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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.

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.

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		

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

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;

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-

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

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.

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.

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

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.

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.

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

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.

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

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.

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.

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.

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 lm 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).

Colorants—most often utilize a classic iron oxide without any surface treatment.

Functional llers —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.

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 llers** : 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:

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

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 modi ers (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),

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).

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

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

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.

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

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.

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.

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

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.

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 I	
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

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

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

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

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

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).

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-

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

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

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.

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

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.

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-

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,

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

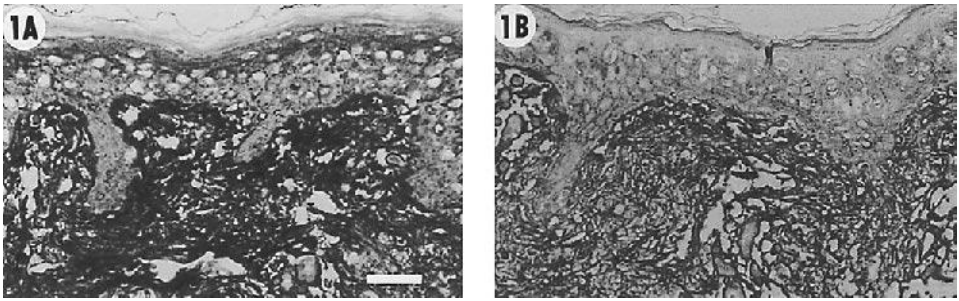

Figure 1 Sections of human skin were stained for HA using a biotinylated HA-binding peptide, derived from bovine cartilage aggrecan. Histolocalization of HA is indicated by the blue color, developed using an avidin-conjugated alkaline phosphatase. Slides were counterstained with Nuclear Fast Red to visualize 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

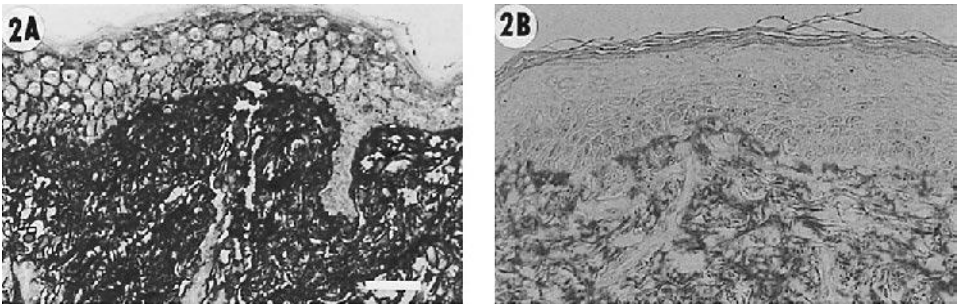

Figure 2 Sections of human skin were incubated in PBS for 24 h at 37°C prior to staining precisely as described in Figure 1. (A) The skin section was fixed in acid-formalin/ethanol, and in (B) formalin/PBS. The bar = 50 μm. To confirm further that the HA in skin fixed in acid-formulin/alcohol is better 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.

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).

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

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.

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-

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

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).

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

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.

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

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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