD&C Red No. 40, FD&C Blue #1, FD&C Yellow #5, and Green #5. Carmine, N.F. is the only natural organic pigment allowed and all of the inorganic pigments and a wide range of pearls may be used. Preservation is very important in eye makeup products. Problems of poor adherence to the skin, color matching, and creasing in the eyelid is common when the binder formulation is ineffective with the type and level of pearls used. High binder levels may result in uneven pressing of the godets. In manufacture, formulas with high pearl content should be allowed to settle to remove entrapped air before pressing.

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Quality Assurance on Powder Products

Color testing is done, where production batch and standard are placed side by side on white paper and pressed flat with a palette-knife. Shades are compared to one another. Shades of eye shadows and blushers are checked on the skin using a brush or wand.

Bulk density is carried out on loose powder to ensure that no entrapped air is present so that incorrect filling weights are minimized.

Penetration and drop tests are carried out on pressed godets. A penetrometer is used to determine the accuracy of the pressure used during filling. A drop test is designed to test the physical strength of the cake. Normally, the godet is dropped onto a wooden floor or rubber matte (1–3 times) at a height of 2 to 3 ft to note damage to the cake.

Glazing and payoff is done where the pressed cake is rubbed through to the base of the godet with a puff and any signs of glazing is noted. Payoff must be sufficient and the powder should spread evenly without losing adhesion to the skin.

Foundation

In general, foundation makeup's chief functions are to hide skin flaws, even out various color tones in the skin, act as a protectant from the environment, and makes the skin surface appear smoother. Requirements for an ideal makeup foundation's application are as follows: (1) moderately fast drying to allow for an even application; (2) *should be* nonsettling, easy pourability, stable in storage; (3) no tacky, greasy, or dry feel; (4) improve appearance, not artificially; (5) have proper ''play time'' and slip. Depending on the formulations, several contain treated pigments and volatile silicones to add water-resistance properties. There should be shade consistency between the bottle and skin tone. Products should be uniform. Coverage or capacity will vary with skin types; finish on the skin may by matte, shiny, or ''dewy.'' Wear is extremely important—product should not peel off, become orangy on the skin, or rub off on clothes.

Foundation makeup is available in the following forms:

1. Emulsions: *oil-in-water*—anionic, nonionic, and cationic. *Water-in-oil* became more popular for water/proofness and contains volatile silicone, hydrocarbons, mineral oil, and light esters.
2. Anhydrous: cream powder and stick.
3. Suspensions: oil and aqueous.

Emulsified Foundations

Composition can vary widely depending on degree of coverage and emolliency desired. Although nonionic (usually not stable), cationic (difficult to make, not

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on market), and water-in-oil systems have been marketed, most emulsified foundations are anionic oil-in-water emulsions due to ease of formulation. Anionics possess the following properties: emulsion stability; pigment wetting and dispersion; easy spreading and blending; good skin feel; slippery (soaplike) feeling.

Formulation Considerations

1. Prolonged skin contact. Minimize emulsifier levels to avoid irritation.
2. Choose oils based on low *comedogenicity*.
3. Foundations may be difficult to preserve if they contain water, gums, etc.

Makeup Manufacturing Equipment

Emulsion Makeup: *pigment extenders*—hammer mill and jet mill; *internal phase*—propeller mixer/SS stream jacketed kettle; *external phase*—colloid mill, homogenizer/sidesweep and SS stream jacketed finishing kettle; *emulsification*—sidesweep, homogenizer, and recirculating mill (i.e., colloid mill); *high-viscosity systems* need a planetary mixer.

MANUFACTURING

The coloration of the emulsion base may be handled in different ways: direct pigment, pigment dispersions, mixed pigment blender, and monochromatic color solutions (14). Each has its advantages and disadvantages. In the direct pigment method, the pigments are weighed directly into the aqueous phase and dispersed or colloid milled, then the emulsion is formed in the usual manner. The major problem is that there are too many color adjustments needed and accurate color matching is difficult. With the pigment dispersion method, the pigment is mixed with talc as a 50:50 dispersion and pulverized to match a standard. This reduces the number of color corrections needed, but storage may be a problem as well as the time taken to make these dispersions. During the mixed pigment blender method the pigments and extenders are premixed, pulverized and matched to a standard. It is then dispersed in the aqueous phase of the emulsion and the emulsion is formed in the normal way. The finished shade is color-matched at the powder blender stage. Chances of error are reduced. In the last method, the monochromatic color solutions require color concentrates of each pigment to be made in a finished formula. It is easy to color match by blending finished base, but much storage space is needed and the possibility for contamination is increased.

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ANHYDROUS FOUNDATIONS

Anhydrous foundations generally are powdery, not fluid, and easy to travel with. Ingredients include:

Emollients—often texturally light and low viscosity (i.e., oils, esters and silicones).

Waxes—**natural:** beeswax, jojoba, orange, carnauba, candelilla, and castor; **beeswax derivatives:** dimethicone copolyol beeswax, polyglyceryl-3 beeswax, butyloctanol and hexanediol beeswax (nice texture, compatibility with silicone material); **synthetic:** paraffins, microcrystalline, polyethylene and ''synthetic wax'' (high branched olefin polymers); **fatty alcohols and fatty alcohol ethoxylates:** unithox and unilin; **fatty esters:** croda (syncrowaxes), koster keunen (kester waxes), pheonix chemical, scher, flora tech, and RTD.

Pigments—often surface treated—**TiO_2:** pigmentary and ultrafine; **ZnO:** pigmentary and ultrafine; **iron oxides:** pigmentary and ultrafine (enhances SPF value).

Texturizing agents—often surface treated; include nylon, PMMA, sericite, talc, mica, boron nitride, teflon®, borosilicates copolymer, polyvinylidene copolymer, spherical silica, starches (oats, rice, wheat, corn, dry flo-starch), BiOCl, microcrystalline cellulose, polyurethane powder and silicone powder.

Wetting agents—small amount to be used; include low HLB emulsifiers, polyglyceryl esters (e.g., polyglyceryl-3 diisostearate, hydrogenated lecithin, lanolin alcohols, polyhydroxy stearic acid and soya sterols)

Basic Formulation

Emollients (fluids, low melting point waxes, gel-like raws)—30–60%
Waxes—5–10%
Wetting agents—0.50–1.00%
Texturing agents—30–60%

Surface-treated raw materials are frequently utilized in these types of formulations for the following reasons: improves dispersibility; enhances solids loading (provides drier texture, creates matte appearance, improves wear, overall improved aesthetics).

MANUFACTURING PROCEDURE

1. Emollients, waxes, and wetting agent(s) are introduced into a jacketed kettle and heated until phase is clear and uniform.

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2. Pigments and texturizing agents are slowly introduced into the oil phase with higher shear mixing. Continue high shear mixing until dispersion is uniform and colorants are completely ''extended.'' *Note:* If surface treatments are temperature sensitive, care must be taken to prevent the displacement of that treatment from the surface of the powder into the oil phase itself.

EYE MAKEUP

Mascara

1. Brings out the contrast between the iris and the white of the eye, sharpens white of the eye.
2. Thickens the appearance of the lashes.
3. Lengthens the appearance of the eye.
4. Adds depth and character to the overall look.
5. Sharpens the color of the eye shadow when worn.

Mascara's performance is usually judged by application, appearance, wear, and ease of removal. It is critical that proper brush is supplied for the chosen formulation. Generally, mascara and eyeliners consist of one or more film formers, pigment, and the vehicle that mostly evaporates to allow the film to set.

Three types of formulations are currently in use. In the past, cake or block mascara was popular. This was basically a wax base with a soap or nonionic emulsifier present so that that color could be applied with a wetted brush. Mascara and eyeliners consist or one or more film formers, pigment, and the vehicle that mostly evaporates to allow the film to set.

1. Anhydrous solvent-based suspension: waterproof but not smudge-proof and difficult to remove.
2. Water-in-oil emulsion: waterproof but not smudge-proof and can be removed with soap and water.
3. Oil-in-water emulsion: ''water-based'' if the film is sufficiently flexible, can be flake-proof and smudge-proof. Water resistance can be achieved with the addition of emulsion polymers (i.e., acrylics, polyvinyl acetates, or polyurethanes).

Oil in Water

Water phase: water; suspending agent: hydroxyethylcellulose; film former/dispersing agent: polyvinylpyrrolidone; pigment; hydrophilic emulsifier: alkali, high HLB nonionic.

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Wax phase: high melting point waxes; lipophilic emulsifier: fatty acid, low HLB nonionic, coemulsifier; plasticizer: lanolin or derivatives, liquid fatty alcohol; petroleum solvent (optional) as extender for water phase; preservative: propyl paraben.

Additional film former: solution polyacrylate (improves flake resistance); emulsion polyacrylate; polyurethane; polyvinyl acetate; rosin derivatives; dimethiconol; proteins: wheat, soy, corn, keratin, oat, silk.

Preservative: formaldehyde donor (not for use in Japan).

Manufacturing: procedure is general oil-in-water emulsification procedure except that iron oxides are first wet and milled in the water phase prior to emulsification and final product goes through a colloid mill, roller mill, or homogenizer.

Solvent-Based

Hard, high melting point waxes; rosin derivative (optional); wetting agent; pigment; suspending agent (organoclay); volatile solvent (to achieve wax solubility)—petroleum distillate; cyclomethicone. Preservatives: parabens; Plasticizer: lanolin or derivative, liquid fatty alcohol.

Water-in-Oil

Wax phase: high melting point waxes (carnauba, candellila, polyethylene); rosin derivative (optional); lipophilic emulsifier (lanolin acids, low HLB nonionic); pigment; preservative: propyl paraben; petroleum solvent, some cyclomethicone.

Water phase: hydrophilic emulsifier (alkali, medium HLB nonionic); preservative: methyl paraben.

Additives: emulsion polymer (optional); preservative: formaldehyde donor (not for use in Japan).

Anhydrous Mascara

Ingredients

Solvents—branched chain hydrocarbons and petroleum distillates, isoparaffinic hydrocarbons, and volatile silicones.

Waxes—beeswax and its derivatives, candelilla, carnauba, paraffin, polyethylene, microcrystalline, castor, synthetic, ceresin, and ozokerite.

Resins (could be introduced, but do not have to be)—include aromatic/aliphatic, hydrogenated aromatics, polyterpene, synthetic, rosin, acrylics, and silicones.

Gellants—clays (stearalkonium hectorite, quaternium-18 bentonite, quaternium-18 hectorite), metal soaps (Al, Zn stearates).

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Colorants—most often utilize a classic iron oxide without any surface treatment.

Functional fillers—Spherical particles (PMMA, silica, nylon), boron nitride, starches, teflon®.

Purpose

Provides body to film to enhance thickening properties.
Improves transfer resistance.
Improves deposit on lashes.

Basic Formulation:

Solvent(s)—40–60%.
Waxes—10–20%.
Resin(s)—3–10%.
Gellant—3–7%.
Colorant(s)—5–15%.
Filler(s)—2–10%.

Procedure

1. Heat waxes, solvents and resins in a jacketed kettle until uniform and clear. Slowly add pigments under high shear and mill until dispersion is uniform.
2. Under high shear, add gellant and mill until uniform. Activate gellant with polar additive like propylene carbonate. Under high shear, add fillers and mill until uniform. Cool to desired temperature.

Mascara Componentry

Bottle: PVC-polyvinyl chloride for solvent-based and H.D. polyethylene/polypropylene for water-based types.

Brush/rod/wiper: works complementarily with each other to deliver required product attributes.

For a thickening mascara, the following are required: larger diameter rod; larger diameter wiper; larger brush with significant spacing between the bristles.

For a defining mascara, the following are suggested: smaller diameter rod; smaller diameter wiper; brush with minimal spacing between the bristles.

Brush materials, fiber diameter, brush shape, fiber shape, fiber length, wire diameter, and the number of turns in the wire all affect performance.

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Creme Eyeshadows

Generally, cream eye shadows are another form of eye shadow not as popular as the pressed form. Care must be taken in formulation to avoid creasing and other wear problems. In the past, stick eye shadows were popular. They are similar to cream eye shadows but contain high melting point waxes to make them moldable. The ***ingredients*** utilized are as follows.

1. **Volatile solvents**: cyclomethicone, hydrocarbons, isoparaffins.
2. **Waxes**: similar to those utilized in the anhydrous waterproof mascaras although at lower concentrations.
3. **Emollients**: esters, oils, silicones.
4. **Gellants**: bentonite derivatives, hectorite derivatives.
5. **Colorants and pearls**: classical.
6. **Fillers**: mica, talc, sericite.
7. **Functional fillers**: boron nitride, PMMA, nylon, starches, silica, teflon®, lauroyl lysine.

For enhanced textural properties, higher solids loading, improved application and coverage, use surface treated raw materials whose coatings are neither temperature nor solvent sensitive. Balance the absorption of fillers to main similar textures throughout the shade range.

Basic Formulation:

Solvent—35–55%
Gellants—1.50–3.50%
Waxes—7–12%
Emollients—3–8%
Colorants/pearls—5–20%
Fillers—10–20%
Functional fillers—5–15%

Manufacturing Procedure: Identical to anhydrous mascaras.

Eyeliners

Eyeliners frame the eye while adding shape or changes the shape of the eye. They give the illusion of a larger or smaller eye bringing out the color contrast between the iris and white of the eye. Last, eyeliners assist in making the lashes appear thicker. Generally, liquid eyeliners are the most popular and will be chiefly outlined. Cake eyeliner was popular in the past and was a wettable pressed cake applied with a wet brush. It contained powder fillers, waxes, resins, and a soap or nonionic. Liquid eyeliners include the following list of ingredients:

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Solvent: water
Gellant: gums (magnesium/aluminum silicate and bentonite)
Wetting agents: water-soluble esters, and high HLB emulsifiers
Polyols: propylene glycol, butylene glycol and 2-Methyl-1,3 propanediol
Colorants: surface treatment is not essential but will enhance ease of dispersibility, maintain fluidity, improve adhesion and may enhance water resistance. Chiefly, iron oxides and other inorganic are utilized.
Alcohol: can solubilize resins and improve dry time
Film formers: PVP, PVA, acrylics, PVP/VA, PVP/urethanes

Basic Formulations

Water—50–70%
Gellant—0.50–1.50%
Wetting agent(s)—1–3%
Polyol—4–8%
Colorants—10–20%
Alcohol—5–10%
Film former—3–8%

Manufacturing Procedure

Gellants are premixed with the polyol and added to a heated water phase that also contains the wetting agent. Disperse with high shear until uniform. Add colorants and disperse until uniform. Cool and add alcohol and film former with low shear.

Pencils

Pencils are used in general for coloring the eyebrows and eyelids, although they are now popular as lipsticks, lip liner, and blushers depending on the hardness of the pencil and the color composition.

Products are nearly always manufactured by a handful of contract manufacturers.

Chemists' responsibility is to evaluate the finished product, rather than create one. Evaluation includes shade, texture, sharpenability, wear, application, stability (freeze-thaw and at 40–45°C) and penetration.

Generally, extruded pencils are less stable than the molded ones.

Raw Materials

Oils, esters, silicones
High melt-point triglycerides

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Stearic acid—helps the extrusion
Synthetic waxes
Japan wax
Bright colorants and pearls in leads increase the variety available in cosmetic pencils
Fillers—mica, talc, sericite
Functional fillers—boron nitride, teflon, PMMA, silicas

Product types: eyeliner, lipliner, eyeshadow, lipstick, brow, blush, concealer

Manufacturing Procedure

Molded and extruded; significant differences exist in how these products are evaluated initially after manufacturing. Molded pencils set up within a few days. Extruded pencil set up slowly over a few weeks. The molded or extruded lead is placed in a slat of wood grooved lengthwise. A second grooved slat is glued onto the first slat and pressed together.

Lipsticks

Lipsticks add color to the face for a healthier look, shape and sometimes conditions the lips. Harmonizes the face between the eyes, hair, and clothes. Creates the illusion of smaller or larger lips depending on the color.

There are two types of lipsticks—classical and volatile based.

Ingredients in a Classical Lipstick

Emollients: castor oil, esters, lanolin/lanolin oil, oily alcohols (octyl dodecanol), organically modified silicones (phenyltrimethicone and alkyl dimethicones), meadowfoam seed oil, jojoba oil and esters and triglycerides
Waxes: candelilla, carnauba, beeswax and derivatives, microcrystalline, ozokerite/ceresein, alkyl silicone, castor, polyethylene, lanolin, paraffin, synthetic and ester
Wax modifiers (plasticizers): work in conjunction with the waxes to improve texture, application and stability include cetyl acetate and acetylated lanolin, oleyl alcohol, synthetic lanolin, acetylated lanolin alcohol and petroleum (white and yellow)
Colorants widely used—D&C's (Red #6 and Ba Lake, Red #7 and Ca Lake, Red #21 and Al Lake- (stains), Red #27 and A1 Lake- (stains),

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Red #33 and Al Lake, Red #30, Red #36, Yellow #10). FD&C's (Yellow #5,6 Al lake, Blue #1 A1 lake). Iron oxides (TiO2, ZnO, pearls.) No Fe Blue, Ultramarines, Mn Violet.

Actives: raw materials are added for claims and moisturization; tocopheryl acetate, sodium hyaluronate, aloe extract, ascorbyl palmitate, silanols, ceramides, panthenol, amino acids, and beta carotene

Fillers (matting and texturizing agents): mica, silicas (classical and spherical), nylon, PMMA, teflon, boron nitride, BiOCl, starches, lauroyl lysine, composite powders, and acrylates co-polymers

Antioxidants/preservatives: BHA, BHT, rosemary extract, citric acid, propyl paraben, methyl paraben and tocopherol

Formula	Gloss	Matte
Emollients	50–70%	40–55%
Waxes	10–15	8–13
Plasticizers	2–5	2–4
Colorants	0.50–3.0	3.0–8.0
Pearl	1–4	3–6
Actives	0–2	0–2
Fillers	1–3	4–15
Fragrance	0.05–0.10	0.05–0.10
Preservatives/antioxidants	0.50	0.50

Procedure

1. Pigments are premilled in either one of the emollients (e.g., castor oil) or the complete emollient phase either by a 3-roller mill, stone mill or a type of ball mill.
2. Grind phase is added to complete emollient phase and waxes, heated and mixed until uniform (approx. 90–105°C).
3. Pearls and fillers are added to above phases and mixed with shear (if necessary) until homogeneous.
4. Add actives, preservatives, fragrance and antioxidants and mix until uniform.
5. Maintain a temperature just above the initial set point of the waxes and fill as appropriate.

Ingredients for Volatile Lipstick

The proper balance of solvents and emollients prevent transfer and allow lipstick not to become too dry on the lips (15).

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Solvents: isododecane, alkyl silicones, cyclomethicone
Emollients: phenyl trimethicone, esters, alkyl silicones (fluids, pastes), vegetable/plant oils
Waxes: polyethylene, synthetic, ceresin, ozokerite, paraffin (not compatible with some silicones), beeswax, alkyl silicones
Fixatives: silicone resins (MQ type from G.E.), silicone plus polymers (SA 70-5, VS 70-5)
Colorants/pearls: identical to classical lipstick
Fillers: identical to classical lipstick
Actives: identical to classical lipstick
Preservatives/antioxidants: identical to classical lipstick

Formula

Solvent—25–60%
Emollient—1–30%
Waxes—10–25%
Fixatives—1–10%
Fillers—1–15%
Colorants/pearls—1–15%
Fragrance—0.05–0.10%

Procedure

Identical to classical lipstick except product should be prepared in a closed vessel to prevent loss of volatile components.

Nail Color

Nail lacquers form the largest group of manicure preparations. They should be waterproof, glossy, adherent, dry quickly and be resistant to chipping and abrasion. The main constituents include a film former, modifying resin, plasticizer, and solvents. Additionally, pigments, suspending agents and ultraviolet absorbers are usually included. Nitrocellulose is the chief film-forming ingredient. Nitrocellulose is derived from cellulose, a polymer made of several anhydroglucose units connected by ether linkages. Nitrocellulose by itself will produce a hard brittle film so it is necessary to modify it with resins and plasticizers to provide flexibility and gloss. The most commonly used modifying resin is para foluene sulfonamide formaldehyde resin, which is contained at 5–10% levels. This resin provides gloss, adhesion, and increases the hardness of the nitrocellulose film. The formaldehyde resin has caused allergies with a small number of consumers so that other modifiers such as sucrose benzoate, polyester

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resin and toluene sulfonamide epoxy resin have been used in its place with varying results. Plasticizers used include camphor, glyceryl diesters (16), dibutyl phthalate, citrate esters and castor oil. Other resins such as polyurethanes and acrylics have been used as auxiliary resins. Variations of plasticizers and resins will change the viscosity, dry time, and gloss of the lacquer. Colorants include titanium dioxide, iron oxides, most organics, and pearlescent pigments. Soluble dyes are never used because of their staining effects on skin and nails. In order to reduce settling of the heavier pigments, treatment such as silicone (17) and oxidized polyethylene (18) have been utilized. Modified clays derived from bentonite and/or hectorite are used to suspend the pigments and make the nail enamel thixotropic and brushable. Solvents that constitute approximately 70% of nail lacquers include *n*-butyl acetate, ethyl acetate, and toluene. Generally, those are cream and pearl nail lacquers. Cream shades may be shear or full coverage with titanium dioxide as the chief pigment. Pearlescent nail polish usually contains bismuth oxychloride and/or titanium dioxide coated micas and may even contain guanine-natural fish scales. The manufacturing of nail lacquer is usually carried out by specialty manufacturing firms that are familiar with the hazards of working with nitrocellulose and solvents.

The manufacture consists of two separate operations: (1) manufacture and compounding of the lacquer base; and (2) the coloring and color matching of shades. Top coats that are used to enhance gloss, extend wear, and reduce dry time are usually made with high solids and low boiling point solvents. Cellulose acetate butyrate (CAB) has been used as a substitute for nitrocellulose in nonyellowing top coats but does not adhere as well to the nail (19). Most top coats are nitrocellulose based. Base coats function to create a nail surface to which nail lacquer will have better adhesion. Different auxiliary resins, such as polyvinyl butyral have been used in nitrocellulose systems. Fibers, polyamide resins, and other treatment items have been added in order to provide advertising claims and some may actually alter the effectiveness of the film. In the evaluation of nail enamels the following criteria are used: color, application, wear, dry-time, gloss, and hardness.

MAKEUP FORMULARY—FACE PRODUCTS

Loose Face Powder (20)

Ingredients	W/W%
1. Zinc stearate	8.00
2. Magnesium carbonate	1.00

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3. Iron oxides	q.s.
4. Bismuth oxychloride and mica	25.00
5. Fragrance	q.s.
6. Talc to 100.00	
7. Preservative	q.s.

Procedure

1. Mix ingredient #3 with a portion of ingredient #6; pulverize.
2. Add the other ingredients; mix in a ribbon or double-cone blender until uniform.

Pressed Powder Foundation (21)

Ingredients	W/W%
PART A:	
Talc	6.60
Titanium dioxide	19.20
Mica (and) titanium dioxide	4.80
Iron oxides	11.20
Zinc oxides	6.20
Barium sulfate	13.70
PART B:	
Dimethicone	5.50
Lanolin	8.20
Petrolatum	1.40
Mineral Oil	1.40
Isopropyl Myristate	1.40
PART C:	
Fragrance	q.s.
PART D:	
Preservatives	q.s.

Procedure

1. Mix all of the pigments in Part A together.
2. Add Part B, Part C, Part D with high shear mixing.
3. Press into suitable container.

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Two-Way Powder Foundation (Wet and Dry)

Ingredients	W/W%
1. Sericite	35.0
2. Talc	24.0
3. Mica	10.0
4. Nylon-12	10.0
5. Titanium dioxide	8.0
6. Zinc stearate	3.0
7. Iron oxide pigments, silicone treated	2.0
8. Cetyl octanoate	q.s.
9. Squalane	2.0
10. Octyldodecyl myristate	2.0
11. Mineral oil	2.0
12. Dimethicone	2.0
13. Propyl paraben	0.05
14. Butyl paraben	0.05
15. Perfume	q.s.

Procedure

Mix all ingredients except liquid oils and perfume in a blender. Spray or add liquid oils and perfume. Mix and pulverize. Press into pans.

Pressed Face Powder

Ingredients	W/W%
PART A:	
1. Polymethyl methacrylate	12.00
2. Talc (and) polyethylene	q.s. to 100.0
3. Sericite	10.00
4. Mica (and) polyethylene	5.00
5. Magnesium stearate	3.00
6. Mica (and) titanium dioxide	5.00
7. Kaolin	8.00
8. Color	q.s.
PART B:	
9. Dimethicone	6.00
10. Glyceryl diisostearate	2.00
11. Tocopherol	0.10
12. Butyl paraben	0.05
13. Propyl paraben	0.05

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Procedure

Mix A well. Heat B to 80°C. Mix until uniform. Add B to A. Mix well until uniform. Pulverize and sieve. Press into pans.

Liquid Compact Foundation

A hot-pour solid crème foundation that seems to ''liquefy'' when touched. Easy to blend to a sheer finish.

Ingredients	W/W%
PART A:	
Titanium dioxide (and) isopropyl titanium triisostearate	12.99
Yellow iron oxide (and) isopropyl titanium triisostearate	0.33
Red iron oxide (and) isopropyl titanium triisostearate	0.33
Black iron oxide (and) isopropyl titanium triisostearate	0.10
Aluminum starch ocetenyl succinate (and) isopropyl titanium triisostearate	15.00
Sericite	6.25
Silica	2.00
PART B:	
Squalene	6.50
Dimethicone (5 Centistoke)	11.00
Octyl palmitate	18.00
Polyglycerol-3 diisostearate	5.50
Mineral oil	3.00
Hydrogenated coco glycerines	2.00
Microcrystalline wax	4.00
Carnauba	1.00
PART C:	
Nylon-12	12.00
	100.00

Procedure

Micronize Part A until the color is fully developed. Heat Part B with stirring to 195–200°F. Continue to stir for ½ h. Add Part A to Part B and mix until homogeneous. Cool to 180°F. Add Part C and mix until homogeneous. Pour into pans at 165–170°F.

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Blusher (Pressed) (22)

Ingredients	W/W%
1. Talc	65.70
2. Zinc stearate	8.00
3. Titanium dioxide	3.50
4. Iron oxides (russet)	12.00
5. Iron oxides (black)	0.20
6. D&C Red No. 6 barium lake	0.30
7. Titanium dioxide (and) mica	6.00
8. Methyl paraben	0.10
9. Imidazolidinyl urea	0.10
10. Fragrance	0.10
11. Pentaerythritol tetraisostearate	4.00
	100.00

Procedure

Mix ingredients 1 through 9 well. Pulverize. Place into ribbon blender spray into batch number 10 than 11. Repulverize. Sieve. Press into pans.

Eye Shadow (Pressed) (23)

Ingredients	W/W%
1. Mica (and) iron oxides (and)	40.5
2. Titanium dioxide	
3. Talc	32.4
4. Cyclomethicone (and) dimethicone	13.6
5. Oleyl erucate	13.5
	100.00

Procedure

1. Mix and mill all ingredients through a 0.027″ herring bone screen.
2. Press into a suitable container.

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Eye Shadow (Pressed) (24)

Ingredients	W/W%
1. Talc	4.20
2. Bismuth oxychloride	10.00
3. Fumed silica	5.00
4. Zinc stearate	5.00
5. Titanium dioxide (and) mica	65.00
6. Methyl paraben	0.10
7. Propyl paraben	0.10
8. Imidazolidinyl urea	0.10
9. Lanolin alcohol	3.75
10. Mineral oil	9.75
11. Isostearyl neopentanoate	1.50
	100.00

Procedure

Mix 1 through 8 in a ribbon blender. Mix binder 9 through 11 in a separate container. Spray binder into 1 through 8. Mix until uniform. Pulverize, if necessary, without a screen. Press into pans.

Solvent Mascara (25)

Ingredients	W/W%
(A)	
Petroleum distillate	q.s. to 100.00
Beeswax	18.00
PEG-6 sorbitan beeswax	6.00
Ozokerite 170-D	4.00
Carnauba wax	6.00
Propylparaben	0.10
Glyceryl oleate (and) propylene glycol	1.50
(B)	
Iron oxides	15.00
(C)	
Petroleum distillate (and) quaternium-18 hectorite (and) propylene carbonate	12.50
(D)	
Deionized water	15.00
Methylparaben	0.30
Sodium borate	0.60
Quaternium-15	0.10

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Procedure

Mill pigment (B) into (A) which has been heated to 90°C. After (C) has been added slowly and heated with (A), emulsify by adding (D) at 90°C to (A), (B) and (C) mixture. Continue mixing until cool.

Emulsion-Resistant Mascara (26)

Ingredients	W/W%
(A)	
Deionized water	41.00
Hydroxyethyl cellulose	1.00
Methylparaben	0.30
Aqueous 0.10% phenyl mercuric acetate	4.00
Triethanolamine	1.00
Ammonium hydroxide, 28%	0.50
(B)	
Iron oxides	10.00
Ultramarine blue	2.00
(C)	
Isostearic acid	2.00
Stearic acid	2.00
Glyceryl monostearate	1.00
Beeswax	9.00
Carnauba wax	6.00
Propylparaben	0.10
(D)	
Quaternium-15	0.10
(E)	
30% Acrylic/acrylate copolymer solution in ammonium hydroxide	20.00
	100.00

Procedure

Mill the pigments of (B) in the water phase (B). Heat to 80°C. Heat the oil phase (C) to 82°C. Emulsify. Cool to 50°C. Add (D), then (E). Cool to 30°C.

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Waterproof Eyeliner (27)

Ingredients	W/W%
1. Beeswax	16.50
2. PVP/eicosens copolymer	5.00
3. Petroleum distillate	35.00
4. Petroleum distillate (and) quaternium-18 hectorite (and) propylene carbonate	33.50
5. Preservative	0.20
6. Titanium dioxide (and) mica (and) ferric ferrocyanide	9.80
	100.00

Procedure

1. Heat ingredients 1 to 70°C and blend in 3 (n.b. flammable).
2. Blend in 4 with low shear mixing.
3. Cool to 50°C whilst continuing to mix.
4. Blend in ingredients 2, 5 and 6 and mix until uniform.

Aqueous Eyeliner (28)

Ingredients	W/W%
PART 1	
1. Ammonium vinyl acetate/actylates copolymer	55.00
2. Polysorbate 80	1.00
3. Isopropyl myristate	4.00
PART 2	
4. Propylene glycol USP	2.50
5. Methylparaben USP	0.25
6. Water, deionized	29.50
7. Hectorite (and) hydroxyethylcellulose	0.25
8. Iron oxides	7.50
	100.00

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Makeup Pencil (29)

Ingredients	W/W%
PART 1	
1a. Cyclomethicone	40.0
1b. Bis phenylhexamethicone	40.0
1c. Diphenyl dimethicone	40.0
PART 2	
2. Beeswax	15.0
3. Carnauba	7.0
4. Ozokerite	7.0
5. Paraffin	20.0
6. Mineral oil	q.s. to 100.0
7. Cetyl alcohol	1.0
PART 3	
8. Pigments	q.s.
9. Titanium dioxide	q.s.

Procedure

1. The ingredients of Part 2 are melted and homogenized at 78–82°C, then maintained by a thermostatic bath regulated to 58–62°C.
2. The ingredients of Part 3 are dispersed in Part 1; the mixture is placed in a thermostatic bath at 58–62°C.
3. Part 3 is then added.
4. After homogenization, the whole is cooled in a silicone-treated mold (with Dimethicone).

Classical Lipstick (30)

Ingredients	W/W%
Carnauba wax	2.50
Beeswax, white	20.00
Ozokerite	10.00
Lanolin, anhydrous	5.00
Cetyl alcohol	2.00
Liquid paraffin	3.00
Isopropyl myristate	3.00
Propylene glycol ricinoleate	4.00
Pigments	10.00
Bromo acids	2.50
Castor oil	q.s. to 100.00

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Solvent Lipstick (31)

Ingredients	W/W%
Synthetic wax	6.00
Ceresin	4.00
Isododecane	10.00
Paraffin	3.00
Cetyl acetate/acetylated lanolin alcohol	5.00
Methylparaben	0.30
Propylparaben	0.10
BHA	0.10
D&C Red No. 7 calcium lake	4.00
FD&C Yellow No. 5 aluminum lake	3.00
Titanium dioxide/mica	5.00
Titanium dioxide/mica/iron oxides	3.00
Bismuth oxychloride	10.00
Cyclomethicone	41.50
Isostearyl trimetholpropane siloxy silicate	5.00
	100.00

Procedure

Mix the dry ingredients with the volatiles and silicone ester wax. The waxes and oils are added with heating. The powders are added next. The mixture is then stirred before pouring into molds and allowed to cool.

Cream Nail Enamel (32)

Ingredients	W/W%
n-Butyl acetate-solvent	28.23
Toluene-diluent	24.54
Nitrocellulose ½ sec wet-film-former	12.00
Ethyl acetate-solvent	11.00
Toluene sulfonamide/formaldehyde resin–secondary resin	10.00
Acrylates copolymer–resin	0.50
Dibutyl phthalate–plasticizer	5.00
Isopropyl alcohol, 99%–diluent	4.25
Stearalkonium hectorite–suspending agent	1.00
Camphor–plasticizer	1.50
D&C Red No. 6 barium lake–color	0.08
Titanium dioxide	0.75
Iron oxides	0.15
	100.00

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Pearlescent Nail Enamel (33)

Ingredients	W/W%
n-Butyl actate	34.04
Toluene	30.00
Nitrocellulose ½ sec wet	14.90
Toluene sulfonamide/formaldehyde resin	7.10
Dibutyl phthalate	4.80
Camphor	2.40
Stearalkonium hectorite	1.20
Benzophenone-1	0.20
D&C Red No. 7 calcium lake	0.08
D&C Red No. 34 calcium lake	0.05
FD&C Yellow No. 5 aluminum lake	0.08
Iron oxides	0.15
Bismuth oxychloride (25%)	5.00
	100.00

Acrylic Nail Hardener (34)

Ingredients	W/W%
Ethyl acetate	41.20
Butyl acetate	30.00
Nitrocellulose ½ sec. wet	14.00
Toluene sulfonamide/formaldehyde resin	10.00
Dibutyl phthalate	4.00
Camphor	0.50
Acrylates copolymer	0.20
Benzophenone-1	0.10
	100.00

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Hyaluronan: The Natural Skin Moisturizer

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INTRODUCTION

Skin is a large and complex tissue with a vast range of functions that interfaces with a hostile environment. The mechanisms that underlie the resilience of skin to the harsh outside world, and the extraordinary ability of the skin to also protect underlying tissues, are just beginning to be understood. Skin retains a large amount of water, and much of the external trauma to which it is constantly subjected, in addition to the normal process of aging, causes loss of this moisture. The key molecule involved in skin moisture is hyaluronan (hyaluronic acid) (HA) with its associated water-of-hydration. Understanding the metabolism of HA, its reactions within skin, and the interactions of HA with other skin components will facilitate the ability to modulate skin moisture in a rational manner, different from the empirical attempts that have been utilized up to now.

Recent progress in the details of the metabolism of HA has also clarified the long-appreciated observations that chronic inflammation and sun damage caused by ultraviolet light cause premature aging of skin. These processes, as well as normal aging, all utilize similar mechanisms causing loss of moisture and changes in HA distribution.

In the past several decades, the constituents of skin have also become better characterized. The earliest work on skin was devoted predominantly to the cells that make up the layers of skin: epidermis, dermis, and underlying subcutis. Now it is beginning to be appreciated that the materials that lie between cells, the matrix components, have major instructive roles for cellular activities. This same

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extracellular matrix (ECM) endows skin with its hydration properties. The components of the ECM, though they appear amorphous by light microscopy, form a highly organized structure of glycosaminoglycans (GAG), proteoglycans, glycoproteins, peptide growth factors, and structural proteins such as collagen and, to a lesser extent, elastin. The predominant component of the ECM of skin, however, is HA. It is the primordial and the simplest of the GAGs, and the first ECM component to be elaborated in the developing embryo. It is the water-of-hydration of HA that forms the blastocyst, the first recognizable structure in embryonic development. Attempts to enhance the moisture content of skin, in the most elemental terms, requires increasing the level and the length of time HA is present in skin and preserving the chain length of this sugar polymer, and inducing expression of the best profile of HA-binding proteins to decorate the molecule.

HISTORICAL PERSPECTIVE

The ''Ground Substance'' Era

The term ''ground substance'' was first attributed to the amorphous-appearing material between cells by the German anatomist Henle in 1841 (1). It is a mistranslation of the German ''Grundsubstanz'' which would be better translated as ''basic,'' ''fundamental,'' or ''primordial'' substance. By 1855, sufficient information had accumulated for its inclusion in a textbook of human histology by Köllicker (2).

The study of ground substance began in earnest in 1928, with the discovery of a ''spreading factor'' by Duran-Reynals (3–7). A testicular extract was shown to stimulate the rapid spreading of materials injected subcutaneously, and functioned by causing a dissolution of ground substance. Thus, a new field of research was founded. The active principle in the extract was later shown to be a hyaluronidase, one of the class of enzymes that degrade HA (8,9). The observed dissolution of ''ground substance'' simulated Duran-Reynals to write the following, which is just as applicable today:

> If the importance of a defensive entity is to be judged by the magnitude of the measures taken against it, nature is certainly pointing its finger to the ground substance, as if to invite us to learn more about it (10).

The ''Mucopolysaccharide'' Period

''Ground substance'' was subsequently renamed ''mucopolysaccharides,'' a term first proposed by Karl Meyer (11) to designate the hexosamine-containing polysaccharides that occur in animal tissues, referring to the sugar polymers alone, as well as when bound to proteins. However, the term ''ground substance'' persisted for many years afterward, and could be found in textbooks of biochemistry, dermatology, and pathology as late as the 1970s. It is now established that HA

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is the predominant ''mucopolysaccharide'' of skin, and the major component of ''ground substance.''

Discovery of Hyaluronic Acid (Hyaluronan)

Hyaluronan, this major constituent of ground substance or mucopolysaccharide component, and the substrate for the ''spreading factor'' was identified 1938 by Karl Meyer (12) as a hexuronic acid-containing material that also provided the turgor for the vitreous of the eye. The name hyaluronic acid was proposed from the Greek *hyalos* (glassy, vitreous) and uronic acid. It required 20 years, however, before the chemical structure of HA was established (13). It was later found to be a polymer present throughout the body, identified in virtually every vertebrate tissue, the highest concentrations occurring in the vitreous of the eye, in the synovial fluid found of the joint capsule, in the umbilical cord as Wharton's jelly. However, over 50% of total body HA is present in skin (14).

The Modern Era

The modern era of HA biology began with the realization that HA is a critical regulator of cell behavior, with profound effects on cellular metabolism, and not merely a passive structural component of the ECM. This was brought into focus by a number of observations.

1. HA is prominent in embryogenesis, in maintenance of the undifferentiated state, with its removal required prior to the onset of differentiation, as was established by the pioneering work of Brian Toole (14).
2. HA has a dynamic turnover rate. In the circulation, HA has a half-life of 2 to 5 min (15).
3. HA is prominent in the earliest stages of adult wound healing (16), with elevated levels occurring over a prolonged period in the scar-free wound healing of fetal repair (17–19).
4. HA is involved in malignant progression (20), and the aggressiveness of tumors correlates with levels of HA on the cancer cell surface (21).
5. HA is a signaling molecule, and fragmented HA has major influences on angiogenesis (22,23) and inflammation (24–26).
6. HA has receptors on cell surfaces. The predominant HA receptors CD44 (27–29) and RHAMM (30,31) have complex variant isoforms, and these receptors have the ability to confer motility upon cells with signaling to the cytoskeleton (32,34).
7. These receptors themselves are regulated and are the substrates for phosphokinases (32).
8. HA is found intracellularly and has intracellular modes of action (35).

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Postmodern

The growth of molecular genetics and progress in the human genome project has facilitated rapid development in the understanding of HA metabolism. The enzymes that synthesize HA, HA synthases (HAS), as well as the enzymes that catalyze the catabolic reaction, the hyaluronidases, are all multigene families of enzymes with distinct patterns of tissue expression. The HA receptors, which also come in myriad forms, owe their diversity to both variant exon expression as well as to post-translational modifications. The multiple sites for the control of HA synthesis, deposition, cell- and protein-association, and degradation is a reflection of the complexity of HA metabolism. Their relationships are becoming clarified through the ability to sequence rapidly using the new techniques of molecular genetics. There promises to be an enormous increase in information and in the understanding of HA biology, as the genes for these enzymes and proteins become sorted out.

BIOLOGY OF HYALURONAN

Overview

Hyaluronan is a high molecular weight, very anionic polysaccharide that promotes cell motility, adhesion, and proliferation processes requiring cell movement and tissue organization (36,37). The tight regulation required of HA expression under such conditions is modulated in part by association of HA with cell surface receptors.

Despite the monotony of its composition, without branch points or apparent variations in sugar composition, HA has an extraordinarily high number of functions. Physicochemical studies indicate that the polymer can take on a vast number of shapes and configurations, dependent on polymer size, pH, salt concentration, and associated cations. Hyaluronan also occurs in a number of physiological states, circulating freely, tissue-associated by way of electrostatic interactions but easily dissociated, and in equilibrium with the HA in the rest of the body.

Hyaluronan may be bound to proteins termed hyaladherins (38,39). The HA can be very tightly associated with hyaladherins through electrostatic interactions. The HA in the ECM of cartilage is an organizer of the matrix, the proteoglycan aggrecan and link proteins decorating the HA in a bottle brush configuration. The Km of such associations are of such magnitude that HA is not easily dissociated and is not in equilibrium with the HA of the surrounding loose connective tissues. HA also occurs covalently bound to proteins such as inter-alpha-trypsin inhibitor (40).

Tissues that contain high molecular weight HA are unusually resistant to invasion and penetration (41). Blood vessels are unable to penetrate joint syno-

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vium, cartilage, and the vitreous of the eye. It is also unusual for tumor metastases to develop in these structures. It may be the large size of the HA polymer that also protects such structures from invasion by parasites. The mechanism by which such high molecular weight structures resist hyaluronidase degradation and avoid the rapid HA turnover characteristic of the rest of the body is not known. Potent hyaluronidase inhibitors are involved, a class of molecules about which little is known.

Structure and Terminology

Hyaluronan is composed of repeating alternating units of N-acetylglucosamine and glucuronic acid, all connected by β-linkages, GlcAβ(1 → 3)GlcNAc β(1 → 4). The β-linkage is of more than passing interest and not merely a curiosity relevant only to carbohydrate chemists. Glycogen is a polymer of α-linked glucose. Changing to a β-linkage converts the polymer to cellulose. A high molecular weight chain of β-linked N-acetylglucosamine is the structure of chitin. Chitin and cellulose are the most abundant sugar polymers on the surface of the earth. Yet such β-linked sugar polymers are rare in vertebrate tissues, and require unusual reactions for their catabolic turnover.

Hyaluronan is the simplest of the GAGs, the only one neither covalently linked to a core protein nor synthesized by way of a Golgi pathway, and it is the only nonsulfated GAG. The current terminology refers to (1) GAGs, the straight chain hexosamine sugars and (2) proteoglycans, referring to GAG chains together with the core protein to which they are covalently bound. Hyaluronan is thus the only GAG to date that is not also a component of a proteoglycan.

Existing models suggest that for high molecular mass HA, super molecular organization consists of networks in which molecules run parallel for hundreds of nanometers, giving rise to flat sheets and tubular structures that separate and then join again into similar aggregates. There is strong evidence that an H_2O bridge between the acetamide and carboxyl groups is involved in the secondary structure. The hydrogen-bonded secondary structure also shows large arrays of contiguous -CH groups, giving a hydrophobic character to parts of the polymer that may be significant in the lateral aggregation or self-association, and for interaction with membranes (42). This same hydrophobic character is perhaps involved in the extrusion of newly synthesized HA chains from the cytoplasmic surface of the plasma membrane where the HA synthases are located, through the membrane to the exterior of the cell (43). The unusually stiff tertiary polymeric structure is also stabilized by such hydrophobic interactions.

Glycosaminoglycans and proteoglycans must be distinguished from ‘‘mucins,’’ the branch-chained sugars and their associated proteins. These occur more often on cell surfaces, though they also accumulate in the intercellular ‘‘ground substance,’’ particularly in association with malignancies. The terms are used

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carelessly, particularly among pathologists and histologists, and ''mucin,'' ''mucinous,'' ''myxomatous,'' ''myxoid'' or ''acid mucoproteins'' unless they have been defined biochemically, may or may not refer to HA-containing materials. This problem has arisen in part because of the ill-defined or unknown nature of histochemical color reactions. A recent example of this ambiguity is the incorrect assumption that the stain Alcian blue has some specificity for HA at pH 3.0 and for the sulfated GAG at pH 1.5 (44).

By electron microscopy, HA is a linear polymer (45). It is polydisperse, but usually has a molecular mass of several millions. In solution at physiological pH and salt concentrations, HA is an expanded random coil with an average diameter of 500 nm. The molecular domain encompasses a large volume of water, and even at low concentrations, solutions have very high viscosity. The HA in high concentrations, as found in the ECM of the dermis, regulates water balance, osmotic pressure, functions as an ion exchange resin, and regulates ion flow. It functions as a sieve, to exclude certain molecules, to enhance the extracellular domain of cell surfaces, particularly the lumenal surface of endothelial cells, to stabilize structures by electrostatic interactions, and also acts as a lubricant.

Hyaluronan also acts as an organizer of the ECM, the central molecule around which other components of the ECM distribute and orient themselves (46). The avidity of HA for certain ECM moieties, such as the NH_2-terminal of the proteoglycan aggrecan approaches that of avidin-biotin. The anomalous ability of HA to be both hydrophobic and hydrophilic, to associate with itself, with cell surface membranes, with proteins, or with other GAGs speaks to the versatility of this remarkable molecule.

Function

General

The large volume that HA occupies including its cloud of solvent, the water of hydration under physiological conditions underlies its ability to distend and maintain the extracellular space, and preserving tissue hydration. Hyaluronan increases whenever rapid tissue proliferation, regeneration, and repair occur (14). Its ability to organize the ECM and its voluminous water of hydration, and its interaction with other macromolecules explain only a portion of the remarkable functions with which it is associated.

For example, bursts of HA deposition correlate with mitosis (47–49). Elevated levels promote cell detachment, in preparation for mitosis, as cells leave tissue organization, and enter the transient autonomy required for the mitotic event to occur. Cells must then degrade that HA, after mitosis has occurred, to regain adhesiveness, and to reenter the ''social contract.'' The prediction is that HA synthesis occurs as cells enter mitosis, and that a hyaluronidase activity is

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activated as cells leave mitosis. To date, such experiments have not been carried out in synchronized cells. The persistent presence of HA also inhibits cell differentiation (50,51), creating an environment that instead promotes cell prolieration. The elevated levels of antiadhesive surface HA that promotes cell detachment also permits the embryonic cell to migrate (52) or the tumor cell to move and metastasize (20,21). The water of hydration also opens up spaces creating a permissive environment for cell movement.

Hyaluronan is generally produced in the interstitium, in the mesenchymal connective tissue of the body, and is thought to be largely a product of fibroblasts. It reaches the blood through the lymphatics. Most of the turnover of HA, approximately 85%, occurs in the lymphatic system. This remaining 15% that reaches the blood stream has a rapid turnover, with a $t_{1/2}$ of 2 to 5 min, being rapidly eliminated by receptors in the liver, and also, by unknown mechanisms in the kidney (15,53,54). When the hepatic or renal arteries are ligated, there is an immediate rise in the level of circulating HA (55). Thus, humans synthesize and degrade several grams of HA daily.

During acute stress, such as in shock or with septicemia, there is a rapid rise in circulating HA (56–59). Such HA may function as a volume expander, as a survival mechanism to prevent circulatory collapse. However, some of this rapid rise in HA represents HA recruited from interstitial stores and from lymphatics, and not entirely a reflection of increased synthesis or decreased degradation (60). However, higher plasma levels of HA does correlate with decreased turnover rates, the $t_{1/2}$ reaching 20 to 45 min in situations of acute stress.

The mean serum and plasma level in healthy young people is 20–40 μg/L (61,62). This value increases with age (63,64), and probably reflects slower clearance, and decreased HA degradative capacity, though this has not been carefully investigated. Hyaluronan also increases in the circulation in liver disease, particularly cirrhosis, and in renal failure reflecting aberrant degradation (65–67), in rheumatoid arthritis (68) and in some malignancies, resulting from increased tissue synthesis (69).

Embryonic Development

The developing embryo is rich in HA. The HA creates the spaces permissive for fetal cell migration and proliferation. The HA concentration is high not only in the fetal circulation, but also in amniotic fluid (70), the fetal tissues, fetal membranes, and in the placenta. The HA levels reach a maximum of 20 μg/mL at approximately 20 weeks of gestation, and then drop until, at 30 weeks gestation, they reach the 1 μg/mL adultlike levels. This corresponds approximately to the time when a ''switch'' from the scar-free fetal wound healing to the adultlike wound healing with scarring occurs (71). The factors in the fetal circulation that support such high levels of HA synthesis have been explored and partially characterized (72), but have not yet been isolated nor fully identified.

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The neural crest cells as they pinch off from the neuroectoderm, migrate through the embryonic body in a sea of HA (52). When these cells reach their particular destination, hyaluronidases remove the HA, and cell migration then ceases. In embryology, as parenchymal glands develop, HA can be found in the stroma immediately ahead of the arborizing tips, creating the spaces into which the growing glands can grow (73–74).

The classic studies of Bryan Toole and his laboratory separate embryology into two stages, a model that can be superimposed on the development of virtually all parenchymal organs and vertebrate structures: (1) a primary HA-rich phase in which undifferentiated stem cells involved proliferate and migrate; followed by (2) removal of the HA and the onset of cellular differentiation and morphogenesis (14).

Wound Healing

The ECM in the earliest stages of wound healing is also rich in HA. There is also an abundance of inflammatory cells, a necessary component for the normal process of wound healing. In the adult, HA levels rapidly reach a maximum and then drop rapidly (19), reminiscent of the stages in embryology. Decreasing HA levels are followed by increasing amounts of chondroitin sulfate, the appearance of fibroblasts, and then deposition of a collagen-rich ECM. In the adult, wound healing results in scar formation. In the fetus, however, wound repair is associated with levels of HA that remain elevated, and the final result is a wound free of scar. Such observations are made in both the experimental fetal rabbit and sheep models, as well as clinically, in infants delivered following *in utero* surgery. It is on this basis that elevated HA in the wound matrix is assumed to be a key to decreased scarring, contractures, and adhesions in adult wound repair. Aspects of wound healing appear to be a strategic retreat to an embryonic situation, followed by a rapid recapitulation of ontogeny.

Carcinogenesis

In malignancy, HA also appears to play a critical role (20,75). Levels of HA on the surface of tumor cells correlate with their aggressiveness (21). In a study of tumor cell-associated HA, the proportion of tumor HA-positive cells, as well as intensity of HA staining, were unfavorable prognostic factors in colorectal cancer (76). However, overexpression of hyaluronidase also correlates with disease progression, as shown recently in bladder (77,78) and in breast tumor metastases (79,80). These apparently diverse scenarios may indicate that HA and hyaluronidase are required at different stages in the multistep progression of cancer.

Aging

HA levels are high in the fetal circulation and fall shortly after birth. After maintaining a steady level for several decades, circulating levels of HA then begin to

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increase again in old age (61,81,82). Elevated levels of circulating HA are also found in the syndromes of premature aging, in progeria (83), and in Werner's syndrome (84).

Increased HA levels in the bloodstream decreases immune competence (85). Various mechanisms have been invoked. An HA coating around circulating lymphocytes may prevent ligand access to lymphocyte surface receptors (86–88). The increased HA may represent one of the mechanisms for the immunosuppression in the fetus. The reappearance of high levels of HA in old age may be one of the mechanisms of the deterioration of the immune system in the elderly. The increasing levels of HA with aging may be a reflection of the deterioration of hydrolytic reactions, including the hyaluronidases that maintain the steady state of HA. This is a far more likely mechanism than an increase in HA synthase activity.

The increased HA that is found often in malignancy in the bloodstream (89–92) as well as on the surface of tumor cells (21) may be one of the cancer's techniques for compromising host immune function. It is the probable basis of the failure to rosette in the classic sheep red blood cell rosette test, a former laboratory procedure used to diagnose malignancy (93,94). The rosetting failure may have been due to the HA coating on the cancer patients' lymphocyte surfaces.

Hyaladherins

Hyaluronan exists in a number of states in the vertebrate body. Within the ECM, it can be firmly intercalated within proteoglycans and binding proteins in a bottlebrush-like configuration. It can be bound to cells by means of cell surface receptors. Some of the HA exists in a free form circulating in the lymphatic or cardiovascular system. However, even in this relatively free form, there are a number of binding proteins that decorate HA. These are referred to collectively as hyaladherins, a term coined by Toole (38,39). The hyaladherins associate with HA through electrostatic or covalent bonds (40). It is likely that some of the unique properties attributed to HA are in fact a function of the hyaladherins that are bound to the HA. Growth factors, collagen (94), and myriad other proteins have been identified.

One of the major challenges and opportunities in dermatology is to identify the profile of hyaladherins specific for the HA of epidermis and dermis, to characterize these proteins, and to understand their function in relation to age-related changes. In an examination of skin as a function of age, the levels of HA do not decrease, as would be expected, but rather the binding of HA to tissue proteins became more tenacious, and the HA became increasingly more difficult to extract (96). Another challenge is to understand how HA as a substrate for degradation by hyaluronidases is affected by associated hyaladherins. It is also reasonable to

assume that the secondary structure of the HA polymer is modulated, in part, by the hyaladherins bound to it.

Hyaluronan in the Extracellular Matrix

The ECM that surrounds cells, and occupies the variable spaces between cells is composed predominantly of structural proteins such as collagen and elastin, as well as proteoglycans, and a number of glycoproteins. The basal lamina or basement membrane that separates dermis and epidermis is composed of similar materials, and is therefore also considered an ECM structure.

A number of growth factors are embedded in the ECM, concentrated by ECM components where they are protected from degradation. Such factors are presented to cells as mechanisms for growth control and modulators of cell function. Heparan sulfate-containing proteoglycans bind members of the FGF and EGF family (97), while HA can bind growth factors such TGF-beta (98). A complex picture is emerging suggesting that the two classes of GAG, HA and heparan sulfate, have opposing functions. An HA-rich environment is required for the maintenance of the undifferentiated, pluripotential state, facilitating motility and proliferation, while the heparan-sulfate proteoglycans promote differentiation. However, the concentration of HA in the ECM can vary widely. Even when the levels are decreased, as in areas of marked fibrosis, HA functions as an organizer of the ECM, as a scaffold about which other macromolecules of the ECM orient themselves. Diameters of collagen fibers can be modulated by levels of HA, the thinner more delicate fibers being favored in regions of high HA concentrations. In fibroblast cultures, the addition of exogenous HA to the medium decreases the diameter of the collagen fibers that accumulate (unpublished observations).

The ability of HA to promote cell proliferation is dependent in part on the concentration of the HA molecule (99), opposite effects being achieved at high and low concentrations. Size is also important. High molecular weight HA is antiangiogenic (41), while lower molecular weight HA moieties are highly angiogenic, stimulating growth of endothelial cells (22), attracting inflammatory cells, and also inducing expression of inflammatory cytokines in such cells (24–26). Partially degraded HA may have the opposite effect, possibly because it is no longer able to retain and release growth factors such as TGF-beta (98).

The intense staining for HA in psoriatic lesions may in part be due to partially degraded HA, and may be the mechanism for the marked capillary proliferation and inflammation that characterizes these lesions (100–102). Attempts to stimulate HA deposition for purposes of promoting skin hydration must use caution that the HA deposited remain high molecular weight, by preventing free radical-catalyzed chain breaks and by carefully restricting the catabolic reactions of the hyaluronidases.

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Intracellular Hyaluronan

The most recent development is the realization that HA and associated hyaladherins are intracellular, and have major effects on cellular metabolism. Much of the recent advance comes from the ability to remove the ECM of cultured cells using the highly specific Streptomyces hyaluronidase. Permeabilizing such cells and using confocal microscopy then makes it possible to use localization techniques for the identification of intracellular HA and its associated proteins (34). They also appear to be a component of the nuclear matrix in a wide variety of cells (103,104). They also have importance in regulating the cell cycle and gene transcription. A vertebrate homologue of the cell cycle control protein CDC37 was recently cloned and found to be an hyaladherin (105), as was a protein that copurified with the splicing factor SF2 (106). An intracellular form of the HA receptor RHAMM was demonstrated to regulate erk kinase activity. Changes in function of these intracellular hyaladherins, depending on whether or not they have HA molecules attached, confers another layer of complexity dependent on intracellular hyaluronidase enzymes.

In the HA-rich vertebrate embryo and fetal tissues, there is minimal intercellular ECM. Most of the HA is intracellular, and the role of such intracellular HA in development is unknown. The HA-rich germinal epithelium and pluripotential basal cells of the bone marrow, as well as basal epithelium keratinocytes contain large amounts of HA that are involved in cell physiology. Such HA should be separated from the HA of the ECM, presumably the more important compartment when dealing with skin moisture.

HYALURONAN RECEPTORS

CD44

There are a variety of HA-binding proteins that are broadly distributed, and with wide variations in locations, in the ECM, cell surface-associated, intracellular, both cytoplasmic and nuclear. The same molecule may occur in multiple locations. However, it is those on that attach HA to the cell surface that constitute receptors. The most prominent among these is CD44, a transmembrane glycoprotein that occurs in a wide variety of isoforms, products of a single gene with variant exon expression (27–29). CD44 is coded for by ten constant exons, plus from zero to ten variant exons, all inserted into a single extracellular position near the membrane insertion site (107). Additional variations in CD44 can occur as a result of post-translational glycosylation, addition of various GAG, including chondroitin sulfate and heparan sulfate. CD44 is able to bind a variety of other ligands, some of which have not yet been identified. CD44 has been shown, however, to interact with fibronectin, collagen, and heparin-binding growth fac-

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tors. CD44 is distributed widely, being found on virtually all cells except red blood cells. It plays a role in cell adhesion, migration, lymphocyte activation and homing, and in cancer metastasis.

The appearance of HA in dermis and epidermis parallels the histolocalization of CD44. The nature of the CD44 variant exons in skin at each location has not been described. The ability of CD44 to bind HA can vary as a function of differential exon expression. It would be of intrinsic interest to establish whether modulation occurs in CD44 variant exon expression with changes in the state of skin hydration. Changes in the profile of CD44 variant exon expression as a result of skin pathologies also await description.

Only one of many possible examples of the importance of CD44-HA interactions in normal skin physiology is given here. The HA in the matrix surrounding keratinocytes serves as an adhesion substrate for the Langerhans cells with their CD44-rich surfaces, as they migrate through the epidermis (108,109). In skin pathophysiology, the effect of local and systemic immune disorders on such interactions between Langerhans cells and keratinocytes awaits explication (110).

RHAMM

The other major receptor for HA is RHAMM (Receptor for HA-Mediated Motility) (111,112) discovered and cloned by Turley. This receptor is implicated in cell locomotion, focal adhesion turnover, and contact inhibition. It also is expressed in a number of variant isoforms. The interactions between HA and RHAMM regulates locomotion of cells by a complex network of signal transduction events and interaction with the cytoskeleton of cells. It is also an important regulator of cell growth (113).

The TGF-β stimulation of fibroblast locomotion utilizes RHAMM. TGF-β is a potent stimulator of motility in a wide variety of cells. In fibroblasts, TGF-β triggers the transcription, synthesis and membrane expression of not only RHAMM, but also the synthesis and expression of the HA, all of which occurs coincident with the initiation of locomotion (114).

Both RHAMM and CD44 may be among the most complex biological molecules ever described, with locations in an unusually wide variety of cell compartments, and associated with a spectrum of activities involving signal transduction, motility, and cell transformation. The apparent inconsistency of observations between different laboratories regarding the receptors CD44, and RHAMM (115) reflects the subtle ways HA exerts its broad spectrum of biological effects and the myriad of mechanisms for controlling levels of HA expression and deposition. Particularly in the experimental laboratory situation, minor changes in culture conditions, differences in cell passage number, length of time following plating,

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variations in growth factors contained in lots of serum, or differences in stages of cell confluence have major repercussions in expression of HA, its receptors or the profile of hyaladherins that decorate the HA molecule.

HYALURONAN IN SKIN

Artifacts of Hyaluronan Histolocalization in Skin

Hyaluronan occurs in virtually all vertebrate tissues and fluids, but skin is the largest reservoir of body HA, containing more than 50% of the total. Earlier studies on the distribution of HA in skin, using histolocalization techniques, seriously underestimated HA levels. Formalin is an aqueous fixative, and much of the soluble tissue HA is eluted by this procedure. The length of time tissue is in the formalin is a variable that may explain the conflicting results that are often encountered. Acidification and addition of alcohol to the fixative causes the HA to become more avidly fixed, so that subsequent aqueous steps are unable to elute HA out of the tissue (44).

Shown below are comparisons HA localization in skin sections fixed with acid ethanol formalin (Fig. 1A), and conventional formalin (Fig. 1B) fixation. Much of the HA, particularly in the epidermis, is eluted during the process of formalin fixation. This suggests that epidermal HA is more loosely associated with cell and tissue structures than is dermal HA. A further incubation of 24 h in aqueous buffer further increases the disparity between the acid alcohol formalin Fig. 2A) and the conventional fixation (Fig. 2B) technique. Once the tissue has been exposed to the acid alcohol formalin, the HA association with tissue becomes permanently fixed, with little loss of apparent HA observed following additional aqueous incubation, while the formalin-fixed tissues demonstrates progressive loss of HA.

Epidermal Hyaluronan

Until recently, it was assumed that only cells of mesenchymal origin were capable of synthesizing HA, and HA was therefore restricted to the dermal compartment of skin. However, with the advent of the specific techniques for the histolocalization of HA, the biotinylated HA-binding peptide (116), evidence for HA in the epidermis became apparent (96,117–120).

In addition, techniques for separating dermis and epidermis from each other permitted accurate measurement of HA in each compartment, verify that epidermis does contain HA (121).

Hyaluronan is most prominent in the upper spinous and granular layers of the epidermis, where most of it is extracellular. The basal layer has HA, but it is

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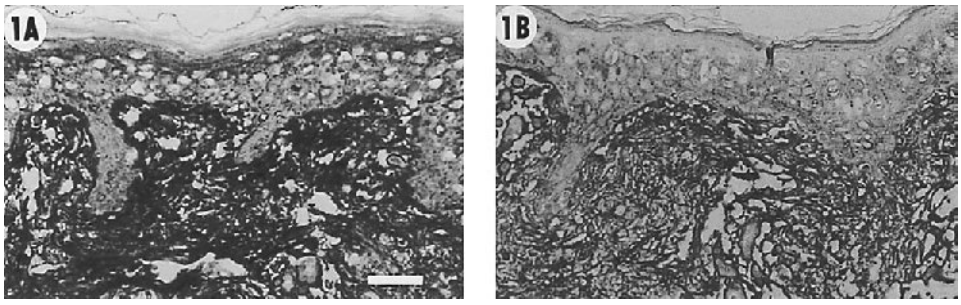

Figure 1 Sections of human skin were stained for HA using a biotinylated HA-binding peptide, derived from bovine cartilage aggrecan. Histolocalization of HA is indicated by the blue color, developed using an avidin-conjugated alkaline phosphatase. Slides were counterstained with Nuclear Fast Red to visualize skin structures. (A) The skin section was fixed in acid-formalin/ethanol, and in (B) formalin/PBS. The bar = 50 μm. HA staining in skin is found predominantly in the dermis, rather than in the epidermis, particularly in the papillary dermis. The most intense staining is observed in the section fixed with the acid-formalin/alcohol (A), compared to the section fixed with the conventional neutral-buffered formalin (B). Of particular interest is that small scattered foci of staining in the epidermal layer are comparable to the intensity of staining found in the dermis using the acid-formalin/alcohol (A). Such foci in the epidermal layer stained less intensely in conventionally fixed samples (B). The staining for HA was blocked by preincubation of the HA binding peptide with HA. In addition, preincubation of the HA-binding peptide with other GAGs, such as chondroitin sulfate, dermatan sulfate, and keratan sulfate at the same concentration did not decrease the intensity of subsequent HA staining (not shown). These results demonstrate that the HA-binding peptide staining reaction is highly specific for HA and that the peptide does not react with other tissue GAGs.

predominantly intracellular, and is not easily leeched out during aqueous fixation. Presumably, basal keratinocyte HA is involved in cell-cycling events, while the secreted HA in the upper outer layers of the epidermis are mechanisms for disassociation and eventual sloughing of cells.

Cultures of isolated keratinocytes have facilitated the study of epithelial HA metabolism. Basal keratinocytes synthesize copious quantities of HA. When Ca^{2+} of the culture medium is increased, from 0.05 to 1.20 mM, these cells begin to differentiate, HA synthesis levels drop (122), and there is the onset of hyaluronidase activity (123). This increase in calcium that appears to simulate in culture the natural in situ differentiation of basal keratinocytes parallels the increasing calcium gradient observed in the epidermis. There may be intracellular stores of calcium that are released as keratinocytes mature.

Alternatively, the calcium stores may be concentrated by lamellar bodies

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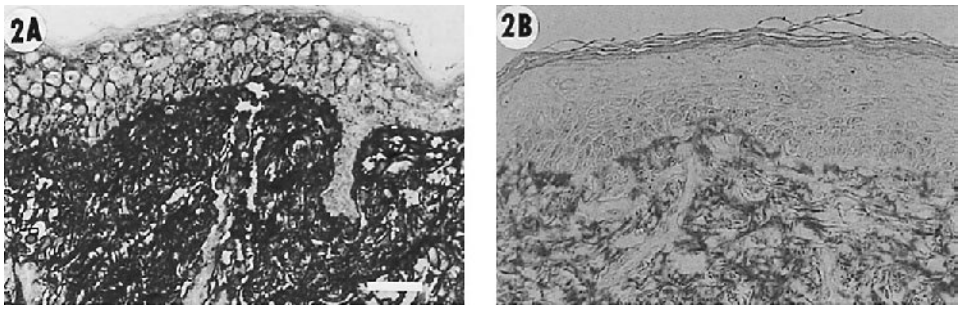

Figure 2 Sections of human skin were incubated in PBS for 24 h at 37°C prior to staining precisely as described in Figure 1. (A) The skin section was fixed in acid-formalin/ethanol, and in (B) formalin/PBS. The bar = 50 μm. To confirm further that the HA in skin fixed in acid-formulin/alcohol is better preserved than in neutral buffered formalin, one set of slides was incubated with PBS overnight prior to the staining reaction. As shown, approximately 80–90% of HA is retained in the section fixed in the acid-formalin/alcohol (A) compared to the section fixed in neutral buffered formalin (B). Notably, the staining of the epidermis is almost unchanged. However, much of the stainable HA in the dermis has leeched out of the sample fixed with neutral formalin during the overnight incubation in PBS (B). Therefore, tissue fixed in acid-formalin/ethanol retained HA far better than that fixed in neutal buffered formalin. (Figures 1 and 2 from Ref. 45.)

from the intercellular fluid that are released with terminal differentiation. The lamellar bodies are thought to be modified lysosomes containing hydrolytic enzymes, and a potential source of the hyaluronidase activity. The lamellar bodies fuse with the plasma membranes of the terminally differentiating keratinocyte, increasing the plasma membrane surface area. Lamellar bodies are also associated with proton pumps that enhance acidity. The lemallar bodies also acidify, and their polar lipids become partially converted to neutral lipids, thereby participating in skin barrier function.

Diffusion of aqueous material through the epidermis is blocked by these lipids synthesized by keratinocytes in the stratum granulosum, the boundary corresponding to the level at which HA-staining ends. This constitutes part of the barrier function of skin. The HA-rich area inferior to this layer may obtain water from the moisture-rich dermis. And the water contained therein cannot penetrate beyond the lipid-rich stratum granulosum. The HA-bound water in both the dermis and in the vital area of the epidermis are critical for skin hydration. And the stratum granulosum is essential for maintenance of that hydration, not only of the skin, but of the body in general. Profound dehydration is a serious clinical problem in burn patients with extensive losses of the stratum granulosum.

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Dermal Hyaluronan

The HA content of the dermis is far greater than that of the epidermis, and accounts for most of the 50% of total body HA present in skin. The papillary dermis has the more prominent levels of HA than does reticular dermis (96). The HA of the dermis is in continuity with both the lymphatic and vascular systems, which epidermal HA is not. Exogenous HA is cleared from the dermis and rapidly degraded.

The dermal fibroblast provides the synthetic machinery for dermal HA, and should be the target for pharmacological attempts to enhance skin hydration. The fibroblasts of the body, the most banal of cells from a histological perspective, is probably the most diverse or all vertebrate cells with the broadest repertoire of biochemical reactions and potential pathways for differentiation. Much of this diversity is site-specific. What makes the papillary dermal fibroblast different from other fibroblasts is not known. However, these cells have an HA synthetic capacity similar to that of the fibroblasts that line the joint synovium responsible for the HA-rich synovial fluid (Stern, unpublished experiments).

Aging Skin

Though dermal HA is responsible for most skin HA, epidermal cells are also able to synthesize HA. The most dramatic histochemical change observed in senescent skin is the marked decrease in epidermal HA (96). In senile skin, HA is still present in the dermis, while the HA of the epidermis has disappeared entirely. The proportion of total GAG synthesis devoted to HA is greater in epidermis that in dermis, and the reasons for the precipitous fall with aging is unknown. The synthesis of epidermal HA is influenced both by the underlying dermis, as well as by topical treatments, such as with retinoic acids, indicating that epidermal HA is under separate controls from dermal HA.

In contrast with previous in vitro (124,125) and in vivo (126,127) observations, recent studies document that the total level of HA remains constant in the dermis with aging. The major age-related change is the increasing avidity of HA with tissue structures with the concomitant loss of HA extractability. Such intercolated HA may have diminished ability to take on water of hydration. This decreased volume of water of hydration HA is obviously a loss in skin moisture. An important study for the future would be to define precisely the hyaladherins, the HA-binding proteins, that decorate the HA in senile skin, and to compare that profile with the hyaladherins of young skin, in both the dermal and epidermal compartments. Progressive loss in the size of the HA polymer in skin as a function of age has also been reported (128).

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The increased binding of HA with tissue as a function of age parallels the progressive cross-linking of collagen and the steady loss of collagen extractability with age. Each of these phenomena contribute to the apparent dehydration, atrophy, and loss of elasticity that characterizes aged skin.

Photoaging of Skin

Repeated exposure to UV radiation from the sun causes premature aging of skin (129,130). UV damage causes initially a mild form of wound healing, and is associated first with elevated dermal HA. As little as 5 min of UV exposure in nude mice causes enhanced deposition of HA (Thiele and Stern, unpublished experiments), indicating that UV-induced skin damage is an extremely rapid event. The initial ''glow'' after sun exposure may be a mild edematous reaction induced by the enhanced HA deposition. But the transient sense of well being in the long run extracts a high price, particularly with prolonged exposure. Repeated exposures ultimately simulate a typical wound healing response with deposition of scar-like type I collagen, rather than the usual types I and III collagen mixture that gives skin resilience and pliability. The biochemical changes that distinguish photoaging and chronological aging have not been identified.

The abnormal GAG of photoaging are those also found in scars, in association with the changes found late in the wound healing response, with diminished HA and increased levels of chondroitin sulfate proteoglycans. There is also an abnormal pattern of distribution (130). The GAG appear to be deposited on the elastotic material that comprises ''elastosis'' and diffusely associated with the actinic damaged collagen fibers. These appear as ''smudges'' on H&E sections of sun-damaged skin, rather than between the collagen and elastin fibers as would be observed in normal skin.

Acute and Chronic Inflammation

Chronic inflammation causes premature aging of the skin, as observed in patients with atopic dermatitis. The constant inflammatory process leads to decreased function of the skin barrier, accompanied by loss of skin moisture. Presumably, the skin of such patients contains decreased levels of HA. Alternatively, the HA may reflect that found in chronological aging, with a change in the ability to take on water of hydration with enhanced association with tissue structures and loss of extractability. Demonstration of such changes and the precise histolocalization of this decreased HA deposition would be of intrinsic interest, a study that has not been performed yet.

The acute inflammatory process is associated initially with increased HA

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levels, the result of the cytokines released by the polymorphonuclear leukocytes, the predominant cells of the acute inflammatory process. The erythema, swelling, and warmth of the acute process are followed later by the characteristic dry appearance and the formation of wrinkles. The precise mechanisms are unknown, but may relate to the differences between acute and chronic inflammatory cells and the attendant chemical mediators released by such cells. Alternatively, initiation of a wound healing response, with collagen deposition, may be a mechanism invoked for the premature aged appearance of the skin in chronic inflammation.

Hyaluronan in Skin Substitutes

There is a requirement for skin substitutes in a great number of clinical situations. In patients with extensive burns, insufficient skin is available for autologous split-thickness skin grafts. Resurfacing of the burned area can occur with autologous cultured epidermal cell autografts. However, this is dependent on a functioning dermal support, a problem that has given rise to a number of reasonable approaches. Cadaver skin dermis has the problem of possible contamination and potential infection. A synthetic dermis has the requirement for an HA content that will support epithelial migration, angiogenesis, and differentiation. Various methods have been examined for modifying natural HA to provide materials with properties similar to the native polymer. Many derivatives of HA have been formulated (131–133). Such materials could provide flat dressings that can be seeded with fibroblasts. These same artificial dressings could also be seeded with cultured autologous keratinocytes, and with laser-drilled microperforations, the keratinocytes can migrate through the membrane onto the wound bed. Such applications are already in use and result in complete healing with a minimum of scarring.

It is anticipated that in the coming years, a number of HA-derivatives will appear for clinical application in dermatology that contain cross-linked HA polymers as well as HA-ester derivatives obtained by the conjugation of the carboxylic acid of HA with various drugs in their alcohol forms. The HA polymer, because of its intrinsic biocompatibility, reactivity, and degradability, will have many uses in the rapidly expanding field of tissue engineering and in the tissue substitutes of the future.

HYALURONAN SYNTHASES

A single enzyme protein is now recognized as being able to synthesize HA, utilizing the two UDP-sugar substrates. In eukaryotes, the enzyme resides on the cytoplasmic surface of the plasma membrane, and the HA product is extruded by

some unknown mechanism through the plasma membrane into the extracellular space, permitting unconstrained polymer growth (43). Such growth could not occur in the Golgi nor on the endoplasmic reticulum where most sugar polymers are synthesized, without destruction of the cell. Recent work has demonstrated that the HA synthases are a multigene family with at least three members, HAS-1, -2, and -3 (135,136), which are differentially regulated.

In situ expression of the HAS-1 and -2 genes are up-regulated in skin by TGF-β, in both dermis and epidermis, but there are major differences in the kinetics of the TGF-β response between HAS1 and HAS2, and between the two compartments, suggesting that the two genes are independently regulated. This also suggests that HA has a different function in dermis and epidermis.

Stimulation of HA synthesis also occurs following PMA (phorbol ester) and PDGF treatment, though a direct effect on HAS has not been demonstrated (136). Glucocorticoids induce a nearly total inhibition of HAS mRNA in both dermal fibroblasts and osteoblasts (137). Extracts of dermal fibroblasts indicate that HAS-2 is the predominant HA synthase therein. This may be the molecular basis of the decreased HA in glucocortcoid-treated skin. However, an additional effect on rates of HA degradation has not been examined.

The parallels between chitin, cellulose and HA structures, all being β-chains of hexose polymers are reflected in the striking similarity in sequence between the HA synthases from vertebrates, cellulose synthases from plants and chitin synthases from fungi. A primordial ancestral gene must have existed from which all of these enzymes evolved that are involved in the biosynthesis of all polymers that contain β-glycoside linkages, an ancient β-polysaccharide synthase.

HYALURONAN CATABOLISM

The Hyaluronidases

Hyaluronan is very metabolically active, with a half-life of 3 to 5 min in the circulation, less than 1 day in skin, and even in an inert tissue like cartilage, the HA turns over with a half-life of 1 to 3 weeks (15,137,138). This catabolic activity is primarily the result of hyaluronidases, endoglycolytic enzymes with a specificity in most cases for the β 1–4 glycosidic bond.

The hyaluronidases family of enzymes have, until recently, been relatively neglected (138), in part because of the great difficulty in measuring their activity. They are difficult to purify and characterize, are present at exceedingly low concentrations, and have very high, and in the absence of detergents, unstable specific activities. New assay procedures have now facilitated their isolation and characterization (123,141). The human genome product has also promoted explication at the genetic level, and a virtual explosion of information has ensued.

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An entire family of hyaluronidase-like genes has been identified (141). There are seven hyaluronidases in the human genome, a cluster of three on chromosome 3p, and a similar cluster of three on chromosome 7q31. This arrangement suggests that an original ancient sequence arose, followed by two tandem gene duplication events. This was followed by a more recent *en masse* duplication and translocation. From divergence data, it can be estimated that these events occurred over 300 million years ago, before the emergence of modern mammals. A seventh and nonhomologous hyaluronidase gene occurs on chromosome 10q (142). All of the hyaluronidase-like genes have unique tissue specific tissue patterns.

The biology of hyaluronidases in skin has not been investigated, nor has it been established which of the various hyaluronidases participate in the turnover of HA in dermis and epidermis.

In vertebrate tissues, total HA degradation occurs by the concerted effort of three separate enzymatic activities, hyaluronidase, and the two exoglycosidases that remove the terminal sugars, a β-glucuronidase, and a β-N-acetyl glucosaminidase. Endolytic cleavage by the hyaluronidase generates ever increasing substrates for the exoglycosidases. Their relative contribution of each to HA turnover in either dermis or epidermis are yet to be established. But each of these classes of enzymes as well as the hyaluronidases represent important potential target for the pharmacological control of HA turnover in skin.

Nonenzymatic Degradation

The HA polymer can be degraded nonenzymatically by a free radical mechanism, particularly in the presence of reducing agents such as thiols, ascorbic acid, ferrous or cuprous ions. This mechanism of depolymerization requires the participation of molecular oxygen. The use of chelating agents in pharmaceutical preparations to retard free radical catalyzed scission of HA chains has validity. However, a carefully monitored effect of such agents on HA chain length in human epidermis has not been attempted. Whether such agents can also effect the integrity of dermal HA in protecting them from free radical damage, and whether these agens have any substantial effect on the moisturizing properties of skin HA remain important questions to be answered.

HYALURONIDASE INHIBITORS

Macromolecular Inhibitors

The extraordinarily rapid turnover of HA in tissues suggests that tightly controlled modes exist for modulating steady state levels of HA. The HA of the vertebrate body is of unique importance, and rapid increases are required in situa-

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tions of extreme stress. Rapid turnover of HA in the normal state indicates constant synthesis and degradation. Inhibition of degradation would provide a far swifter response to the sudden demand for increased HA levels, than increasing the rate of HA synthesis. The ability to provide quickly high HA levels is a survival mechanism for the organism. This might explain the apparent inefficiency for the rapid rates of HA turnover that occur in the vertebrate animal under basal conditions. It can be compared to the need to suddenly drive an automobile much faster in the case of an emergency, not by stepping on the accelerator, but by taking a foot off the break.

If inhibition of HA degradation by hyaluronidase occurs, then a class of molecules that have not been explored, the hyaluronidase inhibitors, are very important. It can be postulated that with extreme stress, hyaluronidase inhibitors would be found in the circulation as acute phase proteins, the stress response products synthesized by the liver. These would prevent the ever present rapid destruction and allow levels of HA to quickly increase.

Circulating hyaluronidase inhibitor activity has been identified in human serum over half a century ago (144,145). Modifications in levels of inhibitor activity have been observed in the serum of patients with cancer (146,147), liver disease (148), and with certain dermatological disorders (149). This area of biology is unexplored, and though some early attempts were made (150–152), and even though a review appeared (153), these hyaluronidase inhibitors have never been isolated nor characterized at the molecular level.

Inhibitors of mammalian origin, such as the serum inhibitor or heparin, are far more potent than the rather mild inhibitors of plant origin. Hyaluronidase inhibitors of animal origin would provide a means for enhancing levels of HA in skin, and represent an important research area in attempting to enhance skin moisture.

Low Molecular Weight Inhibitors

Classes of lower molecular weight inhibitors of hyaluronidase have been identified, some of which come from folk medicines, from the growing field of ethnopharmacology. Some anti-inflammatories as well as some of the ancient beauty aids and practices for freshening of the skin may have as the basis of their mechanism of action, some of these compounds.

Those that have been identified in recent times include flavonoids (154–156), aurothiomalate (157), hydrangenol (158), occurring in the leaves of Hydrangea, tannins (159), derivatives of tranilast (160), curcumin (161), an extract of the spice turmeric, glycyrrhizin (162), found in the roots and rhizomes of licorice (*Glycyrrhiza glabra* L.), used as an effective anti-inflammatory agent used in Chinese medicine.

Clinically, heparin used as an anticoagulant, has potent antihyaluronidase

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activity (163), as does indomethacin (164,165), a classic nonsteroidal anti-inflammatory agent, and salicylates (167).

OXIDATIVE STRESS AND SKIN HYALURONAN

Reactive oxygen species or free radicals are a necessary component of the oxygen combustion that drives the metabolism of living things. Although they are important for generating the life force, they are also extraordinarily harmful. Organisms thus had to evolve protective mechanisms against oxidative stress. Over the course of evolution, different enzymatic and nonenzymatic antioxidative mechanisms were developed, such as various vitamins, ubiquinone, glutathione, and circulating proteins such as hemopexin. Hyaluronan may also be one such mechanism, acting also as a free radical scavenger (168).

Sunlight (ultraviolet) is an additional generator of harmful oxygen-derived species such as hydroxyl radicals. Such radicals have the ability to oxidize and damage other molecules such as DNA causing cross-linking and chain scission. These hydroxyl radicals may also be destructive for proteins and lipid structures, as well as for ECM components such as HA. After a very few minutes of UV exposure, disturbance in HA deposition can be detected (Thiele and Stern, unpublished experiments). The anomalous situation exists, therefore, where HA can both be protective as a free radical scavenger and at the same time a target for free radical stress. This paradox may be understood by a hypothetical model in which HA protects the organism from the free radical stress generated by the oxygen-generated internal combustion, but is itself harmed by the more toxic free radicals generated in the external world by UV irradiation.

The generation of HA fragments by UV may underlie some of the irritation and inflammation that often accompanies long-term or intense sun exposure (169–172). As discussed above, HA fragments are themselves highly angiogenic and inflammatory, inducing the production of a cascade of inflammatory cytokines. Further complications have occurred in this assembly of metabolic attack and counterattack reactions that have been compiled in the selective forces of evolution. An unusually high level of antioxidants are present in skin, such as vitamins C and E, as well as ubiquinone and glutathione. However, these precious compounds are depleted by exposure to sunlight (173–175).

To prevent this sun-induced cascade of oxidative injuries, topical preparations containing antioxidants have been developed in the past several decades. Initially, such antioxidants were added as stabilizers to various dermatological and cosmetic preparations. In particular, lipophilic vitamin E has been a favorite as a stabilizing agent. However, following oxidation, vitamin E is degraded into particularly harmful pro-oxidative metabolites (176).

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In the past several years, increasing concentrations of antioxidants have been used in such skin preparations in an attempt to create complementary combinations, or to create constant recycling pairs that alternatingly oxidize and reduce each other (177). Finally, molecules such as HA should be protected by topical antioxidants to prevent degradation. Topical antioxidants, protecting against free radical damage as well as maintaining HA integrity, may have major effects against natural aging and photoaging (178,179).

ENHANCING SKIN MOISTURE BY MODULATING HYALURONAN

Alpha-Hydroxy Acids

Fruit compresses have been applied to the face as beauty aids for millennia. The alpha-hydroxy acids contained in fruit extracts, tartaric acid in grapes, citric acid in citrus fruits, malic acid in apples, mandelic acid in almond blossoms and apricots are thought to be active principles for skin rejuvenation. Such alpha-hydroxy acids do stimulate HA production in cultured dermal fibroblasts (unpublished experiments). However, the ammonium salts present in most current cosmetic preparations of alpha-hydroxy acids may prevent HA enhancement. The results of such alkaline preparations may depend more on their peeling effects rather than on the ability of alpha-hydroxy acids to stimulate HA deposition.

Lactic acid (180,181), citric acid (180,182), and glycolic acid (180,183–185), in particular, though frequent ingredient in alpha-hydroxy-containing cosmetic preparations, have widely varying HA-stimulating activity in the dermal fibroblast assay. Some of these mildly acidic (pH 3.7–4.0) preparations may owe their effectiveness to their traumatic peeling, astringent properties, with constant wounding of the skin. The cosmetic effects of these preparations of alpha-hydroxy acids, including lactic acid, involve increased skin smoothness with the disappearance of lines and fine wrinkles. Long-term use results in thickening of the skin, in both the epidermal and papillary dermal layers because of the mild fibrous reaction. These results derive from the mild fibrous reaction typical of diffuse wound healing, and may explain the increased thickness and firmness of both dermis and epidermis. The increased collagen deposition documented in skin after prolonged use is consistent with a wound-healing effect (186). Neutral or mildly acidic preparations of alpha-hydroxy acids, as would have been found in the fruit compresses of the ancients have yet to find current cosmetic equivalents, though such vehicles are actively being sought (187).

Upon examining the structure, it is obvious that ascorbic acid is also an alpha-hydroxy acid. This is generally not appreciated. However, ascorbic acid is also present in fruit, and may underlie some of the effects attributed to fruit

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extracts. It has pronounced HA-stimulating effect in the fibroblast assay. But its antioxidant activity confounds the effects it may induce.

Retinoic Acid and Its Derivatives

Topical application of retinoic acid derivatives reduce the visible signs of aging and of photodamage (188) though there is little correlation between the histological changes and the clinical appearance of the skin. Initial improvement in fine wrinkling and skin texture correlates with the deposition of HA in the epidermis.

While vitamin D is considered the ''sunshine vitamin,'' vitamin A has been accepted as an apparent antidote for the adverse effects of sun exposure, and assumed to prevent and repair cutaneous photodamage (188). Application of vitamin A derivatives do reverse some of the sun damage to skin, the roughness, wrinkling, and irregular pigmentation (189,190). For the over-40 generation, brought up in an era of ''suntan chic,'' appropriate preparations to restore or to prevent further deterioration of skin are critically important. Impairment of the retinoid signal transduction pathways occur as a result of prolonged UV exposure. Down-regulation of nuclear receptors for vitamin A occurs (191), resulting in a functional deficiency of vitamin A. Application of vitamin A derivatives would appear to be an obvious treatment modality. Topical application of vitamin A does increase the HA in the epidermal layer, increasing the thickness of the HA meshwork after prolonged treatment (192).

A number of cytokines and growth factors stimulate dermal fibroblasts to increase their production of HA. The presence of vitamin A, surprisingly, does not impede this HA-enhancing ability in dermal fibroblasts cultures (183). A combination of vitamin A and such cytokines or growth factors may provide the requisite treatment to reverse effectively the effects of long-term sun exposure.

Steroids

Topical and systemic treatment with glucocorticoids induces atrophy of skin, bone, as well as a number of other organs, with a concomitant decrease in glycosaminoglycans, in particular HA. In human skin organ cultures, hydrocortisone has a bimodal effect. At low physiological concentrations, 10^{-9} M, hydrocortisone maintains active synthesis and turnover of HA in the epidermis, while at high concentrations, 10^{-5} M, hydrocortisone reduces epidermal HA content. The effect is achieved through both decreased synthesis as well as decreased rates of degradation (193). The high concentrations of cortisone also enhance terminal differentiation of keratinocytes and reduces rates of cell proliferation.

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Hydrocortisone is also a potent inhibitor of HA synthesis in fibroblasts. HA synthase 2 is the predominant synthase of dermal fibroblasts, of the three HA synthase genes. Glucocorticoids induces a rapid and near total suppression of HA synthase 2 mRNA levels. The inhibition of HA deposition thus appears to occur at the transcriptional level. Progesterone inhibits HA synthesis in fibroblasts cultured from the human uterine cervix (194). The steroid effect on HA appears to be system-wide.

Edema is one of the four cardinal signs of acute inflammation. The ability of glucocorticoids to suppress inflammation occurs in part by their ability to suppress the deposition of HA, the primary mechanism of edematous swelling of the inflammatory response.

GENERAL COMMENTS FROM THE DERMATOLOGY AND COSMETIC PERSPECTIVES

The nature moisture of skin is attributed to its HA content. The critical property of HA is its ability to retain water, more than any known synthetic or naturally occurring compound. Even at very low concentrations, aqueous solutions of HA have very high viscosity.

The advantage of using HA in cosmetic preparations was recognized very soon after its discovery. Difficulties in preparing large enough amounts of HA free of contaminating glycoproteins, lipids, and other tissue materials prevented its convenient use in commercial preparations including its use in cosmetics. Initially, HA was isolated from rooster combs. This HA was highly purified, and used in ophthalmology as a viscoelastic to replace fluid loss following cataract surgery. The revolution in biotechnology and molecular genetics made it possible more recently to engineer bacteria with augmented HA production, by amplifying the HA synthase gene. This generates a material much lower in molecular weight that has the additional disadvantage of frequent contamination by residual bacterial pyrogens. Such HA, processed from vast fermentation of engineered bacteria has reduced the price of HA drastically, bringing the price into a range that is reasonable for its use in cosmetics. However, this genetically engineered HA of bacterial origin is not of sufficient purity for injectional use.

Many of the cosmetic preparations than contain HA have a concentration of 0.025 to 0.050%, sufficient to give the preparations a very smooth and viscous feel. Such solutions, applied to the skin form hydrated films that hold water for considerable periods, and confer the properties of a moisturizer.

Currently, research is underway to modify HA in such a way as to make it more stable and to confer very specific properties. Another direction in such research is to combine it with other materials, such as chondroitin sulfate and

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modified sugar polymers, to simulate more closely the associations that HA has in its natural state in vertebrate tissues. Since the low molecular size HA fragments are highly angiogenic, defining the optimal size of the HA polymer for cosmetic purposes is also a major goal of such research.

FUTURE DEVELOPMENTS

Currently, the biology of HA and its metabolic cycle is in its infancy. The enzymatic steps that constitute the extracellular and intracellular HA cycles are beginning to be sorted out. The goals that lie before us are the identification of such reactions, and new modes of modulating these reactions, in order to enhance skin appearance and to increase the moisture content of photodamaged and aging skin.

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