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(54) Title: COMPOSITIONS AND METHODS FOR ENHANCING THE GROWTH OF HAIR AND RESTORING HAIR COLOR

(57) Abstract

The present invention relates to a method for stimulating dormant hair follicles in intact skin, especially intact skin of the scalp of humans and enhancing the growth and restoring the natural hair color of hair which has been diminished in its natural color. The formulations according to the present invention are useful for increasing hair growth by stimulating dormant, weak of dying hair follicles to produce hair. Auxiliary to such hair growth is the unexpected result that the hair's natural color is restored to hair which is diminished in its natural color, for example in graying and/or gray hair, as the hair grows. In addition, the formulations act to produce angiogenesis in epidermal tissue surrounding hair follicles as well as enhance the growth of ungual tissue or revitalize skin in animals. These formulations generally comprise an effective amount of a mixture of non-steroidal anabolic hormones selected from insulin, growth hormone, triiodothyronine or thyroxine, in combination with a minimum essential medium, preferably, an enriched minimum essential medium such as MCDB 153.

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COMPOSITIONS AND METHODS FOR ENHANCING THE GROWTH OF HAIR AND RESTORING HAIR COLOR

FIELD OF THE INVENTION

The present invention relates to methods for stimulating hair follicles and promoting the growth of hair in intact skin, especially the scalp. More particularly, the invention relates to a method for inducing angiogenesis and causing increased vascularization and increased circulation (vasodilation) of intact skin tissue and stimulating dormant, dying or weak hair follicles to produce hair or promote the growth of hair in such skin and reduce hair loss. The present invention also relates to a method for stimulating nail growth and producing stronger nail (ungual) tissue. In addition, the present invention also relates to a method for promoting melanogenesis and restoring the natural color to hair in which the color is diminished or diminishing, such as in gray or graying hair. The compositions of the present invention which are used to enhance hair and nail growth and promote melanogenesis and restore natural hair color are compositions which are based on a minimum essential medium in combination with preferably at least two non-steroidal anabolic hormones one of which hormones is insulin and the other which is selected from triiodothyronine, thyroxine and growth hormone. Three anabolic hormones, including insulin, triiodothyronine or thyroxine and growth hormone in combination with minimum essential media may be preferably used. Preferably, an enriched growth media such as MCDB 153 or related enriched growth media is used in combination with insulin, triiodothyronine or thyroxine and growth hormone. Compositions for promoting hair and nail growth may also contain an effective amount of a penetration enhancement agent for promoting the penetration of the active constituents of the compositions through the surface of the skin where angiogenesis, melanogenesis and vascularization may be promoted.

BACKGROUND OF THE INVENTION

Hair follicles produce different types of hair at different stages in an animal's life. The hairs formed may change quite dramatically in thickness, color, and length. Thus, hair follicles have a transforming ability which allows animals to adjust to seasonal changes and to life cycle changes (i.e., hormonal or metabolic changes) over the course of an animal's life. This allows the rapid distinction between young animals and adults and between the adult sexes. Thus, hair growth plays a major role in social and sexual communication in mammals and explains why hair disorders such as hirsutism, androgenetic alopecia and alopecia areata often create psychological problems.

To enable hair follicle life cycle changes, hair follicles pass through regular cycles of hair development and growth (anagen) followed by periods of resting (telegen). The hair produced after a resting period may be very similar to the previous one produced, as seen in many hair follicle cycles on the eyelids and the young human scalp; or, it may be slightly or even markedly different. In certain instances, for example during aging, stress conditions and the like in a cycle known as catagen, the hair may actually regress in quality and cease growing. The precise mechanism of how alterations occur in the type of hair produced by a hair follicle are unknown. Methods which are shown to stimulate hair follicles and enhance the growth of hair may be used to regulate hair follicle disorders and increase hair production by domesticated animals such as sheep.

The hair follicle is composed of epithelial components (the matrix and outer root sheath) and dermal components (the dermal papilla and connective tissue sheath). Hair growth is effected by the division of the hair follicle matrix cells under the control of the dermal papilla. Three distinct stages of hair growth can be identified, an active phase (anagen) during which hair growth occurs, an intermediate regressive phase (catagen) during which hair actually ceases growing or even regresses and a resting phase (telogen) during

which no cell proliferation occurs. The factors that regulate cell division are poorly understood, although growth factors, steroid hormones, dermoepithelial interactions and the immune system have been implicated. Philpott, et al., J. Cell Sci., 97(Pt3), 463 (1990), recently demonstrated that growth factors such as epidermal growth factor (EGF) mimic the in vivo depilatory action of EGF resulting in the formation of a club hair-like structure; and that transforming growth factor (TGF)-β_1 may serve as a negative growth regulatory factor for the hair follicle.

One area of investigation into hair growth involves the use of androgens (steroidal hormones) where it has been shown that androgens modulate the type of hair produced by hair follicles in humans.

A number of recent publications evidence that human scalp cells and hair may be grown in vitro. Several publications report the use of hormonally supplemented serum-free cell culture medium of the MCDB series as an optimal medium for growing human hair cells in culture. Kitano, et al., J. Dermatol., 19(11), 793, (1992), used MCDB 153 supplemented with amino acids, hydrocortisone, insulin, EGF and bovine pituitary extract to grow isolated human scalp hair follicles in vitro. Tanigaki, et al., Arch. Dermatol. Res., 282(6), 402, (1990), used MCDB supplemented with 7 growth factors to induce hair cell differentiation of C3H mice hair cells in culture. M.P. Philpott, et al., Ann.NY Acad. Sci., 642, 148 (1991), reported that serum inhibited hair follicle cell growth in vitro while serum-free medium supported its growth. Tobin, et al., Arch. Dermatol. Res., 285(3), 158 (1993), maintained human hair follicles in culture in serum-free medium.

The main source of energy for hair follicle cells in the culture medium was reported by Williams, et al., J. Invest. Dermatol., 100(6), 834 (1993). That group observed that the glucose-glutamin cycle supports human hair follicle growth rate. Philpott, et al., J. Cell Sci., 97(Pt3), 463 (1990), demonstrated that growth factors such as TGF-beta in vitro and TGF-alpha and EGF in vivo had negative effects on

hair follicle growth. Thyroxine participation in in vivo scalp hair growth in hyper- and hypothyroidism was reported by Kiesewetter, et al., Z. Hautkr., 65(12), 1120 (1990). Li, et al., Proc. Natl. Acad. Sci. USA, 89(18), 8764 (1991), reported success in maintaining intact human scalp skin in culture. The organ culture technique reported in this reference evidenced hair elongation, thymidine incorporation and a continued hair growth cycle. The presence of mast cells was associated with the anagen phase while macrophages were seen during the catagen phase of the cycle, suggesting the role of the circulatory cells in the process of the hair growth cycle.

In vivo experiments investigating hair growth recently have been reported by a number of investigators. Skoutelis, et al., J. Invest. Dermatol., 95(2), 139 (1990) indicated that neovascularization is a necessary component in the anagen phase of hair growth, suggesting that adequate blood supply to the skin is vital for hair growth. Randall, et al., J. Invest. Dermatol., 101(1), 114s-120s (1993), described the ebb and wax of the human hair growth annual cycle. These cyclical changes must be taken into account when conducting in vivo experiments in order to ensure accuracy.

Studies on the in vitro culturing of follicular cells invariably refer to the usage of serum-free medium. In some cases, MCDB 153 and Williams media are specifically mentioned. Where serum supplement was added to the media, the result was an inhibition of hair growth. In the in vitro studies in the art, only intact hair follicles were capable of growing hair and then only for a limited period of time whereupon senescence of the culture cells ensued. In studies where the cells were dispersed, either by trypsinization or other mechanical methods, hair growth did not occur. This may suggest that intact hair follicles may be capable of being revitalized.

In other studies, the effect of proteoglycans in the hair growth cycle was determined. Chondroitan 6 sulphate, unsulphated condroitin, dermatan sulphate and heparin sulphate were studied for their effects of hair growth. A direct correlation between the presence of chondroitan proteoglycans and

hair growth was established. Still other studies have shown that keratin growth factor may stimulate hair follicles as well as sebaceous glands.

The importance of the vascularization of the dermis to the process of hair growth is well known. Thus, any factor which negatively affects the skin microvasculature will most likely also undermine hair growth.

The presently available product Minoxidil^{tm} has been shown to be efficacious in enhancing hair growth. Its mechanism of action is based upon its vasodilatory effect which causes shifting of the fluid volume from the vascular compartment into the extracellular compartment, especially in the dermal area. This, in turn, may cause an increase in blood circulation in the dermis and increased supply of nutrients to the dermis affecting the hair follicles.

In experiments on wound healing, MCDB 153 supplemented with non-steroidal anabolic hormones was used as treatment on surgical wounds. The skin on the dorsum of all the animals was depilated prior to the extirpation of the skin patches. During the change of bandages it was noted that the depilated hair on the skin adjacent to the wound edges was growing at a faster rate than the hair on areas further away from the treated wound. The application of MCDB 153 including anabolic hormones induced vascularized granulation tissue formation as well as epithelialization. The barrier for penetration through the skin, the keratinized layer, was overcome by the opening in the wound area. This condition allows for unhindered contact between the medium and the deeper layers of the skin including the hair follicle cells and the capillary endothelial cells.

Unpublished data by the present inventor on angiogenic activity of supplemented MCDB 153 (with insulin, growth hormone and triiodothyronine or thyroxine) performed on the CAM (chorioallantoic membrane) indicated that the application of the gelled medium did not elicit vascular response. The gel did not appear to penetrate the external epithelial layers

of the CAM. However, clinical experience has repeatedly shown that in healing wounds, either burn wounds or surgical wounds, where angiogenic activity is evident, faster rate of hair growth was found adjacent to the wound area.

Additional unpublished data by the present inventor using supplemented MCDB 153 gel (insulin, growth hormone and triiodothyronine/thyroxine) on surgically induced corneal wounds yielded pronounced and unexpected angiogenic response in all experimental test animals. The angiogenic response was expressed by coiled corneal neovascularization of branches of the superficial and deep anterior ciliary arterial plexus and by engorgement of all the surrounding vascular bed. When the gel was applied to the intact eye, no irritation or angiogenic activity was found.

BRIEF DESCRIPTION OF THE INVENTION

The present invention relates to formulations and methods for promoting hair or nail (ungual tissue) growth in intact skin of animals, especially including the scalp of humans. The formulations according to the present invention are also useful for promoting angiogenesis in the dermis and for stimulating dormant, dying or weak hair follicles to produce hair or promote the growth of hair in such skin. In concert with such enhanced growth, the natural hair color of the growing hair is restored in hair diminished in its natural color. This is an unexpected result. Compositions according to the present invention are also useful for enhancing the growth and increasing the strength of ungual tissue as well as correcting irregularities in such tissue. Methods for enhancing angiogenesis and increasing vascularization and blood flow in intact skin, restoring the natural hair color to growing hair, as well as methods for revitalizing intact skin are also embraced by the present invention. The formulations according to the present invention are useful for enhancing hair growth by causing increased vascularization of intact skin tissue and stimulating dormant, dying or weak hair follicles to produce hair or promote the growth of hair in such skin. The formulations according to the present invention are also useful for

promoting melanogenesis in hair follicles and for restoring the hair color in hair which exhibits diminished hair color such as in gray, graying and damaged hair.

The present formulations comprise a hair or nail growth enhancing effective amount of a non-steroidal anabolic hormone selected from insulin, growth hormone, triiodothyronine and thyroxine (T_3 or T_4), and mixtures thereof, most preferably a mixture of all three hormones, in combination with a hair or nail growth enhancing effective amount of minimum essential medium, preferably a supplemented medium such as MCDB 153. The formulations may be adjusted to enhance penetration of the individual components through intact skin in order to promote angiogenesis of the underlying dermal layers and consequently, to stimulate hair follicles and ungual tissue and promote hair or ungual tissue growth. All of the components used in the compositions according to the present invention are included in amounts effective for an intended use generally- enhancing or stimulating hair and/or nail growth or restoring hair color in hair exhibiting diminished color.

In preferred embodiments according to the present invention, the non-steroidal anabolic hormone is a mixture of insulin and at least one or more anabolic hormones such as triiodothyronine, thyroxine and growth hormone. It has been unexpectedly discovered that a mixture of the anabolic hormones insulin and triiodothyronine, thyroxine or growth hormone produces a synergistic enhancement in hair growth when combined with a minimum essential medium. In more preferred embodiments according to the present invention, the anabolic hormone comprises a mixture of an amount of insulin, growth hormone and triiodothyronine or thyroxine in amounts effective to substantially enhance the growth of hair or ungual tissue, also synergistically. Embodiments in which the anabolic hormone is a mixture of effective amounts of triiodothyronine or thyroxine and growth hormone or insulin and growth hormone are also contemplated by the present invention. Generally in concert with enhanced hair growth, hair color restoration occurs in hair which is diminished in hair color. Composi-

tions which restore hair color are generally made of the same componentry and such components are included in the same general amounts which are useful for promoting hair growth.

In general, insulin is included in compositions according to the present invention at concentrations ranging from about 5 ng/ml (nanograms/ml) to about 100 ug/ml (micrograms/ml) (preferably, at least about 50 ng/ml within this range), more preferably about 500 ng/ml to about 20 ug/ml. When a non-steroidal anabolic hormone other than insulin is included in compositions according to the present invention, for example, triiodothyronine, thyroxine or human growth hormone, among others, each of these other hormones is included in an amount effective to enhance the growth of hair or ungual tissue, i.e., in an amount of at least about 0.05 ng/ml of the formulation, with a preferred range of about 0.5 ng/ml to about 100 ng/ml. In compositions which are delivered in solid or concentrated form, i.e. as a gel, creme or the like, the anabolic hormone is included in concentrations similar to those contained in the solutions (based upon the general assumption that 1 ml of solution is approximately equal to about 1 gram in weight of the final composition). Percent weights may fall outside of these ranges, depending upon the ability to deliver the individual components of the formulations through the skin, the level of stability of the hormone and other factors, as well recognized by one of ordinary skill in the art.

In addition to insulin, the compositions according to the present invention preferably include at least one anabolic hormone selected from triiodothyronine, thyroxine, and growth hormone and most preferably both growth hormone and triidothyronine or thyroxine, in combination with at least a minimum essential medium, preferably a supplemented minimum essential medium such as MCDB 153. When growth hormone is used, the preferred growth hormone is human growth hormone, preferably in combination with triiodothyronine (T_3), thyroxine (T_4) or insulin and more preferably in combination with both insulin and triiodothyronine (T_3) or thyroxine (T_4).

The preferred amount of anabolic hormone other than insulin used will generally depend on the extent and rate of hair or nail growth desired or damage to hair, nail or skin tissue which is to be corrected, but in most of the cases the amount of hormone will fall within a preferred range of about 0.5 ng/ml and about 100 ng/ml by weight or more of the composition. In the case of compositions which are delivered as a gel, growth hormone, preferably human growth hormone, is included in an amount ranging from about 0.5 ng/ml to about 50 ng/ml by weight or more, more preferably about 0.5 ng/ml to about 10 ng/ml. Triiodothyronine (T_3) or thyroxine (T_4) is preferably included in amounts ranging from about 0.5 ng/ml to about 100 ng/ml or more. Triiodothyronine (T_3) may be preferred over thyroxine (T_4) because it has greater potency and the same general activity as thyroxine. Thyroxine (T_4), however, is more storage stable than is triiodothyronine (T_3) and thyroxine's stability should be taken into account and is preferred when formulating compositions which are to be stored for at least several weeks or more. Triiodothyronine (T_3) and thyroxine (T_4) may be readily substituted for each other, however, with the general rule that at least about three to five times the amount of thyroxine (T_4) is substituted for triiodothyronine (T_3).

BRIEF DESCRIPTION OF THE FIGURES

Figures 1-2 represent the results of the experiments performed and described in Example 3. These graphs show the relative degree of increase in blood flow measured by Laser-Doppler Flow changes on burn wounds treated with a composition of the present invention and conventional therapy (Silverol).

DETAILED DESCRIPTION OF THE INVENTION

In describing the present invention in the specification, a number of terms will be used.

The term "angiogenesis" is used throughout the specification to describe processes which result in the development of new blood vessels and the vasculature (neovasculature).

The term "melanogenesis" is used throughout the specification to describe processes which result in the development or restoration of pigment or color in the hair as it grows.

The term "revitalizing skin" is used throughout the specification to describe a process in which skin becomes rejuvenated, i.e., smoother, more moist, softer, oily/waxy, and more cosmetically pleasing to the vision and touch.

The term "delivery polymer" or "gelling agent" is used throughout the specification to describe a polymer or other gelling agent which can be used in combination with minimum essential medium and non-steroidal anabolic hormones selected from insulin, triiodothyronine or thyroxine and growth hormone and mixtures thereof, and optionally, a penetration enhancement agent, the amount of delivery polymer included in an amount effective to gel the composition and hold it in place on the intact skin to be treated without running off. As used herein, the term delivery polymer and gelling agent are synonymous where the amount of delivery polymer or gelling agent included is effective to gel the composition. These delivery polymers include, for example, numerous hydrogels in hydrated or unhydrated form, such as those derived from hydroxyethylmethacrylate (HEMA), glycerolmethacrylate (GMA) and polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), various carbohydrates, cellulose, cellulose ethers, including methyl cellulose, hydroxyethyl cellulose and hydroxypropyl cellulose, dextran, polythyleneoxide, dextran-polyethylene, acrylamide, polyacrylamide, amylose, collagen, gelatin, sepharose, agarose (for example, as an agarose saturated gel), related polymers and mixtures thereof, among numerous others.

The cellulose ethers, especially including methyl cellulose, hydroxymethyl cellulose, hydroxyethylcellulose and hydroxypropylcellulose are preferred and are preferably included at a weight ratio of about 0.1% to about 20% by weight, more preferably about 0.5% to about 10% by weight of the hair growth compositions. Methylcellulose and hydroxyethyl cellulose are preferred cellulose ethers for use

in the hair gel compositions according to the present invention, with hydroxyethylcelllulose being more preferred. One of ordinary skill in the art will recognize to vary the type and amount of delivery polymer in compositions according to the present invention to provide enhanced delivery of the hair growth composition appropriate for topical delivery and penetration of the individual components through intact skin. The term delivery polymer is also used to describe polymers which instill slow-release or sustained release characteristics to the hair and nail growth formulations of the invention.

The term "minimum essential medium" is used throughout the specification to describe a medium or mixture which contains no serum, and in combination with anabolic hormone and optionally, a penetration enhancement agent, comprises the compositions according to the present invention. The term minimum essential medium is readily understood by those in the art to comprise a nutrient media which supports cellular growth. The minimum essential medium according to the present invention preferably comprises the following elements: (a) essential amino acids; (b) non-essential amino acids; (c) vitamins selected from the group consisting of biotin, folate, lipoate, niacinamide, pantothenate, pyridoxine, riboflavin, thiamin and vitamin B_{12} and mixtures thereof, preferably a vitamin mixture comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin; (d) glucose; and (e) a mixture of inorganic ions selected from the group consisting of calcium, sodium, potassium, magnesium, chloride and mixtures thereof, preferably a mixture comprising calcium, sodium, potassium, magnesium and chloride. Optionally, vitamin C may be included as one of the preferred vitamins in the present formulations. It is noted that a minimum essential medium for use in the present invention may exclude non-essential amino acids (b), but preferably, non-essential amino acids are included in combination with essential amino acids. Especially preferred amino acids include glutamine, serine and cysteine.

All of the above-described elements (a), (b), (c), (d) and (e) are included with the anabolic hormone mixture in con-

centrations and/or amounts effective for enhancing or stimulating the growth of hair and/or nails or revitalizing skin tissue. It is noted that amounts of these components which stimulate or enhance the growth of hair also quite unexpectedly restore the natural hair color to the growing hair in many instances where the hair color is diminished and is characterized as being gray or graying. The preferred concentration of essential and optional non-essential amino acids used in the present invention ranges from about 5.0 um (10^{-6} mole) to about 50 mmol. (10^{-3} mole). The preferred concentrations of vitamins used in the present invention ranges from about 1 nanomole (10^{-9} mol.) to about 10 um. The preferred concentrations of glucose used in the invention ranges from about 1 umol. to about 10 or more mmol. In the case of element (e), these inorganic ions are preferably included in the present compositions at a concentration range of about 1 umol to about 50 mmol. Optionally, a penetration enhancing agent is also included in the formulations.

In addition to the elements (a), (c), (d) (e) and optionally (b), the nutrient medium according to the present invention may also contain any one or more of the following elements: (f) purines and pyrimidines; (g) other organic compounds; (h) other inorganic ions; (i) trace elements; and (j) buffers and indicators. All of these optional elements (f), (g), (h), (i) and (j), when they are included in the nutrient medium according to the present invention, are included in amounts effective, in combination with the anabolic hormone(s), for promoting angiogenesis, enhancing vascularization and/or stimulating dormant, dying or weak hair follicles to promote hair growth and, in certain instances, to restore the growing hair to its natural pigment or color. In the case of nail growth compositions, these components are added to stimulate or enhance the growth of ungual tissue, including nail tissue of humans. In the case of skin revitalizing compositions, these components are added in amounts effective to stimulate, enhance and revitalize the growth and appearance of intact skin tissue of animals, including humans. Preferably, components (f), (g), (j) and (k) range in concentration from about 1 nmol. to about 10 mmol. In the case of components (h)

and (j), the concentration preferably ranges from about 1 umol. to about 50 mmol. One of ordinary skill in the art will be able to readily modify the type and amount of the components of the minimum essential medium consistent with the teachings of the present invention.

In addition to serum free minimum essential medium, the present invention may also make use of medium containing serum, although the use of a serum containing cellular nutrient medium is generally less preferred than is serum free medium. Examples of such nutrient medium include, among numerous others, DMEM, HAM F12 and HAM F10, all containing serum. The term "minimum essential medium" is used throughout the specification to describe all types of nutrient medium contemplated for use in the present invention which contain at least the basic elements as described hereinabove, as well as supplemental components.

MCDB 153 is a preferred supplemented minimum essential medium for use in the present invention. MCDB 153 is believed to contain the components which are associated with an enhanced rate of hair growth as well as other components which enhance the rate of angiogenesis and vascularization of tissue surrounding the hair follicle and consequently, the growth of hair from the follicle and, in many instances, the restoration of natural hair color in growing hair.

The minimum essential medium according to the present invention may include one or more commercially available media in solution or lyophilate (solid) form. The cellular nutrient medium used may be in the form of a lyophilate which may be reconstituted with water, preferably sterilized, distilled water and then supplemented with an anabolic hormone such as insulin, triiodothyronine, thyroxine, growth hormone or mixtures thereof, and optionally, certain penetration enhancing agents or other additives. Lyophilized forms of insulin, growth hormone and triiodothyronine or thyroxine may also be used in the present compositions. Alternatively, the medium may be used directly in formulations according to the present invention in the form of a lyophilate, or related solid-type

material, rather than a solution, especially when a gel is to be used for delivery. It is clearly preferred when utilizing solid-type materials for delivering the wound healing compositions according to the present invention that the delivery system in the form of a gel or other form contain moistening quantities of water.

Many of the commercially available media (preferably, serum free) which may be used in the instant invention are available from suppliers such as Collaborative Research Incorporated, Bedford Massachusetts, USA, GIBCO (Grand Island Biological Company), USA or Biological Industries, Beth HaEmek, Israel. These media may be used as purchased or modified within the scope and practice of the present invention.

The term "non-steroidal anabolic hormone" is used throughout the specification to describe the primary hormones which are included in the instant invention in combination with minimum essential media to enhance or stimulate hair or nail growth. These primary hormones include insulin, triiodothyronine, thyroxine, and growth hormone, among others. When growth hormone is used, it is preferred to use it in combination with triiodothyronine or thyroxine or insulin or most preferably with a mixture of triiodothyronine or thyroxine and insulin. As used herein, the term non-steroidal anabolic hormone includes naturally isolated (preferably, human) or synthetically produced versions of these hormones which are known to function substantially the same as the naturally occuring hormones and includes, where relevant, compounds produced by genetic engineering processes and recombinant techniques. While not being limited by way of theory, it is believed that the inclusion of at least one non-steroidal anabolic steroid selected from insulin and triiodothyronine or thyroxine (preferably, insulin) serves to enhance the effect of the minimum essential media in unexpectedly increasing the rate and quality of hair (particularly, with respect to restoration of hair color) and nail growth. A combination of at least two anabolic hormones, e.g., insulin and growth hormone, insulin and triiodothyronine or thyroxine, or growth

hormone and triiodothyronine or thyroxine is preferably used because a combination of non-steroidal anabolic hormones and minimum essential medium creates an enhancement of tissue growth (hair, ungual tissue, skin revitalization) which is greater than the sum of the individual parts. Three anabolic hormones (insulin, triiodothyronine or thyroxine and growth hormone) in effective amounts in combination with minimum essential medium acts synergistically to promote angiogenesis, vascularization and consequently, hair growth and in many instances, melanogenesis and hair color restoration. Thus, it is believed that the non-steroidal anabolic hormone actually enables the cells to utilize or process the nutrients in the media, which action results in angiogenesis, and an enhancement of vascularization and rate of hair and nail growth and skin revitalization. Restoration of hair color is an auxiliary beneficial result which occurs along with enhanced hair growth in many instances.

The term "penetration enhancement agent" is used throughout the specification to describe compounds which are used to treat the skin before or during treatment with the hair or nail growth promoting compositions according to the present invention or which are added to the other components in the present formulations in amounts effective to enhance the penetration through the skin of the non-steroidal anabolic hormones and the individual components of the minimum essential medium. It is noted that compositions according to the present invention may be used with or without the inclusion of a penetration enhancement agent. It is an unexpected result that compositions which exclude a penetration enhancement agent will be absorbed into intact skin and evidence a surprising degree of activity (i.e., hair or nail growth enhancement or skin revitalization). While not being limited by way of theory, it is believed that the components used in the present composition are absorbed through the skin by way of hair roots and pores in the skin in a manner sufficient to evidence significant activity.

Exemplary penetration enhancement agents which may be used to treat the skin before applying the present formula-

tions include, for example, soaps and detergents (solvents for removing natural skin oils), depilatories, epilatories such as trichloroacetic acid and phenol, enzymes which detach an epithelial layer from the dermal substratum including trypsin, collagenase, hyaluronidase, elastase and dispase and keratin solvents such as urea and DMSO. In certain preferred instances, water may be used as a penetration enhancement agent, as hydration of the skin is associated with better penetration. All of these agents are used in concentrations and amounts which are effective for treating the skin in order to enhance the penetration of the individual components in the hair and nail growth compositions according to the present invention through intact skin.

In the case of the enzymes trypsin, collagenase, dispase, hyaluronidase and elastase, these enzymes are used to treat intact skin in an amount ranging from about 0.1 to about 10 mg/ml., more preferably about 1 mg/ml. In the case of trichloroacetic acid, this epilatory should be used in dilute concentrations in water- generally about 1% by weight or less. In the case of the use of urea, this is generally used as a mixture of 20% by weight urea in lanolin (water may be substituted for the lanolin). DMSO may also be used, generally as a dilute solution in water (0.25% to about 5% by weight, preferably about 1% to about 2% by weight of the solution). Water may also be used preferably as a penetration enhancer in certain instances. Where water is used, the amount used is that which will wet or hydrate the surface of the skin or scalp to which the compositions of the present invention are applied.

Other penetration enhancement agents which may be used in the present compositions include, for example, ionophoresis agents such as the mucopolysaccharides, for example chondroitin, chondroitin-6-sulphate and dermatan sulphate, among others. These compounds are generally included in compositions according to the present invention in effective amounts to enhance penetration of the individual components through intact skin.

In addition to the above agents, a negatively charged synthetic membrane (which generates a static charge) may also be used for enhancing the individual components in compositions according to the present invention.

The amount of each penetration enhancement agent which is used in the formulations according to the present invention will depend upon the requirement for hair or nail growth or skin revitalization, but each component is included in an amount effective for producing the intended results, for example, substantially enhancing the rate of penetration of the formulations through the skin. This amount will vary according to the type of agent used. One of ordinary skill in the art will know to vary the amount and type of agent within the weight ranges defined above to enhance penetration through intact skin and promote the efficacy of compositions according to the present invention.

In general, in embodiments according to the present invention, the formulations include an anabolic hormone other than insulin at a concentration of at least about 0.05 ng/ml, preferably about 0.5 ng/ml to about 100 ng/ml or more. In the case of formulations containing insulin, the amount of insulin generally falls outside of this range. Preferably, the anabolic hormone comprises a mixture of insulin, triiodothyronine or thyroxine and growth hormone because of the known benefits these hormones have in promoting the growth and elaboration of cells and their general absence of toxicity. In addition, it is this combination of anabolic hormones which evidences unexpected synergistic activity in promoting angiogenesis, vascularization and hair or nail growth in the instant invention.

The preferred insulin is human insulin (more preferably human recombinant or genetically engineered insulin), which is a well-known protein which is readily available commercially from a number of sources (for example, Novo Nordisk, Copenhagen, Denmark, among others). It is constituted from a number of amino acids (approximately 51) with a total molecular weight of about 5,500. Human insulin for

use in the present invention is generally prepared using genetic engineering techniques. Depending upon the manufacturer, the insulin may have slightly different activity based upon weight, however the activity of insulin defined in units is, of course, standard. While not being limited by way of theory, in the present invention, it is believed that the insulin promotes hair or nail growth at least in part by enhancing and stimulating the transport and utilization of glucose as an energy source by the hair or nail growing cells.

Growth hormone may also be used in the present invention, preferably in combination with insulin or triiodothyronine or thyroxine and most preferably in combination with both triiodothyronine or thyroxine and insulin. The preferred human growth hormone is a well-known defined protein which is readily available and results from a pituitary secretion into the blood system. It is constituted from a number of amino acids with a total molecular weight of about 193,000. The human growth hormone which may be used in the present invention can be obtained from a variety of sources, including genetic engineering processes and techniques. While not being limited by way of theory, it is believed that the growth hormone serves to stimulate and enhance angiogenesis, i.e., the elaboration and development of the vascular system which is believed to also enhance the blood supply and the delivery of nutrients to hair follices and nail tissue so as to produce an enhancement or stimulation in hair or nail growth and, in certain instances, restoration of natural color. The action of growth hormone is believed to be synergistic with insulin and/or triiodothyronine or thyroxine.

The present invention also contemplates the inclusion of effective amounts of triiodothyronine or thyroxine either alone, but preferably in combination with other non-steroidal anabolic hormones. The preferred triiodothyronine is human triiodothyronine, which is a well-known defined hormone and readily available commercially. Triiodothyronine and thyroxine are naturally occurring amino acids of the thyroid gland which exert a stimulating effect on metabolism. Although virtually identical in metabolic effects,

triiodothyronine is more potent than is thyroxine but is less stable. Where thyroxine is substituted for triiodothyronine, to obtain the same effect as triiodothyronine, thyroxine is added in an amount between about three and five times that of triiodothyronine. Thyroxine, being more stable, is preferred for use in the present invention in those compositions which advantageously have storage stable characteristics. While not being limited by way of theory, it is believed that the triiodothyronine or thyroxine utilized in the present invention stimulates vascularization and facilitates the resupply of blood borne components in cells responsible for the growth of hair.

A particularly preferred composition according to the present invention comprises a mixture of an effective amount of human growth hormone in the presence of an effective amount of insulin and triiodothyronine (T_3) or thyroxine (T_4), preferably in a serum free cellular nutrient medium, most preferably MCDB 153. In this preferred embodiment of the instant invention, the anabolic hormones other than insulin, i.e., growth hormone or triiodothyronine, are generally included in the final composition in a concentration range of about 0.05 ng/ml to about 100 ng/ml, preferably about 0.5 ng/ml to about 20 ng/ml or more and most preferably about 1 ng/ml to about 20 ng/ml. In the case of the inclusion of thyroxine as a substitute for triiodothyronine, thyroxine is generally included in an amount ranging from about 0.5 ng/ml to about 100 ng/ml or more, generally at a concentration of at least about three-five times that of triiodothyronine.

In the most preferred embodiment which includes three anabolic hormones, insulin is also included in an effective amount, generally an amount which is substantially greater than the other anabolic hormones. The amount of insulin is preferably included in amounts ranging from about 5ng/ml to about 100 ug/ml more preferably about 50 ng/ml to about 20 ug/ml and even more preferably about 500 ng/ml to about 20 ug/ml. One of ordinary skill in the art will know to vary the amount of anabolic hormones within effective ranges based upon the type and potency of the preparation of the compound in

order to enhance or stimulate the growth of hair or nail tissue according to the present invention.

Compositions according to the present invention preferably comprise effective amounts of minimum essential medium in combination with effective amounts of insulin, triiodothyronine or thyroxine and growth hormone. It is a combination of these three anabolic hormones in combination with minimum essential medium which has exhibited the greatest synergistic activity in promoting angiogenesis, vascularization, increased blood flow and hair or nail growth. Supplmented minimum essential medium is preferably used and MCDB 153 is the most preferred media to be used in the instant invention. In addition to the other components, the inclusion of effective concentrations of selenide are optional. In addition, the inclusion of effective amounts of vitamin C (ascorbic acid) is preferred. The compositions are preferably transferrin-free, although transferrin may be added.

The minimum essential medium which is used in the present invention is any medium having the effect of promoting hair growth when used in effective amounts in combination with the non-steroidal anabolic hormones. In preferred embodiments according to the present invention, the nutrient media, comprised of the componentry set forth hereinabove, is mixed with an effective amount of the non-steroidal anabolic hormone(s) to form the compositions according to the present invention.

The term "minimum essential medium" is readily recognized by those of ordinary skill in the art. Minimum essential medium is known to preferably comprise effective amounts of the following constituents: (a) essential amino acids; (b) non-essential amino acids; (c) vitamins as previously described; (d) inorganic ions as previously described and (e) glucose; and optionally, (f) purines and pyrimidines; (g) other organic compounds; (h) other inorganic ions; (i) trace elements; (j) buffers and indicators and (k) other supplements. Preferably, the medium used herein also contains effective amounts of elements (f) through (k). Serum free nutrient medium is preferred. It is noted that non-essential

amino acids (b) are preferably added, but are not required. The preferred serum free nutrient medium is modified MCDB 153, a well-known medium. Mixtures of standard commercial nutrient media may also be used with favorable results in the instant invention.

While not being limited by way of theory, it is believed that one plausible explanation of the mechanism of the accelerated growth of hair and nails, hair color restoration and skin revitalization is that the presence of the anabolic hormones, and in particular, the combination of insulin, triiodothyronine or thyroxine and human growth hormone in the formulations according to the present invention, synergistically promotes the utilization of the nutrients from the nutrient medium and consequently, the promotion of angiogenesis and melanogenesis and vascularization of the tissue surrounding the hair follicle and nail growth tissue. The result is the stimulation of hair follicles and related nail tissue and consequently enhanced rate of growth of hair and nails and hair color restoration. In addition, not only is the rate of growth of hair enhanced, but also the number, quality and in most instances, melanogenesis of active hair-growing follicles growing hair also increases, primarily due to the stimulation of dormant, dying or weak follicles to produce hair. In effect, the quality of the growth of hair, nail and skin tissue is enhanced using the compositions of the present invention. Hair color restoration is an auxiliary effect.

With regard to the enhancement in angiogenesis and the promotion of vascularization, the mechanism which might be assumed is that new capillaries appear in the tissue surrounding the hair follicles and related nail tissue from the first day on and reach their maximum levels after one week or so. The new vessels in granulation tissue originate as budlike structures on nearby vessels, enhance vascularization, become canalized and ramify throughout the dermis in proximity to the hair follicles and nail growth tissue. The resulting increase in vascularization provides more nutrition and stimulatory factors for the hair follicle and nail and skin tissue, the

consequence of which is the stimulation of the hair follicle and related nail and skin tissue and the enhancement of production of hair, nail and skin tissue.

It is further believed that the function of the medium is to provide nutrients to normal, distressed and injured follicles of the dermis in order to stimulate the follicle and enhance the growth of hair. In this way, the medium functions with the non-steroidal anabolic hormone to promote the normal processes of stimulation of hair follicles and the enhanced growth of hair. In addition, in many instances, as in the case of gray or graying hair, the color of the hair returns to its natural color.

A number of nutrient media, preferably serum free, alone or in combination, may be used in the present invention, including commercially available media or other media well known in the art. Examples of such media (all without serum or having had the serum removed) include ADC-1, LPM (Bovine Serum Albumin-free), F10 (HAM), F12 (HAM), DCCM1, DCCM2, RPMI 1640, MCDB 105, MCDB 110, MCDB 202, MCDB 402, MCDB 153, BGJ Medium (with or without Fitton-Jackson Modification), Minimum Essential Medium Eagle, Basal Medium Eagle (BME-with the addition of Earle's salt base), Dulbecco's Modified Eagle Medium (DMEM-without serum), Glasgow Modification Eagle Medium (GMEM), Leibovitz L-15 Medium, McCoy's 5A Medium, Medium M199 (M199E- with Earle's salt base), Medium M199 (M199H- with Hank's salt base), Minimum Essential Medium Eagle (MEM-E- with Earle's salt base), Minimum Essential Medium Eagle (MEM-H- with Hank's salt base) and Minimum Essential medium Eagle (MEM-NAA- with non-essential amino acids), among numerous others. These and other useful serum-free media are available from Biological Industries, Bet HaEmek, Israel, among others.

In addition, serum-containing nutrient media may also be used in compositions according to the present invention, but the use of serum-containing media is less preferred because of the possibility that the serum may be contaminated with microbial agents and because the patient may develop immunological reactions to certain antigenic components con-

tained in the serum.

While a large number of serum free nutrient media may be used in the present invention, a preferred nutrient media for use in the present invention is modified MCDB 153.

Hereafter are enumerated the particular constituents and concentrations of the above groups for the preferred medium, MCDB 153:

Group (a):	Concentration (M)
Arginine	1.0 x 10-3
Cysteine or Cystine	2.4 x 10-4
Glutamine	6.0 x 10-3
Histidine	8.0 x 10-5
Isoleucine	1.5 x 10-5
Leucine	5.0 x 10-4
Lysine	1.0 x 10-4
Methionine	3.0 x 10-5
Phenylalanine	3.0 x 10-5
Threonine	1.0 x 10-4
Tryptophan	1.5 x 10-5
Tyrosine	1.5 x 10-5
Valine	3.0 x 10-4
Group (b):	
Alanine	1.0 x 10-4
Asparagine	1.0 x 10-4
Aspartate	3.0 x 10-4
Glutamate	1.0 x 10-4
Glycine	1.0 x 10-4
Proline	3.0 x 10-4
Serine	6.0 x 10-4
Group (c):	
Biotin	6.0 x 10-8
Folate	1.8 x 10-6
Lipoate	1.0 x 10-6
Niacinamide	3.0 x 10-7
Pantothenate	1.0 x 10-6

Pyridoxine	3.0 x 10-7
Riboflavin	1.0 x 10-7
Thiamin	1.0 x 10-6
Vitamin B12	3.0 x 10-7
Group (d)	
Glucose	6.0 x 10-3
Group (e):	
Magnesium	6.0 x 10-4
Postassium	1.5 x 10-3
Sodium	1.5 x 10-1
Chloride	1.3 x 10-1
Calcium	0.1 mmol.
Group (f):	
Adenine	1.8 x 10-4
Thymidine	3.0 x 10-6
Group (g):	
Acetate	3.7 x 10-3
Choline	1.0 x 10-4
i-Inositol	1.0 x 10-4
Putrescine	1.0 x 10-6
Pyruvate	5.0 x 10-4
Group (h)	
Phosphate	2.0 x 10-3
Sulfate	4.5 x 10-6
Group (i):	
Copper	1.0 x 10-8
Iron	1.5 x 10-6
Zinc	3.0 x 10-6
Group (j):	
Bicarbonate	1.4 x 10-2
HEPES	2.8 x 10-2

Weights of each of the above components in the medium

may be varied within the concentrations described hereinabove (in hair or nail growth enhancing effective amounts) to provide formulations workable within the description of the present invention.

Preferably, the non-steroidal anabolic hormone to be incorporated into the modified MCDB 153 composition, according to the present invention, is a mixture of at least two hormones selected from insulin, triiodothyronine/thyronine and growth hormone at hair growth enhancing effective concentrations. Most preferably, the anabolic hormone includes a mixture of human growth hormone, insulin (containing transferrin or transferrin-free) and triiodothyronine (T_3) or thyroxin (T_4), each hormone included in a hair growth enhancing effective amount. The three hormone combination exhibits an unexpected synergistic effect in promoting hair growth. Hormones other than insulin are included in an amount ranging from at least about 0.05ng/ml, preferably at least about 0.5ng/ml to about 100 ng/ml, and more preferably about 1 ng/ml to about 100 ng/ml. In the case of thyroxine, it is generally substituted for triiodothyronine at a concentration of at least about three to five times the concentration of triiodothyronine used. In the case of insulin, the effective amount of insulin generally ranges from about 5ng/ml to about 100ug/ml and more preferably about 50 ng/ml to about 20ug/ml, even more preferably about 500 ng/ml to about 20 ug/ml within this range.

Insulin is a desirable constituent anabolic hormone, found to impart a maturing stimulus of the growing culture. Insulin may be commercially obtained and is generally provided in mU quantities (about 41 ng of insulin). The International Unit of Insulin (SI= System International) is the activity contained in 0.04167 mg (41.67 ug) of the 4th International Standard Preparation (1958). The Standard Preparation is a quantity of purified Zinc Insulin crystals extracted 52% from Bovine and 48% from Porcine pancreas (See, Martindale Pharmacopoeia, 26th Ed.).

In addition to effective amounts of non-steroidal

anabolic hormones and media as described above, formulations according to the present invention may also contain transferrin (which is believed to improve iron transport) in amounts which preferably range from about 500 ng/ml to about 50 ug/ml, more preferably about 5-10 ug/ml and selenite (preferably, in the form of sodium selenite) in amounts preferably ranging from about 0.5 to about 50 ng/ml, more preferably about 5-10 ng/ml. The inclusion of transferrin is not critical to the use of the instant invention and in certain cases may be less preferred.

The formulations according to the present invention may also include an effective amount of an anti-dandruff agent or other ingredients which are commonly applied to the scalp or hair, including antimicrobial agents, where desirable, generally in amounts found useful in topical applications. In the case of nail growth formulations, the inclusion of an anti-fungal agent for use in preventing the outgrowth or further growth of nail fungus infections is also optional. One of ordinary skill in the art can easily determine the type and amount of anti-dandruff or antimicrobial agent chosen for use in formulations according to the present invention.

In certain embodiments according to the present invention, the formulations as described herein are further formulated with gelation agents or related delivery polymers for gelling the formulations according to the present invention for delivery to the area of intact skin to be treated. In these embodiments, the formulations comprising effective amounts of anabolic hormone(s) and nutrient media, either alone or in addition to other optional components, especially including an effective amount of a penetration enhancement agent, are admixed with amounts of a delivery polymer effective for producing a gel, for example a hydrogel polymer derived from HEMA (hydroxyethylmethacrylate) or NVP (N-vinylpyrrolidone), polyethylene glycol (PEG), polyethylene, gelatin, various carbohydrates, sepharose, agarose, methylcellulose and related hydrophilic cellulose ethers including cellulose, hydroxymethyl cellulose, hydroxyethyl cellulose and hydroxypropylcellulose, dextran, polyethyleneoxide, dextran-

polyethylene, acrylamide, polyacrylamide, amylose or collagen to promote the delivery of the components to the surface of the skin. The inclusion of a cellulose ether gellation agent and in particular, the use of methyl cellulose or hydroxyethyl cellulose, is clearly preferred. In general, the amount of delivery polymer which is added to the formulations to produce a gel is that amount which solidifies the composition to a point where the composition does not easily flow off of the intact skin to be treated and generally ranges from about 0.1% by weight to about 20% by weight, preferably about 0.5% to about 10% by weight, depending upon the type of delivery polymer used. The gel compositions according to the present invention preferably contain sufficient water or moisture to maintain the area to be treated in a relatively moist state- a condition shown to be beneficial for penetration enhancement of the individual components of the formulations through the skin.

In addition to solution, gel or hydrogel forms, compositions according to the present invention also may be formulated as creams, elixirs, powders and the like for delivery to the scalp or other area of intact skin to be treated for the hair growth enhancing effects of the present compositions. The various components of the compositions according to the present invention may have to be varied in order to maintain effective concentrations. When compositions according to the present invention are formulated, these compositions may also contain an amount of a pharmaceutically acceptable excipient and, in addition, other additives such as diluents, compounding agents, bulking agents, surfactants and the like. One of ordinary skill in the art will recognize to vary the concentrations of the individual components as a function of the type of delivery vehicle used for the hair growth compositions.

In a method for enhancing the growth of hair and/or restoring the natural color of the hair in intact skin (scalp), especially including hair of the human scalp, the formulations as described hereinabove are topically applied to the skin tissue to be treated as a liquid or gel at least once

a day and preferably at least twice a day and up to six or more times a day. In many instances it may be advisable to apply the compositions to the scalp or other area of the skin to be treated at least once a day after showering or bathing. In the case of formulations containing a delivery polymer, preferably a moistened delivery polymer, the formulations may be administered less frequently than when the formulations are applied as a liquid. One of ordinary skill in the art will readily be able to determine the amount and frequency of administering the formulations according to the present invention.

One possible regimen for utilizing the compositions according to the instant invention is to first treat the skin with a penetration enhancement agent in an amount (i.e., at a concentration) and for a time effective for enhancing penetration of the components of the hair or nail growth composition. In this method, the penetration enhancement agent is generally applied to the intact skin to be treated with the hair growth composition for 30 seconds up to one half hour or more (preferably from about 1 to about 5 minutes) to condition the skin for penetration. It is noted that the inclusion of a penetration enhancement agent is unexpectedly not required in order to deliver effective amounts of the present compositions to promote hair growth, nail growth and skin revitalization. In cases where water is the penetration enhancer, the skin to which the composition is to be applied is first wet with water, after which time, the composition is applied. A covering, such as a polyethylene shower cap, may be used to keep the composition in contact with the area to be treated.

In other methods according to the present invention the scalp is simply washed with soap or detergents to remove all natural oils from the scalp before application of the hair growth enhancing composition. After a period of time sufficient for treating the skin, the treated skin is then exposed to the hair growth composition (preferably, after wetting with water to promote penetration) and massaged into the scalp. In certain preferred embodiments, the hair growth composition is a gel which is rubbed onto the area of the skin to be treated

and is left on the skin for a convenient period, generally at least 1-3 hours and preferably for an eight hour period such as overnight. A covering is preferably placed over the gel in order to keep the gel in contact with the treated skin area. To remove the composition, it can be washed off with clear water.

The amount of material which is to be spread on the skin or massaged into the scalp to be treated will be readily apparent. The formulations should be applied in a manner which is cosmetically appealing. In general, in solution or gel form, about 0.5-2.0 cc of formulation is applied per 5-10 cm^2 to the area of the scalp. Depending upon the requirement of hair growth and the extent of baldness, an amount greater or less than 0.5-1 cc of formulation per 5-10 cm^2 of the wound surface may be utilized. In many instances, the depth of the formulation on the skin should be at least about 0.05-0.1 mm. Greater depths may also be used, but generally at the expense of the cosmetic appeal of the product. Obviously, the greater the amount of composition and the greater the depth of gel on the skin surface, the greater will be the delivery of composition to the surface of the skin. In certain cases where the hair is already quite thick, for example, when treating the hair to restore the natural color of the hair, more material may have to be added to the hair in order to assure the individual that a sufficient amount of the composition will come into contact with the underlying skin or scalp area.

In the case of treating hair to restore natural color, the treatment regimen and compositions used are virtually identical to the method used for enhancing the growth of hair. Restoration of hair color generally will occur as an auxiliary result of enhanced growth of hair. Obviously, where the hair color is diminished in color, for example, in gray, graying or even white hair, the methods of use will be the same as if the desired result is enhanced hair growth, but in such a case, the more clearly desired result will be restoration of hair color. Enhanced hair growth most likely will also occur.

In the case of treatment of nails or ungual tissue or

skin tissue of animals, including humans, to strengthen and enhance the growth of the nail tissue or revitalize the skin, treatment may be at night or during the day. In the case of a preferred night treatment, the tissue of the patient is treated by applying the compositions according to the present invention directly on the tissue after first wetting the tissue and, in the case of ungual tissue, to the cuticle area of the nail or tissue where the nail or ungual tissue is attached to the nail or tissue bed. During this night treatment, about 1 ml per cm^2 is placed on the tissue as described above and the composition and tissue is covered with gauze and adhesive. A cover may be advantageously applied to keep the composition in contact with the treated tissue.

In the case of treatment during the day (following a night treatment as described hereinabove), a preferred treatment includes washing off the remainder of the gel under running water and then drying the tissue. After the drying step, the compositions according to the invention may be applied as for the night treatment, as above, or alternatively, the compositions are applied to the tissue liberally and is allowed to be absorbed into the nail and surrounding skin. Preferably, the skin is first moistened with water before applying the composition. A protective covering may be used in order to keep the composition in contact with the treated scalp.

Preliminary bioassays to determine the acceleration of hair growth which were carried out on rats, guinea pigs and on selected clinical cases indicated that the formulations according to the present invention exhibited a significant beneficial result relative to traditional therapies, including the use of Minoxidil. It is noteworthy that unlike Minoxidil, which evidences hepatotoxicity, tachycardia and contact dermatitis in vivo, the present invention exhibits little, if any, toxicity. In the case of nail growth, clinical use of the preferred formulation evidenced enhanced growth of both finger and toe nails.

The invention will be described hereinafter by a num-

ber of Examples which illustrate some actual tests carried out on skin to enhance the growth of hair utilizing the compositions according to the present invention. It should be understood that the Examples are not exhaustive nor limiting and are presented only for a better understanding of the invention.

EXAMPLES

Materials and Methods

1. Preparation of Hair Growth Gel

The whole procedure was performed under sterile conditions.

a. Delivery System

Four grams of Methyl cellulose (Methocel MC 4000 cp, Fluka AG) in 90 ml of double distilled water was autoclaved.

b. Media

The preferred media contained essential and non-essential amino acids, vitamins, other organic constituents, major inorganic salts, trace elements and buffers and was supplemented with CaCl and L-glutamine and with the non-steroidal anabolic hormones, insulin, thyroxin, growth hormone and insulin-like growth factor (IGF) at the concentrations as indicated below.

Component	Concentration in M
Amino Acids (L-enantiomers)	
Alanine	1.0×10^{-4}
Arginine HCl	1.0×10^{-3}
Asparagine	1.0×10^{-4}
Aspartic Acid	3.0×10^{-5}
Cysteine HCl or Cystine	2.4×10^{-4}
Glutamic Acid	1.0×10^{-4}
Glutamine	6.0×10^{-3}
Glycine	1.0×10^{-4}
Histidine HCl	6.0×10^{-5}
Isoleucine	1.5×10^{-5}
Leucine	5.0×10^{-4}

Lysine HCl	1.0×10^{-4}
Methionine	3.0×10^{-5}
Phenylalanine	3.0×10^{-5}
Proline	3.0×10^{-4}
Serine	6.0×10^{-4}
Threonine	1.0×10^{-4}
Tryptophan	1.5×10^{-5}
Tyrosine	1.5×10^{-5}
Valine	3.0×10^{-4}
Vitamins	
d-Biotin	6.0×10^{-8}
Folic Acid	1.8×10^{-6}
DL-a-lipoic acid	1.0×10^{-6}
Niacinamide	3.0×10^{-7}
D-pantothenate 1/20a	1.0×10^{-6}
Pyridoxine HCl	3.0×10^{-7}
Riboflavin	1.0×10^{-7}
Thiamin HCl	1.0×10^{-6}
Vitamin B12	3.0×10^{-7}
L-Ascorbic Acid	9.9×10^{-5}
Other Organic Constituents	
Acetate	3.7×10^{-3}
Adenine	1.8×10^{-4}
Choline chloride	1.0×10^{-4}
D-glucose	6.0×10^{-3}
i-Inositol	1.0×10^{-4}
Putrescine 2HCl	1.0×10^{-6}
Na Pyruvate	5.0×10^{-4}
Thymidine	3.0×10^{-6}
Major Inorganic Salts	
$CaCl_2$	3.0×10^{-5}
KCl	1.5×10^{-3}
$MgCl_2$	6.0×10^{-4}
NaCl	1.2×10^{-1}
Na_2HPO_4	2.0×10^{-3}
Trace Elements	
$CuSO_4$	1.1×10^{-8}
$FeSO_4$	5.0×10^{-6}
H_2SeO_3	3.0×10^{-8}
$MnSO_4$	1.0×10^{-9}
Na_2SiO_3	5.0×10^{-7}
$(NH_4)_6Mo_7O_{24}$	1.0×10^{-9}
NH_4VO_3	5.0×10^{-9}
$NiCl_2$	5.0×10^{-10}

$SnCl_2$	5.0×10^{-10}
$ZnSO_4$	5.0×10^{-7}
Buffers	
Hepes	2.8×10^{-2}
$NaHCO_3$	1.4×10^{-2}
Phenol Red	3.3×10^{-6}
Supplements	
CaCl2	14.7 ug/ml (Merck)
L-Glutamin	0.877 mg/ml
$NaHCO_3$	1.176 mg/ml
Human Growth Hormone	5 ng/ml (Biotechnology General)
Insulin	5-10 ug/ml (Novo Nordisk) (about 143 mU/ml)
Thyroxine(T_4)	1.0×10^{-7}M (Sigma) (77.69 ng/ml)
Transferrin	5 ug/ml (Sigma)
Sodium Selenite	5 ng/ml (Sigma)
Vehicle	
Methylcellulose (Methocel 4000 cp, Fluka AG)	4% or
Hydroxyethylcellulose	6%

c. Preparation of Hair Growth Formulation

1. 4 grams of the methyl cellulose is autoclaved in 90 ml of double distilled water. The water/methyl cellulose mixture is cooled to 4°C and stirred until the gel dissolves and the solution clears (overnight with a magnetic stirrer in the cold room). To the water/methyl cellulose mixture is added 10 ml of the concentrated MCDB 153 solution (X 10 concentrated media available from Biological Industries, Beth Haemek, Israel) containing the supplements as described above (adjusted to a final volume of 100 ml of formulation). The solution is then mixed well and then decanted into 50 ml sterile test tubes. The formulation is stored at 4°C.

2. Autoclave 6 grams of hydroxyethyl cellulose, middle viscosity 1 (Fluka Cat. #54290) in 83 ml double distilled water. Separately, prepare 10 ml of 10 times concentrated medium containing the supplements as described above (adjusted to a final volume of 100 ml of formulation). Cool the hydroxyethyl cellulose solution to room temperature and stir

until the gel dissolves and the solution clears (overnight with magnetic stirrer, in refrigerator). Add the medium solution with all the supplements to the gel solution, mix well and decant into 20 ml. sterile test tubes and store at 4°C.

d. Instructions for Use in Clinical Settings

The scalp is treated once a day, preferably before going to sleep. If the scalp is oily or if other hair grooming products are in use, the hair is hydrated (in certain cases, also shampooed) to remove the oil or other deposits and then towel dried. Massage the gel into the scalp and leave on overnight. Preferably, the scalp is first moistened before applying the composition. In the morning, wash out the remains of the gel with clear water.

Example 1
Clinical Use of Hair Growth Formulations

The following represent results of the use of the above-described hair growth formulation (formulation 1c.2. in materials and methods section, above) in the clinical setting. Essentially, 15 individuals used the hair growth gel according to the instructions provided in the materials and methods section (1d.), as described above. The following set of questions was answered by each of the individuals who participated in the clinical experiment. The results obtained from patients answering the questionnaire appear in Table 1, below.

1. Did you suffer any loss of hair during combining before you started using the formulation?
2. If so, did the hair loss stop after you started the treatment?
3. If so, how long after the start of the treatment did it stop?
4. Is you hair growing now, after the start of the treatment, at a faster rate?
5. If so, how long after you started the treatment did you notice that?

6. Do you or anybody else see new growth of hair?

7. if so, how long after you started the treatment did you notice that?

PRELIMINARY CLINICAL TRIALS OF HAIR GROWTH FORMULATION

Patient	Sex	Age	Alopecia Type	Alopecia Duration	Etiology of Defect	Pattern Progress	Dynamics
M.M.	M	25	Diffuse	2 yrs.	Heredity	Combination	Slow
B.A.	M	20	Diffuse	2 yrs.	Male Pattern	Combin.	Rapid
D.M.	M	47	Areata	26 yrs.	Male Patt.	Bald/RHL*	Slow
S.L.	M	40	Areata	15 yrs.	Heredity	Bald/RHL*	Slow
D.T.	M	10	Areata	3 mos.	Infection	Bald pate	Static
E.Z.	M	16	Diffuse	1 yr.	Heredity	Combin.	Rapid
A.W.	M	46	Areata	16 yrs.	Heredity	Bald/RHL*	Slow
M.L.	M	44	Areata	6 yrs.	Male Patt.	Bald pate	Slow
S.P.	M	28	Areata	3 yrs.	Herdity	Bald/RHL*	Rapid
L.L.	F	38	Diffuse	5 yrs.	Unknown	Combin.	Slow
F.B.	F	46	Diffuse	20 yrs.	Heredity	Combin.	Slow
M.G.	F	67	Diffuse	5 yrs.	Heredity	Combin.	Slow
M.L.	F	43	Diffuse	10 yrs.	Unknown	Combin.	Slow
R.G.	F	53	Diffuse	20 yrs.	Unknown	Combin.	Slow
T.B.	F	14	Areata	3 mos.	Trauma	Bald pate	Static

* Receding Hair Line

Table 1
RESULTS OF TREATMENT

Patient	Cessation of Loss	Hair Growth Increase	De Novo Hair Growth
M.M.	1 week	2 weeks	1 month
B.A.	2 weeks	1 month	1 month
D.M.	2 weeks	1 month	2 months
S.L.	2 weeks	2 months	2 months
D.T.	1 week	1 week	1 week
E.Z.	2 weeks	3 weeks	1 month
A.W.	2 weeks	1 month	2 months
M.L.	2 weeks	1 month	1 month
S.P.	2-3 weeks	3-4 weeks	3-4 weeks
L.L.	2 weeks	1 month	1 month
F.B.	2 weeks	1 month	1 month
M.G.	2 weeks	6 weeks	6 weeks
M.L.	3 weeks	6 weeks	6 weeks
R.G.	3 weeks	1 month	1 month
T.B.	1 week	1 week	1 week

In all cases, hair loss stopped within a period of 2-3 weeks. In addition, all of the patients showed an increase in hair growth and all patients experienced de novo hair growth.

Example 2
Further Clinical Results Using Hair Growth Formulation

The individuals treated in example 1 were studied for a further period to determine the effects of the instant invention on hair growth and cessation of hair loss. All of the individuals, prior to the study suffered from alopecia androgenetica or traumatic alopecia of variable severity. The individuals were instructed to use the hair growth formulation of the instant invention as described in example 1 (except that transferrin was excluded from the formulation) and this use continued for more than six months. Essentially, the volunteers were instructed to apply the hair growth formulation to the scalp once a day, before retiring, by massaging it into the scalp area. The gel was left on during the night, and washing of the remains of the gel was performed the following morning. The results of the study are presented in Tables 2 and 3, below. As shown in Table 1 of Example 1, the reaction to the treatment was seen 1-3 weeks after the start of the treatment, when cessation of hair loss was reported by all participants. In addition, generally within 4-6 weeks, the increase in the rate of existing hair growth was reported as well as the start of new hair growth. In the further results, after four months of treatment, all of the participants in the study evidenced moderate or dense hair growth, with most show moderate hair growth. After more than 6 months of treatment several individuals within the treatment group evidenced dense hair growth and two individuals exhibited a complete cover of the deficit area.

Table 2
FURTHER RESULTS OF TREATMENT

Pat.	Sex	Age	HAIR GROWTH None 4m	None 6m+	Slight 4m	Slight 6m+	Moderate 4m	Moderate 6m+	Dense 4m	Dense 6m+
M.M.	M	25					X	X		
B.A.	M	20					X	?		
D.M.	M	47					X			X
S.L.	M	40					X	X		
D.T.	M	10							X Comp.	
E.Z.	M	16					X	X		
A.W.	M	46					X	X		
M.L.	M	44					X	X		
S.P.	M	28					X	X		
L.L.	F	38					X	X		
F.B.	F	46					X			X
M.G.	F	67					X			X
M.L.	F	43					X	*		
R.G.	F	53					X	*		
T.B.	F	14							X Comp.	

4m - After 4 months of treatment.

6m+- After more than 6 months of treatment.

Comp- Complete cover of the deficit area.

*- Cessation of loss (main complaint).

Example 3
Effects of Hair Growth Formulation on Hair Growth in Mice

To test the efficacy of the instant invention in inducing hair growth in the animal mode, the model of Paus, et al. (1991) was used.

In this experiment, ninety-six (96) C57BL mice aged 5-6 weeks were divided into five groups. Two experimental groups were treated with the instant invention (formulation 1c.2 with transferrin excluded) three times daily by application of the gel to the dorsal depilated area of the mouse. Three control groups were used, two of which were treated with vehicle placebo in the same manner as the two experimental groups. The third group was untreated.

Group No. 1: Treatment with Hair Growth Composition starting on Day 0 (three times daily) and continued for 30 days.

This group consisted of 20 mice, which were depilated manually on Day 0 of the experiment. On Day 7, anagen ensued (as judged by gray coloration produced by melanogenesis). On Day 10, an injection of the cytoxic agent cytophosphan (0.5 ml per gram) was made ip. On day 15, catagen resulted form the administration of the cytophosphan. On Day 16-17, anagen was seen and on that day blood samples were taken for CBC analysis, as well as punch biopsies. On Day 18, the second cytophosphan injection was given. On Day 26-27, catagen occurred and on Day 28, anagen was present. Second blood samples were taken on Day 30, at which time punch biopsies were also taken.

Group No. 2: Treatment with Hair Growth Composition starting on Day 10 (three times daily) after cytophosphan injection and continued for 30 days.

This group consisted of 20 mice, which were depilated manually on Day 0 of the experiment. On Days 7-9, anagen occurred (Day 7 - 30%, Day 8 - 60%, Day 9 - 70%). On Day 10, cytophosphan (0.5 ml per gram) was administered, all animals were in anagen (100%) at that time. On Day 14, catagen occurred and on Days 16-17, anagen was seen and on Day 17 blood samples were taken for analysis and biopsies were also taken. On Day 18, the second cytophosphan injection was given. On Day 26-27, catagen occurred and on Day 28-29, anagen occurred. Second blood samples were taken on Day 30, at which time punch biopsies were also taken.

Group No. 3: Treatment with vehicle placebo starting on Day 0 (three times daily) and continued for 28 days.

This group consisted of 20 mice, which were depilated manually on Day 0 of the experiment. On Days 7-8, anagen occurred. On Day 9, cytophosphan (0.5 ml per gram) was administered and on Day 15, catagen occurred. On Days 16-17, anagen was seen and on Day 20 vellus hair was seen. On Day 24, terminal hair was observed and on Day 28, the number of animals that had terminal hair was 4 (mild), 10 (moderate) and 6 (extensive terminal growth).

Group No. 4: Treatment with vehicle placebo starting on Day 9 following cytophosphan injections and continued for 20 days.

This group consisted of 20 mice which were depilated on Day 0 and angagen was observed on Day 9, when the mice were injected with cytophosphan. Catagen was observed on Day 15, and anagen followed on Day 17-18. Vellus hairs were seen on Day 20 and Day 24. On Day 28, terminal hair growth was mild in five naimals, moderate in three animals and extensive in two animals.

Group No. 5: No Treatment given to this control group- used as a baseline for the other studies.

This group consisted of 16 mice, which were depilated on Day 0 and anagen ensued on Days 9-10. On Day 10, cytophosphan was injected and catagen occurred on Days 15-17. On Days 18-21, anagen occurred and on Day 21, punch biopsies were taken.

The various experimental procedures performed on the 5

groups of mice as described above are detailed in the flow chart set forth in Table 3, below.

Results: In the experimental mice, the use of the present invention produced significant enhancement of the growth of hair in experimental animals under conditions in which the hair growth composition was used to treat the animals prior to injection with cytophosphan and after cytophosphan. (Table 4, below). Although the standard deviation of the results of the test animals was rather large, nonetheless, the overall results evidenced significant enhancement of hair growth in the test animals which had been treated with the present invention compared to untreated control animals. In addition, the use of the present invention did not produce mortality in any of the test animals (total of 234 animals).

Table 3
FLOW CHART OF EXPERIMENTAL PROCEDURES

Treated Group	Pre-Inj. 1	Post-Inj. 2	Pre-Inj. 3	Post-Inj. 4	None
Day No.					
0	Depil.	Depil.	Depil.	Depil.	Depil.
1-6					
7	1st Anagen	1st Anagen	1st Anagen		
8		Anagen 60%	Anagen 95%		
9		Anagen 70%	1st Inject.	1st Anag. 100% 1st Inject.	1st Anag.
10	1st Inject.	1st Inject.			Anagen 100% 1st Inject.
11-13					
14		1st Catagen			
15	1st Catagen	2nd Anagen 30%	1st Catag.	1st Catag.	1st Catag. 38%
16	2nd Anagen 50%	Anagen 100%	2nd Anagen 20%	2nd Anagen 30%	Catagen 50%
17	Anagen 100% + 1st CBC + Biopsy	1st CBC + Biopsy	Anagen 100%	Anagen 100%	Catagen 100%
18	2nd Inject.	2nd Inject.			2nd Anagen 100%
19					
20			Vellus Hair	Vell. Hair	
21					Biopsy
22-23					
24			Term. Hair	Term. Hair	
25					
26	2nd Catagen 50%	2nd Catagen 50%			
27	Catagen 100%	Catagen 100%			
28	3rd Catagen 100%	3rd Categen 50%			
29		Anagen 100%			
30	2nd CBC + Biopsy	2nd CBC + Biopsy			

Table 4
HAIR LENGTH IN MICE

CONTROL No Treatment	PRE-INJECTION TREATMENT With Invention	POST-INJECTION TREATMENT With Invention
	1st	1st
	Day 7	Day 7
	0.121 ± SD 0.206	0.084 ± 0.12
	(29.4%)	(66.6%)
Day 11		
0.097 ± SD 0.13		
(41%)		
	2nd	2nd
	Day 12	Day 12
	0.79 ± 0.25	0.65 ± 0.31
	(100%)	(100%)

% Number of Animals with Hair

Example 4
Restoration of Hair Color

The following four cases evidence that the present invention may be used to restore the natural hair color in hair treated using the present invention. Auxiliary to enhanced hair growth, the same hair evidenced an unexpected hair color restoration consistent with the enhancement or restoration of melanogenesis by the present composition.

Case #1

A man in his 40's, who had a diffuse vertex hair loss of 5 years duration has been using the present composition (composition 1c.2) for 5 and 1/2 months. His natural hair coloring is light-reddish brown, but in the vertex area, where hair loss appeared, the color had faded to yellowish-white. After using the present composition, the natural hair coloring of hair in the vertex area was restored. The hair has the same reddish-brown coloring as the res of the hair on the head.

Case #2

A woman in her late 40's suffered diffuse, rapid progressive hair loss throughout the frontal and vertex areas. Application of the present composition for 4.5 months has resulted in cessation of hair loss, new hair growth, increased rate of hair growth and resoration of her original dark-brown pigmentation.

Case #3

A 69 year old female suffered from rapid progressive hair loss in the frontal and vertex areas. She has been using the present composition as recommended for the last 6 months. The treatment has induced cessation of hair loss, increased the rate of hair growth and the growth of new hair. Restoration of her original red pigmentation was observed in hair that has been white for years (notwithstanding the artificial hair rinses she uses).

Case #4

A 54 year old caucasian male who has a beard in which the original dark coloration became streaked with white used the present composition as recommended for 1 month (nighttime application sufficient to allow contact of the formulation with the underlying skin, washing off the composition in the morning). The treatment has induced pigment change in which the original coloration has returned in the newly growth hair, and in the white hair, the roots have become black, evidencing restoration of hair color in the newly grown portion of the hair.

Example 5

Experiments Determining Angiogenesis in Animals

The material as defined in the materials and methods section, above, in liquid or gel form was used (with the exception that triiodothyronine at 13.02 ng/ml was substituted for the thyroxine, the amount of $CaCl_2$ used was 4.0×10^{-5} and no Phenol Red was included in the formulation- in liquid form, no thickener was used- in gel form, 4 g of methyl cellulose was used) to treat corneal ulcers in Hartley derived female guinea pigs.

Hartley derived female guinea pigs weighing 350-400g were housed in individual cages and fed guinea pig chaw and vitamin C enriched water ad libitum.

General anasthetics of the animals was obtained by injection of Imalgene (Katamin, Rhone Merieux, France) 20 mg/kg, and local anasthetics with 1 drop of Localin (Benoxinate HCl 0.4%, Fischer Pharmaceuticals, Ltd.).

Under Zeiss dissecting Microscope, using a 3mm diameter biopsy punch to delineate the ulcer margins, the corneal epithelium was carefully scraped off the corneas of both eyes.

Experiment 1

Following the injury, the right eyes of 7 animals were treated with the formulation as described above in liquid form using two drops every two hours and in liquid form in the evening. After 24 hours and 48 hours, the eyes were examined under the microscope and photographed.

Experiment 2

Six animals were used in this experiment. Following the injury, in three animals both right and left eyes were treated with the formulation as described above in liquid form using one drop every three hours (until 11 PM and again starting at 6:30 AM). In the other three animals, both eyes were not treated. After 24 hours and 48 hours the corneas of both eyes of all of the animals were examined under the microscope and photographed.

Experiment 3

Six animals were used in this experiment. Following injury, in three animals both eyes were treated with the formulation as described above in liquid form, 2 drops every 2 hours and in gel form, in the evening. In the other three animals, both eyes were left untreated.

Results

A total number of 19 guinea pig eyes were treated with Cariel while a total number of 16 eyes were used as a control (untreated).

The result of the treatment yielded the following:

1. A strong angiogenic reaction which was seen in and around all treated corneas:

a. The limbal capillary bed was dilated.

b. Very fine radially oriented capillaries were found adjacent to the corneal ulcer in every case where treatment occurred.

2. Engorgement of the surrounding deep and superficial blood vessels.

3. Edema of the cornea and swelling of the stroma surrounding the ulcer.

4. The results of the untreated controls yielded no angiogenic response in and around the corneal ulcers.

Conclusions

1. Treatment with the above-described formulations, whether in liquid or gel form induced an angiogenesis response in the corneal ulcers.

2. The mechanism of action of the formulation in enhancing hair growth is partially due to its strong angiogenic activity and increased blood flow.

Example 6
Experiments Performed on Guinea Pigs
Thermal Burn Wounds

Hartley-derived albino female guinea-pigs weighing 250 g were used in this study. The animals were housed in individual cages and fed regular guinea-pig chow and water enriched with Vitamin C ad libitum. All surgical procedures to impart burn wounds to the animals were performed under general anaesthesia of Katamin HCl 150 mg/kg i.m./d,1-2-(chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride, Parke Davis).

The animals were divided into two groups. In the first group, eight guinea pigs were treated with Silverol, which is currently used as a preparation for burn wounds in all military trauma units in Israel. The second group of eight animals was treated essentially with composition 1c1. of the materials and methods section (5 ug/ml insulin, methyl cellulose as gelling agent). The experiment was repeated on three different occasions.

Laser Doppler Flowmeter measurements on burn wounds were performed on days 2 and 8 after injury. Figures 1 and 2 present the data obtained from the Laser Doppler Flowmeter measurements in graph and hologram form. The results evidence that the preferred embodiment evidences effective increase in blood flow compared to conventional therapy. This increased blood flow is believed to be at least partially responsible for the enhanced growth to hair and nail tissue and the unexpected skin revitalization activity these compositions evidence.

While the invention has been described in its preferred embodiment, it is to be understood that the words which have been used are words of description rather than limitation and that

changes may be made within the purview of the appended claims without departing from the true scope and spirit of the invention in its broader aspects.

CLAIMS

1. A composition for use in stimulating or enhancing the growth of hair or ungual tissue in intact skin in animals comprising a hair or ungual tissue growth stimulating effective amount of at least one non-steroidal anabolic hormone selected from the group consisting of insulin, triiodothyronine and thyroxine in combination with a minimum essential medium, said minimum essential medium comprising hair or ungual tissue growth stimulating effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

2. The composition according to claim 1 further including a hair or ungual tissue growth enhancing effective amount of human growth hormone.

3. The composition according to claim 1 or 2 in the form of a gel produced by including in said composition an effective amount of a gelling agent.

4. The composition according to claim 3 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

5. The composition according to any of claims 2 through 4, wherein the human growth hormone is included at a concentration range of about 0.5 ng/ml to about 50 ng/ml.

6. The composition according to any of claims 1 through 5 wherein said non-steroidal anabolic hormone is insulin included at a concentration of about 50 ng/ml to about 100 ug/ml.

7. The composition according to claim 6 wherein said insulin is included at a concentration of about 500 ng/ml to about 20 ug/ml.

8. The composition according to any of claims 1 through 7 including triiodothyronine or thyroxine in an amount ranging from about 0.5 ng/ml to about 100 ng/ml.

9. A composition for stimulating or enhancing the growth of hair or ungual tissue in intact skin in animals comprising a non-steroidal hormone selected from the group consisting of insulin, growth hormone, triiodothyronine, thyroxine and mixtures, thereof, said insulin being included in said formulation in an effective amount ranging from about 500 ng/ml to about 100 ug/ml, said growth hormone being included in an amount ranging from about 0.5 ng/ml to about 50 ng/ml and said triiodothyronine or thyroxine being included in said formulation in an amount ranging from about 0.5 ng/ml to about 100 ng/ml in combination with a minimum essential medium, said minimum essential medium comprising hair or ungual tissue growth stimulating effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

10. The composition according to claim 9 which includes a hair or ungual tissue growth enhancing effective amount of human growth hormone.

11. The composition according to claim 9 or 10 in the form of a gel produced by including an effective amount of a gelling agent.

12. The composition according to claim 11 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

13. The composition according to any of claims 9 through 12 wherein said formulation includes insulin at a concentration of about 500 ng/ml to about 20 ug/ml.

14. A composition for use in revitalizing skin in animals comprising a skin revitalizing effective amount of at least one non-steroidal anabolic hormone selected from insulin,

triiodothyronine and thyroxine in combination with a minimum essential medium, said minimum essential medium comprising skin revitalizing effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

15. The composition according to claim 14 which includes a skin revitalizing effective amount of human growth hormone.

16. The composition according to claim 15, wherein the human growth hormone is included in a concentration range of about 0.5 ng/ml to about 50 ng/ml.

17. The composition according to any of claims 14 through 16 in the form of a gel produced by including an effective amount of a gelling agent.

18. The composition according to claim 17 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

19. The composition according to any of claims 14 through 18 including insulin at a concentration of about 50 ng/ml to about 100 ug/ml.

20. The composition according to any of claims 14 through 18 including insulin at a concentration of about 500 ng/ml to about 20 ug/ml.

21. The composition according to any of claims 14 through 22 including triiodothyronine or thyroxine at a concentration ranging from about 0.5 ng/ml to about 100 ng/ml.

22. A composition for restoring natural color to hair in intact skin wherein the color of said hair has been diminished, comprising a hair color restoration effective amount of at least one non-steroidal anabolic hormone selected from the group consisting of insulin, triiodothyronine and thyroxine in combination

with a minimum essential medium, said minimum essential medium comprising hair or ungual tissue growth stimulating effective amounts of essential amino acids, a mixture of vitamins comprising folate, niacinamide, pantothenate, pyridoxine, riboflavin and thiamin, a mixture of inorganic ions comprising calcium, sodium, potassium, magnesium and chloride and glucose.

23. The composition according to claim 22 wherein said minimum essential medium further includes non-essential amino acids.

24. The composition according to claim 22 or 23 including human growth hormone.

25. The composition according to any of claims 22 through 24 further including a gelling agent in an amount effective to gel said formulation.

26. The composition according to claim 25 wherein said gelling agent is selected from the group consisting of methyl cellulose and hydroxyethyl cellulose.

27. The composition according to any of claims 22 through 25 including insulin at a concentration ranging from about 50 ng/ml to about 100 ug/ml.

28. The composition according to any of claims 22 through 26 including insulin at a concentration ranging from about 500 ng/ml to about 20 ug/ml.

29. The composition according to any of claims 22 through 29 including human growth hormone at a concentration ranging from about 0.5 ng/ml to about 50 ng/ml.

30. The composition according to any of claims 22 through 29 including triiodothyronine or thyroxine at an effective concentration concentration ranging from about 0.5 ng/ml to about 100 ng/ml.

31. The composition according to any of claims 1 through

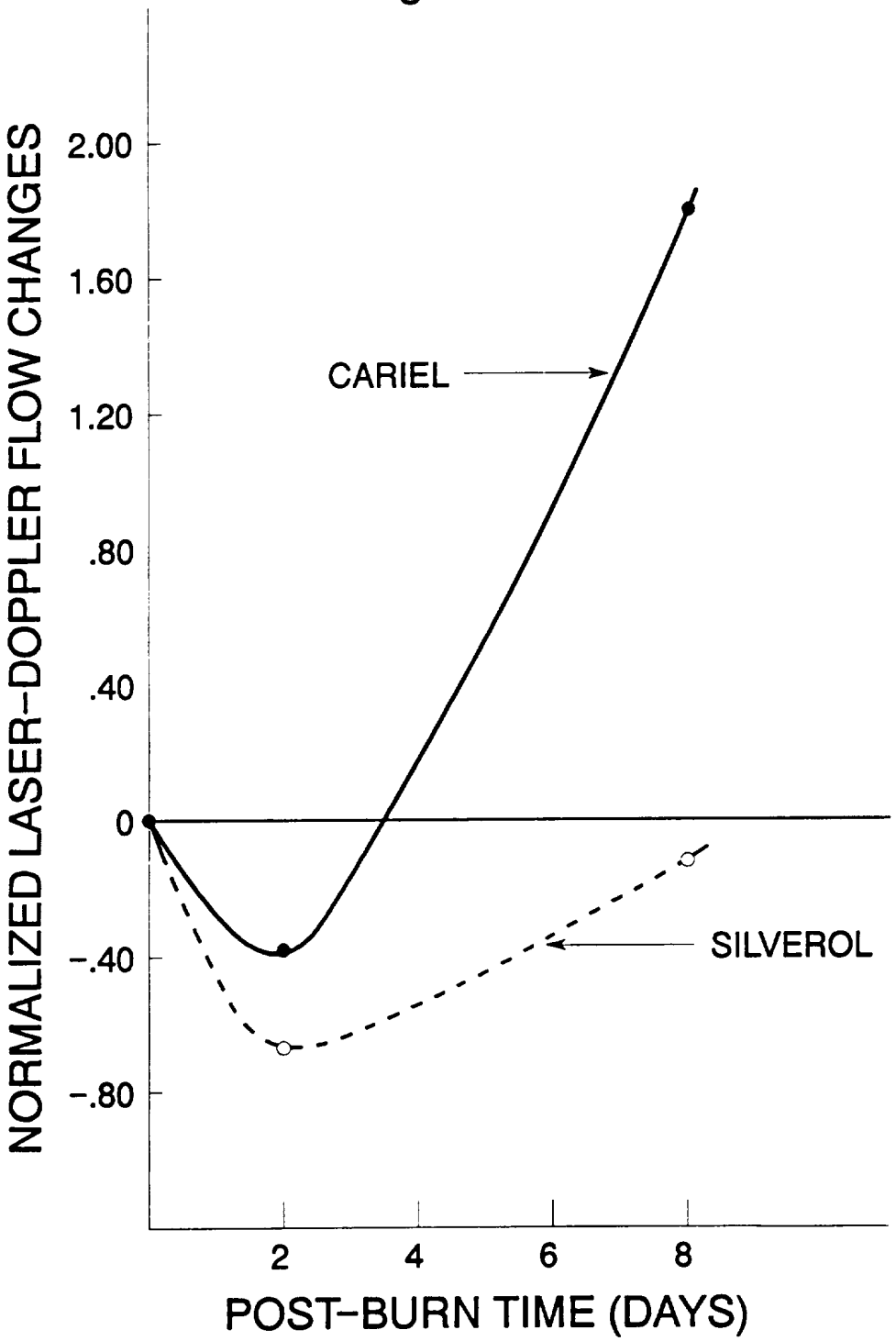
30 wherein said minimum essential medium is a medium selected from the group consisting of ADC-1, Albumin-free LPM, F10, F12, DCCM1, DCCM2, BGJ Medium with or without Fitton-Jackson Modification, Basal Medium Eagle with the addition of Earle's salt base, Dulbecco's Modified Eagle Medium without serum) Glasgow Modification Eagle Medium, Leibovitz L-15 Medium, McCoy's 5A Medium, MCDB 105, MCDB 110, MCDB 202, MCDB 402, MDCB 153, Medium M199 with Earle's salt base, Medium M199 with Hank's salt base, Minimum Essential Medium Eagle, Minimum Essential Medium Eagle with Earle's salt base, Minimum Essential Medium Eagle with Hank's salt base, Minimum Essential Medium Eagle with non-essential amino acids and mixtures thereof.

32. The composition according to any of claims 1 through 31 wherein said medium is MDCB 153.

33. The composition according to any of claims 1 through 32 including an amount of a penetration enhancement agent agent effective for enhancing penetration of said composition through said skin.

34. The composition according to claim 33 wherein said penetration enhancement agent is selected from the group consisting of chondroitin, chondroitin-6-sulphate and dermatan sulphate.

Figure 1


NORMALIZED LASER-DOPPLER FLOW CHANGES
2.00
1.60
1.20
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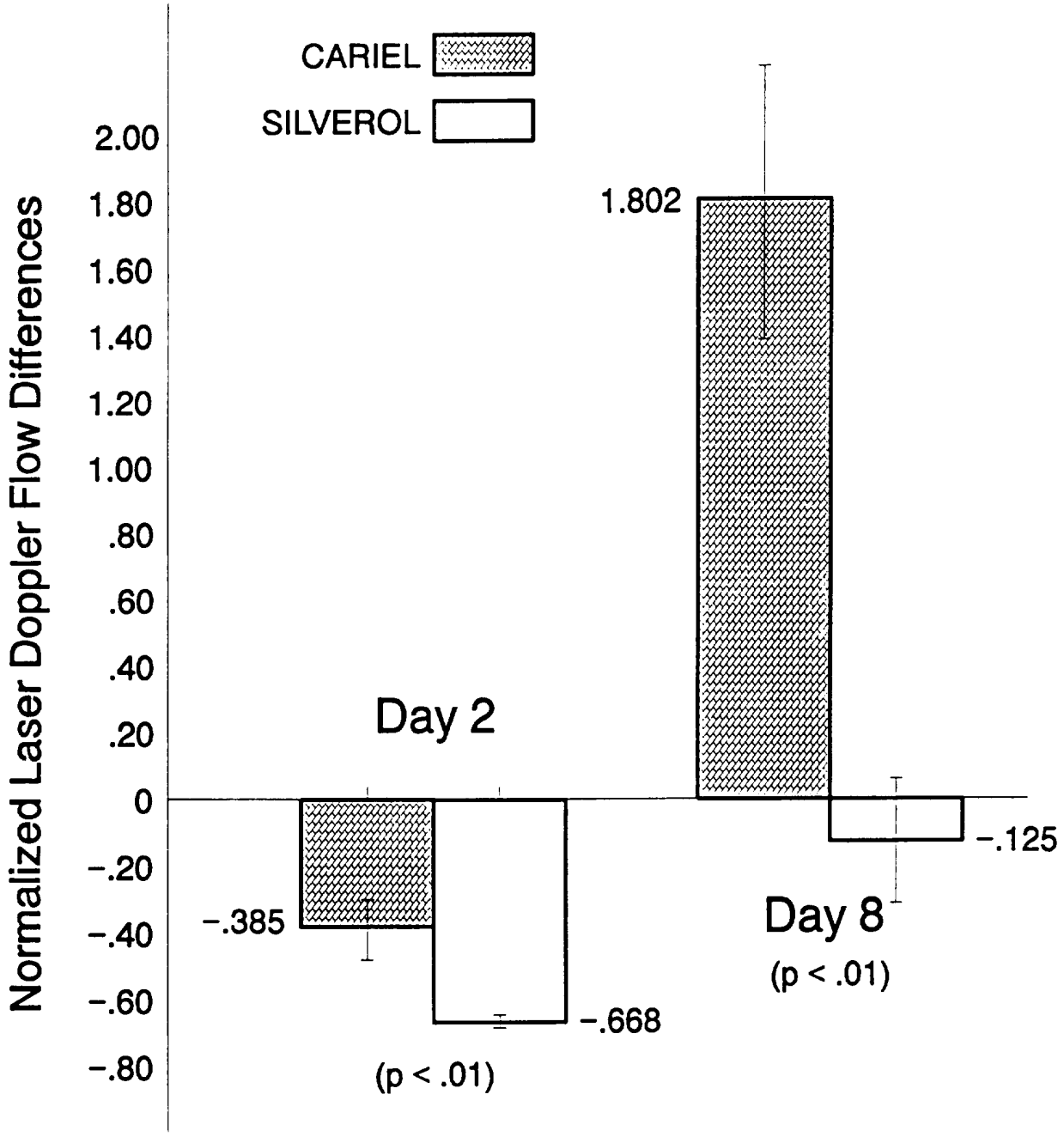
.40
0
-.40
-.80
CARIEL
SILVEROL
2
4
6
8
POST-BURN TIME (DAYS)

Figure 2


CARIEL
SILVEROL
Normalized Laser Doppler Flow Differences
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1.80
1.60
1.40
1.20
1.00
.80
.60
.40
.20
0
-.20
-.40
-.60
-.80
Day 2
-.385
-.668
(p < .01)
1.802
-.125
Day 8
(p < .01)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02341

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/28, 38/00, 31/195
US CL :514/3, 4, 5, 12, 567
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/3, 4, 5, 12, 567

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIDS, HCAPLUS- Compositions containing ingredients herein

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, 4,205,126 A (CARTAYA) 27 May 1980, see the abstract particularly.	1-4, 9-12, 14-16 and 22-24
Y	Database WPIDS, Derwent Information Ltd. , AN 93-100651 [12] , DNC C93-044367, WO 9304691 A1 (LINDENBAUM, E.), abstract.	1-4, 9-12, 14-16, and 22-24
Y	Database HCAPLUS, AN 1984:403559, BE 897760 A1 (KELLEY, P.R. et al.), abstract.	1-4, 9-12, 14-16, and 22-24.
Y	Database HCAPLUS, AN 1992:91451, WO 9119480 A1 (HOELGAARD, A.), abstract.	9-12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
07 JUNE 1996

Date of mailing of the international search report
27 JUN 1996

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02341

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-8, 13, 17-21, and 25-34
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02341

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claim(s)1-4, 14-16, 22-24 and 9-12 (in part) , drawn to a hair growth or ungual tissue growth composition in combination with certain media.
Group II, claim(s) 9-12 (in part), drawn to another hair growth or ungual tissue growth composition with growth hormone.

The inventions listed as Groups I-II do not relate to a single inventive concept under PCT Rule 13.1 because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons: The inventions are not so linked as to form a single general inventive concept. Applicant has claimed multiple distinct composition products with varying uses, each of which represents a separate invention. See 37 C.F.R. 1.475(d).