Chapter-2

Review of Literature
Literature Review

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Hair cycle and parts of murine skin:

Paus et al in 1994 explored distribution and hair cycle-dependent numerical changes of gamma-delta T cells in murine skin. Gamma-delta T cells (gdTC) are recognized as the predominant intraepidermal T-cell population in murine skin, although their physiological functions are still unclear [173]. Little is known of the exact distribution of gdTC in the other epithelial skin compartments of normal mice. Using selective gdTC-receptor antibodies in immunohistology (alkaline phosphatase technique), the distribution and density of gdTC was analysed morphometrically in cryostat sections of full-thickness back skin of normal, adolescent C57 BL-6 mice in all the different stages of the depilation-induced hair cycle. It was found that, during the entire hair cycle, V gamma 3-TCR-bearing lymphocytes are restricted to the epidermis, and to the epithelial hair bulb in, and distal to, the bulge area. No gdTC were seen in the sebaceous glands. During early anagen development, the number of pan-gdTC receptor-positive cells increased significantly \((P<0.005)\) in the interfollicular epidermis and the supainfundibular portion of the hair bulb, whereas the number decreased in the infrainfundibular region \((P \leq 0.005)\). As gdTC are thought to migrate into the skin only during embryogenesis, this finding suggests hair cycle-dependent, differential intraepithelial proliferation of gdTC in murine skin. Thus it is concluded that only skin of defined hair cycle stages in immunological studies on murine skin must be used, and the significance of the C57 BL-6 model for assessing the functions of gdTC in skin and hair biology is discussed here.

Paus et al in 1994 investigated restricted and hair cycle-dependent expression of classical and non-classical MHC class I antigens in normal mouse skin [174]. Not all keratinocytes in human and rat hair follicles express MHC class I antigens (MHC I). In the present study, the first immunohistological profile of classical and non-classical MHC I expression in the skin of adolescent C57 BL-6 mice during the induced hair cycle is reported. MHC I immunoreactivity \((H-2^b, H-2D^b)\) is absent in the matrix and inner root sheath of growing (anagen) hair follicles, and the dermal papillae are \(H-2^b\) negative.
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during catagen and telogen. This lack of normal MHC I expression may serve to sequester potentially damaging autoantigens from immune recognition. In addition, the first evidence of non-classical MHC class I antigen expression in normal mammalian skin was presented: during the entire hair cycle, the distal hair follicle shows strong Qa-2 immunoreactivity, which appears to be restricted to an epithelial follicle compartment densely populated by gamma-delta T cells with which Qa-2 molecules may interact as part of a primitive antibacterial defense system of the follicle. The murine hair cycle is an attractive model for dissecting the functional roles of H-2b and Qa-2 molecules in hair biology and in related tissue-interaction systems.

Paus et al in 1994 conducted correlation of proteolytic activities of organ cultured intact mouse skin with defined hair cycle stages [175]. The cyclic growth activity of the hair follicle is characterized by substantial remodelling of the extracellular matrix, yet, little is known about the proteolytic activities regulating this process. In murine skin, hair cycle is highly synchronized and is associated with dramatic remodelling of all skin compartments. Proteolytic activities of murine skin from various stages of the depilation-induced hair cycle have been assessed in this pilot study. The defined proteolytic activities displayed by organ cultured intact mouse skin differ between hair cycle stages. The C57BL/6 mouse offers an attractive model for dissecting and manipulating hair cycle-associated proteolysis in a physiologically relevant system.

Paus et al in 1994 examined mast cell involvement in murine hair growth [30]. Using the murine hair cycle as a model, the number, localization, and granulation status of skin MC during the hair cycle of C57BL/6 mice were studied. Shortly after the induction of hair growth (anagen) on the back skin of mice with resting (telogen) follicles, a sharp decline in the number of Giemsa-stainable MC was detected by morphometry. This was evident in depilation-induced, pharmacologically induced, and spontaneous anagen. By light and electron microscopy, the anagen-associated decline was correlated with the occurrence of substantial MC degranulation. In vivo, the IgE-independent MC secretagogues, compound 48/80 and ACTH induced anagen in mouse telogen follicles after intracutaneous administration, while inhibitors of mast cell degranulation (cromoglycate,
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tiacrilast) and antagonists of selected MC products (clemastin, ranitidine, ketanserin) significantly retarded the induced development of anagen follicles in these mice. It is suggested that MC act via their secretory products as stimulators of anagen development in mice and that the murine hair cycle is an excellent model for studying growth regulatory functions of MC in developmentally regulated systems.

McDonagh and Messenger in 1996 identified genetic factor associations including HLA class II in alopecia areata [176]. They suggested candidate cell types including the dermal papilla cells, the keratinocytes of the matrix and presumptive cortex and the hair bulb melanocytes. The pathogenesis was known to involve disturbance of immune function but there is no proof that an autoimmune mechanism is fundamental. They proposed a pathogenetic model incorporating polygenic determination of disease susceptibility and severity with additional, possibly environmental, factors as triggers for disease expression.

Furkert et al in 1997 identified and measured β-endorphin levels in the skin during induced hair growth in mice [177]. β-endorphin peptide in mammalian skin demonstrate significant hair cycle-dependent fluctuations in both the skin concentration and the in situ expression pattern of β-endorphin (sebaceous glands) during the entire murine hair cycle. The observed anagen (growth phase)-associated increase in β-endorphin concentration and its decline during the follicle involution (catagen) or resting (telogen) phase raise the possibility of a regulatory function of this neuropeptide in cyclic changes of skin physiology.

Hofmann et al in 1998 declared that the anagen hair cycle induces systemic immunosuppression of contact hypersensitivity in mice [178]. Contact hypersensitivity (CHS) to picryl chloride (PCI) is depressed in C57BL/6 mice when CHS is induced via early anagen skin. This phenomenon has now been further dissected in vivo. The elicitation phase for CHS was suppressed when anagen was induced 4 days after PCI sensitization of telogen animals. Sensitization of mice via abdominal skin with all hair follicles in telogen, and back skin follicles in anagen, significantly reduced the magnitude
of the ear swelling response. Consecutive applications of two sensitizing doses of hapten, first on induced anagen back skin and then on telogen abdominal skin 7 days later, failed to induce tolerance. Furthermore, spleen cell transfer of sensitized anagen mice into telogen mice did not inhibit CHS response in the recipients. The current study suggests that a temporary state of systemic hyporesponsiveness, mediated, e.g., by hair cycle-dependent production of immunosuppressive cytokines rather than hapten-specific T suppressor cell activities, plays a critical role.

Rokhsar et al in 1998 reviewed the efficacy and safety of the use of topical sensitizers in the treatment of alopecia areata [179]. It has been more than 2 decades since the first report of the use of dinitrochlorobenzene to induce hair growth in 2 patients with alopecia areata. Other topical sensitizers, namely squaric acid dibutylester and diphenylcyclopropenone, have been used with variable success.

Slominski et al in 1998 investigated hair cycle-dependent production of ACTH in mouse skin [180]. The functional determinants of the cutaneous expression of elements of the hypothalamic-pituitary-adrenal axis were determined. Here, the presence of adrenocorticotropin (ACTH) peptide in skin of C57/BL6 mouse was demonstrated by reversed-phase HPLC analysis combined with specific radioimmunoassay. ACTH concentration that was low in telogen, increased during anagen in two steps: a rapid phase in anagen I, and a slower rise that reached its peak in anagen VI. Immunofluorescence localized the ACTH antigen to the basal layer of epidermis, outer root sheath of hair follicle and subcutaneous muscle of anagen VI skin. At physiological plasma concentration (10^{-6} M), ACTH selectively stimulated DNA synthesis in dermis, while pharmacological doses (10^{-7}-10^{-6} M) inhibited DNA synthesis in both dermis and epidermis. In conclusion, it was suggested that local production of ACTH may represent a regulatory element in the control of skin functions including hair growth.

Botchkareva et al in 1999 found a role for p75 neurotrophin receptor in the control of hair follicle morphogenesis [181]. During hair follicle (HF) morphogenesis, p75 neurotrophin receptor (p75NTR) reportedly is the first growth factor receptor found to be
expressed by those fibroblasts that later develop into the dermal papilla (DP) of the HF. However, the functional role of p75NTR in HF morphogenesis is still unknown. Studying HF development in fetal and neonatal C57BL/6 murine back skin, it is shown that p75NTR-immunoreactivity (IR) is prominently expressed by DP fibroblasts as well as by skin nerves during the early steps of HF development. In contrast, p75NTR-IR disappears from the DP in the fully developed HF and it is expressed only in the epithelial outer root sheath of the HF. Compared to age-matched wild-type animals, p75NTR knockout (−/−) mice show significant acceleration of HF morphogenesis, and DP fibroblasts of p75NTR knockout mice show reduced proliferative activity in situ, indicating alterations in their transition from proliferation to differentiation. Although no significant differences in the expression of adhesion molecules (NCAM), selected morphogens (TGFβ-2, HGF/SF, FGF-2, KGF), or their receptors (TGFβR-II, m-met, FGFR-1) were seen between DP of p75NTR knockout and wild-type mice, p75NTR mutants showed a prominent upregulation of FGFR-2, a high-affinity receptor for KGF, in both follicular DP and epithelium. Furthermore, the administration of anti-KGF neutralizing antibody significantly inhibited acceleration of HF morphogenesis in p75NTR knockout mice in vivo. These observations suggest that p75NTR plays an important role during HF morphogenesis, functioning as a receptor that negatively controls HF development, most likely via alterations in DP fibroblast proliferation/differentiation and via downregulation of KGF/FGFR-2 signaling in the HF.

Foitzik and Dotto et al in 1999 theorized that TGF-β2 isoform is both a required and sufficient inducer of murine hair follicle morphogenesis [182]. Hair follicle development serves as an excellent model to study control of organ morphogenesis. Three specific isoforms of TGF-β exist which exhibit a distinct pattern of expression during hair follicle morphogenesis. To clarify the still elusive role of these factors in hair follicle development, a combined genetic and functional approach was used: analysis of hair follicle development in mice with disruptions of the TGF-β1, 2, and 3 genes was coupled with a direct functional test of the effect of added purified factors on fetal hair follicle development in skin organ cultures. TGF-β2 null mice exhibited a profound delay of hair follicle morphogenesis, with a 50% reduced number of hair follicles. In contrast to hair
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Follicle development, growth and differentiation of interfollicular keratinocytes proceeded unimpaired. Unlike TGF-β2−/− mice, mice with a disruption of the TGF-β1 gene showed slightly advanced hair follicle formation, while lack of the TGF-β3 gene did not have any effects. Treatment of wild-type, embryonic skin explants (E14.5 or E15.5) with TGF-β2 protein in either soluble form or slow release beads induced hair follicle development and epidermal hyperplasia, while similar TGF-β1 treatment exerted suppressive effects. Thus, the TGF-β2 isoform plays a specific role, not shared by the other TGF-β isoforms, as an inducer of hair follicle morphogenesis and is both required and sufficient to promote this process.

McMichael in 2000 stated that in the new millennium, more focus will be on the hormonal modulation of hair loss in women [183]. With the research interest in the United States in women's health and the aging population, drug therapies will be geared towards the active healthy woman with hair loss. There will be improved specificity of the future drugs modeled after the 5-alpha-reductase inhibitors, with more specificity in enzyme activity and improved efficacy. The new millennium will see laser hair removal that is a treatment for all skin types, locations on the body, with improved hair targeting and less generalized damage to skin cells. The psychological stigma associated with hair loss or with unwanted hair may remain, but dermatologists will have the tools to offer patients superior alternatives to reduce these stigmas.

Mecklenburg et al in 2001 explored the role of foxn1 in hair follicle development and cycling [184]. The original nude mouse mutation has proven to be an incredibly valuable biomedical tool since its discovery in 1966. Initially its value was as a tool to study the immune system. The immunodeficiency in this mutant mouse made nude mice valuable as hosts for xenografts, primarily for cancer research. More recently, the most obvious clinical feature of this mutant mouse, lack of hair, has been capitalized on to define the role of Foxn1 in normal and pathological skin. The original nude mouse mutation has proven to be an incredibly valuable biomedical tool since its discovery in 1966. Initially its value was as a tool to study the immune system. The immunodeficiency in this mutant mouse made nude mice valuable as hosts for xenografts, primarily for cancer research.
More recently, the most obvious clinical feature of this mutant mouse, lack of hair, has been capitalized on to define the role of Foxn1 in normal and pathological skin and hair follicle physiology.

Yano et al in 2002 analysed the expression of cutaneous lymphocyte-associated antigen on the peripheral blood and cutaneous lymphocytes of alopecia areata patients [185]. Alopecia areata has been reported to be accompanied by abnormal autoimmune dysfunction. A chronological study showed that the percentage of CLA-positive peripheral blood lymphocytes, CD4+ or CD8+ lymphocytes decreased in parallel with the patients’ good clinical course. The CLA-positivity in peripheral blood lymphocytes, CD4+ or CD8+ lymphocytes of patients with alopecia areata who did not respond to oral corticosteroid therapy remained higher than in those who responded well to the treatment. In the affected scalp skin, many infiltrating lymphocytes around the hair follicles, which were CD4+ or CD8+ lymphocytes, expressed CLA. These findings suggest that the CLA-positivity correlates with clinical activity and that CLA-positive CD4+ or CD8+ lymphocytes may play an important role in alopecia areata.

Paus et al in 2005 studied how selected tissue sites establish immune privilege (IP) [186]. IP is of interest to both basic immunology and clinical medicine: it provides novel insights into autoimmunity, fetal and allograft rejection and tumor escape from immuno surveillance. Here, it was reviewed why the hair follicle can serve as a uniquely accessible, widely available and instructive model for studying the establishment, maintenance, collapse and restoration of IP. The hair follicle epithelium rhythmically generates, maintains and deconstructs an area of relative IP, characterized by very low expression of MHC I -A and suppressed MHC II-dependent antigen presentation, accompanied by the local production of potent immuno suppressants capable of down regulating MHC I (e.g. transforming growth factor-beta1, alpha-melanocyte-stimulating hormone). The physiological functions of hair follicle IP, illustrate its clinical and therapeutic relevance by focusing on alopecia areata, an autoimmune hair loss disorder, and outline important unanswered questions for future research into one of nature's most intriguing and abundant, yet commonly ignored sites of IP.
Tosti et al in 2006 conducted a long term follow up study of alopecia [187]. The purpose of this study is to better assess the long-term evolution of AA and the possible relationship between disease severity and treatment response with long-term prognosis. In children, however, this trend was not statistically significant. Patients with severe AA who responded to topical immunotherapy seem to have a better prognosis than nonresponders. Severity of AA at time of first consultation is an important prognostic factor. Response to therapy (topical immunotherapy) may be associated with better prognosis. In children, the prognosis is worse; the study found that AA worsens over time.

Types of Alopecia:

Kossard et al in 2005 described progressive frontal hairline recession associated with scarring and frequently involving the eyebrows in postmenopausal women [188]. Progressive frontal fibrosing alopecia is a clinically distinct variant of lichen planopilaris, which affects elderly women. No effective treatment has emerged for this condition but the alopecia may stabilize with time.

Madani and Shapiro in 2000 defined alopecia areata as a nonscarring hair loss condition [189]. Among the many factors under investigation in the pathogenesis of AA, the main areas of concentration have been genetic constitution as well as nonspecific immune and organ-specific autoimmune reactions. Treatment with intralesional corticosteroid injections for localized patchy AA and topical immunotherapy for extensive AA has proven successful in the majority of patients, although all treatments are palliative and do not change the prognosis of the disease.

Lenane et al in 2005 studied congenital alopecia areata [190]. Alopecia areata, the alleged autoimmune process leading to nonscarring hair loss, is not uncommon. It has been classified as an acquired cause of alopecia; however, recently it has been reported in the neonatal period. 4 cases of congenital alopecia areata with follow-up from 3 to 5 years were reported. The diagnosis was made clinically in all cases. All patients had
prolonged periods of quiescence of hair loss ranging from 6 to 24 months. Treatments used included minoxidil 2% and a range of topical steroids including hydrocortisone 1%, betamethasone valerate 0.05%, fluocinonide 0.05%, and clobetasol propionate 0.05%. The best regrowth observed resulted from the use of clobetasol propionate 0.05%, giving full regrowth in 50% of those treated. Alopecia areata can occur at all ages and, thus, can be classified as both an acquired and a congenital disorder resulting in hair loss.

Lew et al in 2009 determined the clinical course and prognosis of Acute Diffuse and Total Alopecia (ADTA) through precise clinical observations [191]. The histopathology of lesion revealed infiltration of mononuclear cells around the hair follicles and prominent pigment incontinence. Patients experienced hair regrowth within about 6 months, without regard to the method of treatment. These cases can be categorized as having “acute diffuse and total alopecia,” a new subtype of AA that is associated with a favorable prognosis and rapid and spontaneous recovery even without treatment.

Barahmani et al in 2009 investigated the association between history of atopy or autoimmune diseases and risk of Alopecia Areata [192]. The analysis revealed that a history of atopy and autoimmune disease was associated with an increased risk of AA and that the results were consistent for both the severe subtype of AA (i.e. alopecia totalis and alopecia universalis) and the localized subtype (i.e. AA persistent).

Duque- Estrada et al in 2009 studied the possible risks of mesotherapy as a therapeutic technique for hair loss [193]. Mesotherapy has recently become an advertised method for the treatment of different types of alopecia despite the lack of any data regarding its efficacy and possible side effects. The substances injected into the scalp include “cocktails” of natural plant extracts, homoeopathic agents, vitamins, vasodilators, and drugs that may stimulate hair growth, such as finasteride and minoxidil. In one patient, alopecia developed after injections of the heparinoid vasodilator mesoglycan; the 3-month follow-up examination revealed a small residual area of cicatricial alopecia. Another patient developed reversible alopecia after multiple scalp injections of homeopathic agents.
Welsh and Guy in 2009 explored experiences of individuals living with alopecia areata and alopecia universalis and investigated their accounts of adjusting to, and coping with, such conditions [194]. Whilst previous research has primarily focused on the adverse psychosocial impact of alopecia, this investigation used Interpretive Phenomenological Analysis to provide a more holistic perspective. Results revealed that strategies used by participants evolved over time and that there were clear gender differences. In the early stages, participants did not want to contemplate that their hair loss would be lasting and managed the condition via concealment. Later coping strategies reflected an embodied acceptance with participants managing the effects of AA/AU and becoming more optimistic about living with the condition.

Harries et al in 2009 studied primary cicatricial alopecia (PCA) representing uncommon inflammatory disorder that results in permanent loss of scalp hair [195]. Cutaneous autoimmunity, most prominently chronic cutaneous lupus erythematosus (CCLE), can result in this kind of scarring hair loss. Hair follicle (HF) cycling and regeneration are abolished in PCA due to irreversible, epithelial hair follicle stem cell (eHFSC) damage, triggered by major, yet unclear pro-inflammatory events (e.g. type I interferon-associated cytotoxic inflammation, loss of HF immune privilege, loss of immunosuppressive “no danger” signals). Therefore, immuno-protection of eHFSC and restitution of their immune privilege are attractive future therapeutic strategies in PCA. Chronic cutaneous lupus erythematosus-associated PCA may serve as a model system for other diseases where epithelial stem cells undergo immuno-destruction.

Treatments for Alopecia:
Happle and Echternacht in 1977 evaluated induction of hair growth in alopecia areata with dinitrochlorobenzene (D.N.C.B.) [196]. 43 patients with alopecia areata were treated with weekly applications of D.N.C.B. dissolved in acetone, on one side of the head, with the other side serving as control region. The therapeutic aim was a mild contact dermatitis. A significant difference of hair growth between the treated and untreated sides was observed in 33 patients. 21 patients showed regrowth of hair exclusively on the
treated side, and in 12 patients' regrowth was considerably faster and denser on the
treated side. In the majority of patients the difference was noted within 3 months.

Mitchell and Krull in 1984 researched alopecia areata and its pathogenesis and
treatment [197]. Although its etiology remains unknown, evidence has accumulated to
support an autoimmune pathogenesis for alopecia areata. This review summarizes the
immunologic data and also examines the role of genetics, atopy, and psychological stress
in this disorder. Until etiology is better understood, treatments for alopecia areata are
likely to remain palliative. Nevertheless, newer therapies such as photochemotherapy,
topical immunotherapy, and perhaps systemic immunotherapy (e.g., inosiplex) offer new
hope for patients with extensive disease.

Case et al in 1984 investigated topical therapy of alopecia areata with squaric acid
dibutylester [198]. The efficacy of the topical allergen squaric acid dibutylester (SADBE)
was investigated in the treatment of twenty-six patients with alopecia areata or alopecia
totalis. The patients had their disease for a mean duration of 8.3 years (range, 3 months to
27 years). Sensitization was attempted with 2% SADBE in acetone. Five individuals
could not be sensitized. The twenty-one sensitized patients were treated with topical
applications of 0.001% to 1.0% SADBE in acetone adjusted to produce and maintain
dermatitis for an average of 21 weeks. Eleven (52%) had excellent responses consisting
of complete regrowth in six and cosmetically acceptable regrowth in five. The average
treatment time for regrowth was 11 weeks (range, 5-20 weeks). Ten (48%) had no
clinical response after an average of 19 weeks of therapy (range, 4-42 weeks). Topical
SADBE is an effective therapy for some patients with long-standing alopecia areata.

Tosti et al in 1986 reported therapies versus placebo in the treatment of patchy alopecia
areata [199]. The results of a controlled trial on 119 patients affected by patchy alopecia
areata involving less than 40% of the scalp were reported. The statistical analysis showed
no differences between the results obtained using squaric acid dibutylester,
diphenycprone, minoxidil, and placebo in the treatment of this form of alopecia.
Fiedler-Weiss et al. in 1986 evaluated the efficacy of topical minoxidil solution (1% and 5%) in the treatment of alopecia areata [200]. Topical minoxidil solution can induce hair regrowth in alopecia areata. A dose-response effect was demonstrated when 48 patients treated with topical 1% minoxidil were compared with 47 patients treated with topical 5% minoxidil. A total of 66 patients were enrolled, 26 of them participating in both study groups. Patients with extensive (75% or greater) scalp hair loss showed a response rate of 38%, defined as terminal hair regrowth, with 1% minoxidil versus an 81% response rate with 5% minoxidil, the current 2% formulation is most likely to elicit cosmetically acceptable regrowth in those with patchy alopecia areata. Occlusion of the treated area appears to be necessary to achieve and maintain maximum results. Non responders are most likely to be found among those with the most extensive scalp hair loss. No other clinical features correlate with response to treatment. However, a finding of increased T cell blastogenesis before treatment may predict response. In patients with severe alopecia areata, hair loss generally recurs after treatment is stopped and may recur during treatment. Systemic absorption of topically applied and occluded minoxidil solutions (1% and 5%) was minimal; no clinically significant changes in blood pressure, weight, cardiovascular status, electrocardiogram, electrolytes, complete blood count, or urinalysis were seen. Mild local irritation occurred, and two of the 66 patients developed allergic contact dermatitis to minoxidil, as confirmed by patch tests.

Price in 1987 conducted a double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata [201]. Effective treatment of female androgenetic alopecia involves cessation of hair shedding and promotion of normal anagen hair growth. The topical use of hormones such as progesterone or an androgen receptor-binding drug such as spironolactone has not been associated with significant hair regrowth. In contrast, the topical use of minoxidil has resulted in decreased hair shedding and hair growth promotion, particularly in men with androgenetic alopecia. To investigate the usefulness of topical minoxidil therapy in female androgenetic alopecia, the efficacy and safety of 3% topical minoxidil in 25 affected women was studied. Results were correlated with disease extent and activity.
Fiedler-Weiss in 1987 assessed potential mechanisms of minoxidil-induced hair growth in alopecia areata [202]. In vivo, topical minoxidil therapy is associated with changes in the follicular epithelium, tissue and blood lymphocyte populations, lymphocyte blastogenic response to mitogens, and perifollicular vasculature. Biopsy specimens taken from areas of terminal hair regrowth show a dose-dependent increase in hair follicle length, a decrease in tissue lymphocyte populations associated with a simultaneous increase in peripheral blood lymphocyte counts, and reopening of previously closed lumina of perifollicular vessels. Responder lymphocytes show pretreatment-increased concanavalin A and phytohemagglutinin-induced blastogenesis, which decrease toward control values after treatment. In vitro, at concentrations approximating the range of tissue levels in patients treated topically with the 5% solution, minoxidil affects both epithelial cells and lymphocytes in tissue culture. Cultured murine epithelial cells show increased cell proliferation and delayed senescence. Cultured human lymphocytes show suppression of mitogen-induced blast transformation. Differential effects on responder, nonresponder, and control lymphocytes are seen. Minoxidil may induce hair regrowth in alopecia areata by a synergistic stimulatory effect on follicular epithelium and a suppressive effect on lymphocyte-mediated immunologic phenomena. A contributing role for its vasodilatory properties must also be considered.

Milgraum et al in 1987 studied alopecia areata, endocrine function, and autoantibodies in patients 16 years of age or younger [203]. Forty-five children with alopecia areata were prospectively studied by means of both clinical and laboratory evaluation for evidence of endocrine diseases and autoantibodies. Twenty-four percent had an abnormality as determined by one or more thyroid function studies (thyroxine, triiodothyronine, and thyroid-stimulating hormone) and/or elevation of microsomal antibody levels. In 16%, smooth muscle antibody was present, and in 4%, parietal cell antibody was present. Routine thyroid function testing is recommended for all children with alopecia areata.

Abell in 1988 characterized histologic response to topically applied minoxidil in male-pattern alopecia [204]. The histopathology of androgenetic alopecia is characterized by
reductions in follicular size and mean hair shaft diameter without significant reduction in follicular density. The proportion of hairs found in anagen is diminished with a corresponding increase in hairs in normal telogen, plus a marked increase in a persistent stage of telogen. The telogen germinal unit is the term given by Headington to the structure that represents a persistence of telogen after shedding of the club hair but in which anagen has not been re-established. Regrowth of scalp hair following topical application of minoxidil has been demonstrated in both primates and humans suffering from androgenetically determined alopecia. As part of a nationwide multicenter trial of topical minoxidil in male-pattern alopecia, scalp biopsy specimens were obtained before and after treatment to determine what histopathologic changes might be found and if this might help us understand the way in which the clinically observed regrowth of hair might occur.

Hordinsky et al in 1988 studied the efficacy of three percent topical minoxidil therapy for female androgenetic alopecia [205]. Effective treatment of female androgenetic alopecia involves cessation of hair shedding and promotion of normal anagen hair growth. The topical use of hormones such as progesterone or an androgen receptor-binding drug such as spironolactone has not been associated with significant hair regrowth. In contrast, the topical use of minoxidil has resulted in decreased hair shedding and hair growth promotion, particularly in men with androgenetic alopecia. To investigate the usefulness of topical minoxidil therapy in female androgenetic alopecia, the researchers studied the efficacy and safety of 3% topical minoxidil in 25 affected women. Results were correlated with disease extent and activity.

Holland in 1988 studied animal models of alopecia [206]. A genetic model of androchronogenic alopecia has yet to be described in the rat or mouse, two of the better characterized species. It may be relevant that the best current animal model of androchronogenic alopecia, the stumpetailed macaque, is a primate. The age of onset and the pattern of hair loss closely resemble human male-pattern baldness and morphologically, as well as mechanistically, may be analogous to the corresponding process in humans. Since genetically controlled regional hairlessness is a phenomenon
relatively unique to *Homo sapiens*, it may be too much to expect to find an analogous process among rodents.

Sawaya in 1998 studied the recent approval in the United States of two new products, Propecia (Merck Co, Rahway, NJ) and Rogaine Extra Strength 5% (Pharmacia & UpJohn Co, Kalamazoo, MI), which promoted scalp hair growth in men [207]. These have added a new dimension to treatment options offered by physicians in treating androgenetic alopecia (AGA). The search for new and effective agents to treat many different hair loss problems has been intensified by the increase in hair biology research taking place worldwide, from university-academic institutions to the pharmaceutical companies. All have a desire to profit from marketing such drugs that have been termed, "cosmeceuticals." Millions of men and women of every race suffer from various forms of alopecia, the most common being AGA where the target tissue active androgen, 5 alpha-dihydrotestosterone (DHT) aggravates genetically programmed scalp hair follicles that results in short, fine, miniaturized hairs. Currently available to treat alopecia are drugs indicated for other disease processes because no other agents are accessible; some have severe side-effects and many are minimally effective. These prescription drugs were not originally indicated for alopecia and have not been adequately tested in controlled clinical trials to assess for efficacy, safety, and toxicity. These agents continue to be used clinically to treat patients with various forms of alopecia. As a result, a variety of new agents are emerging in the patient application process to gain protection and approval specifically for various forms of alopecia. This report reviews the most recently approved products, some of the more promising compounds in clinical trial development, as well as those in the over the counter (OTC) "natural" treatments category.

Bergfeld in 1988 evaluated etiology and diagnosis of androgenetic alopecia [208]. Androgenetic alopecia is the most common form of baldness observed in humans. It involves genetically predisposed individuals from puberty to senescence, with prominent central-pattern scalp alopecia induced by androgens. The many synonyms of androgenetic alopecia include androgenic alopecia, male-pattern baldness, female-pattern baldness, diffuse alopecia, common baldness, hereditary alopecia, and baldness.
Naldi et al in 1990 surveyed the role of topical immunotherapy in the treatment of alopecia areata [209]. A quality analysis of articles was published between January 1977 and January 1988 about three treatments. A survey of clinical trials to assess the scientific evidences presented for the practical use of dinitrochlorobenzene, squaric acid dibutylester, and diphencyprone in the treatment of alopecia areata was conducted. Twenty-six papers published between January 1977 and January 1988, in English, French, and Italian were selected. A standardized protocol of evaluation was used, which focused principally on the reporting of methods. In light of these results, further and better-designed studies are needed for acceptance of dinitrochlorobenzene, squaric acid dibutylester, and diphencyprone in current therapy.

O'goshi K et al in 2000 analyzed the stratum corneum of scalp skin in patients with alopecia areata and androgenetic alopecia [210]. A functional study of the SC of lesional scalp skin of patients with alopecia areata and of patients with androgenetic Alopecia was conducted. The scalp with the cheek and the flexor surface of the forearm (volar forearm) were compared. These characteristics seem to be dependent, at least to some extent, on the amount of sebum-derived skin surface lipids because these were abundant on the scalp skin. Moreover, removal of skin surface lipids led to a significant decrease in skin surface hydration.

Jiang et al in 1995 investigated the effect of topical application of FK506 on the normal hair cycle of C57BL/6 mice [211]. FK506, a macrolide antibiotic, is a T cell specific immunosuppressant. Another T cell specific immunosuppressant, Cyclosporine A, also stimulates hair growth in animals and humans. When telogen mice (7 weeks of age) were treated topically with 1μmol FK506 on days 0 and 3, 50% of the tested mice entered anagen by day 9 and 100% by day 16. With 0.1 μmol of FK 506, 50% tested mice entered anagen by day 13 and 80% by day 19, indicating that the effect of FK506 is dose dependent. Histologic studies revealed that FK506 markedly stimulated the skin and thickened it. The data on hair growth support the contention that FK506 induces early onset of anagen and stimulates hair growth. These results indicate that the hair growth stimulating effect of FK506 is due at least in part to its promoting effect on the hair cycle.
Wade et al in 2002 reported persistent depigmented regrowth after alopecia areata [212]. A case of persistent depigmented hair regrowth from the site of a patch of alopecia areata was reported. It is well known that hair may regrow unpigmented from a site of alopecia areata; however, it was previously thought that this was temporary, lasting no longer than the first hair cycle.

Botchkarev et al in 2006 studied epithelial growth control by neurotrophins [213]. Neurotrophins (NTs) exert many growth-regulatory functions beyond the nervous system. For example, murine hair follicles (HF) show developmentally and spatio-temporally stringently controlled expression of NTs, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4, and their cognate receptors, tyrosine kinase A-C (TrkA-C) and p75 neurotrophin receptor (p75NTR). Follicular NT and NT receptor expression exhibit significant, hair cycle-dependent fluctuations on the gene and protein level, which are mirrored by changes in nerve fiber density and neurotransmitter/neuropeptide content in the perifollicular neural networks. NT-3/TrkC and NGF/TrkA signaling stimulate HF development, while NT-3, NT-4 and BDNF inhibit the growth (anagen) of mature HF by the induction of apoptosis-driven HF regression (catagen). p75NTR stimulation inhibits HF development and stimulates catagen. Since the HF is thus both a prominent target and key peripheral source of NT, dissecting the role of NTs in the control of HF morphogenesis and cyclic remodeling provides a uniquely accessible, and easily manipulated, clinically relevant experimental model, which has many lessons to teach. Given that the most recent data also implicate NTs in human hair growth control, selective NT receptor agonists and antagonists may become innovative therapeutic tools for the management of hair growth disorders (alopecia, effluvium, and hirsutism). Since, however, the same NT receptor agonists that inhibit hair growth (e.g., BDNF, NT-4) can actually stimulate epidermal keratinocyte proliferation; NT may exert differential effects on defined keratinocyte subpopulations. The studies reviewed here provide new clues to understanding the complex roles of NT in epithelial tissue biology and remodeling in vivo, and invite new applications for synthetic NT receptor ligands for the treatment of epithelial growth disorders, exploiting the HF as a lead model.
Literature Review

Langbein and Schweizer in 2005 reviewed keratins of the human hair follicle [214]. Substantial progress has been made regarding the elucidation of differentiation processes of the human hair follicle. This review first describes the genomic organization of the human hair keratin gene family and the complex expression characteristics of hair keratins in the hair-forming compartment. Also, the role and fate of hair keratins in the diseased hair follicle, particularly hereditary disorders and hair follicle-derived tumors are described. A report on the actual state of knowledge concerning the regulation of hair keratin expression is also incorporated in this review.

Connolly et al in 2003 presented disorders of hair in children [215]. The patterns of presentation of pediatric hair problems can be divided into localized absence or localized abnormality of hair from birth, abnormality of hair pattern or character from birth, and acquired disorders resulting in a localized or generalized loss of hair. Hypertrichosis is uncommon in children and as a result will not be discussed in any detail. The evaluation of a child with a hair problem begins with a detailed history, followed by a thorough examination of the scalp, eyebrows, eyelashes, general body hair, nails and teeth. A general physical examination is indicated where the diagnosis is not immediately apparent and localized. Management is dependent on the condition but, in many cases, apart from infections or infestations, treatment options are limited. Often, a ‘wait and see’ approach with reassurance needs to be adopted.

Hrs et al in 2005 researched psychological effect of hair loss [216]. Many people view their hair as an important part of their identity, so hair loss can represent a source of anxiety, depression and loss of self esteem. This problem is of great interest for both pharmaceutical and cosmetic industry. The other substances are Saw palmetto, emu oil, vitamin B, Polysorbate 80, retinoic and azelaic acid. Saw palmetto (*Serenoa repens* L.) is an effective antiandrogen which acts in a similar way that finasteride does. Emu oil is an excellent emollient, with skin cell proliferation properties claimed for it. Vitamin B appears to have an important role in the treatment of androgen related disorders such as acne and androgenetic alopecia. Polysorbate causes the histamine release which is thought to be the main mechanism by which it promotes hair growth. Retinoic acid
Literature Review

(tretinoin) alone or in combination with minoxidil can result in moderate to good hair growth. Azelaic acid is a competitive inhibitor of 5(a)-reductase and is commonly used in the treatment of acne and other skin conditions. Some drugs like cyproterone (progestogen with antiandrogenic properties), spironolactone (potassium sparing diuretic), cimetidine (antiulcer drug) and ketoconazole (antifungal agent) have also antiandrogen activity. In some cases zinc, unsaturated fatty acids, immunosuppressive drugs, irritants and PUVA treatment proved to be useful as well.

Paus in 2006 explored therapeutic strategies for treating hair loss [217]. Here, it was studied why significant progress in the as yet very unsatisfactory pharmacological management of hair loss demands more rational strategies for ‘hair drug’ development, which effectively target defined key events in hair follicle cycling and transformation. Chiefly, drugs need to be identified that serve as inhibitors of catagen, exogen and/or the terminal-to-vellus transformation, or that induce anagen. For this, identification of the relevant molecular controls of human hair follicle cycling is an essential prerequisite.

Tiede et al in 2007 studied hair follicle stem cells [218]. The discovery of epithelial stem cells (eSCs) in the bulge region of the outer root sheath of hair follicles in mice and man has encouraged research into utilizing the hair follicle as a therapeutic source of stem cells (SCs) for regenerative medicine, and has called attention to the hair follicle as a highly instructive model system for SC biology. Under physiological circumstances, bulge eSCs serve as cell pool for the cyclic regeneration of the anagen hair bulb, while they can also regenerate the sebaceous gland and the epidermis after injury. More recently, melanocyte SCs, nestin+, mesenchymal and additional, as yet ill-defined “stem cell” populations, have also been identified in or immediately adjacent to the hair follicle epithelium, including in the specialized hair follicle mesenchyme (connective tissue sheath), which is crucial to wound healing. Thus the hair follicle and its adjacent tissue environment contain unipotent, multipotent, and possibly even pluripotent SC populations of different developmental origin. It provides an ideal model system for the study of central issues in SC biology such as plasticity and SC niches, and for the identification of reliable, specific SC markers, which distinguish them from their
immediate progeny (e.g. transient amplifying cells). The current review attempts to provide some guidance in this growing maze of hair follicle-associated SCs and their progeny, critically reviews potential or claimed hair follicle SC markers, highlights related differences between murine and human hair follicles, and defines major unanswered questions in this rapidly advancing field.

Randall in 2007 evaluated hormonal regulation of hair follicles [219]. Hair's importance for insulation and camouflage or human communication means that hairs need to change with season, age or sexual development. Regular, regenerating hair follicle growth cycles produce new hairs which may differ in colour and/or size, e.g., beard development. Hormones of the pineal-hypothalamus-pituitary axis coordinate seasonal changes, while androgens regulate most sexual aspects with paradoxically different effects depending on body site; compare beard growth and balding. Hormones affect follicular mesenchymal-epithelial interactions altering growing time, dermal papilla size and dermal papilla cell, keratinocyte and melanocyte activity. Greater understanding of these mechanisms should improve treatments for poorly controlled hair disorders, alopecia and hirsutism.

Waters et al in 2007 reviewed hair follicle stem cells [220]. The increasing use of the hair follicle as a stem cell paradigm is due in part to the complex interplay between epithelial, dermal and other cell types, each with interesting differentiation potential and prospective therapeutic applications. This review focuses on research into the environmental niche, gene expression profiles and plasticity of hair follicle stem cell populations, where many recent advances have come about through novel technological and experimental approaches. Major developmental pathways involved in the establishment and control of the epithelial stem cell niche, and evidence of plasticity between stem and transit amplifying cell populations were discussed.

Schlake in 2007 determined the structure and shape of hair [221]. The hair follicle attracted significant attention as a model for the investigation of diverse biological problems. Whereas its morphology and the structure of the hair shaft are known in detail, the molecular biology of this miniorgan is significantly less characterised. Many efforts
Literature Review

focused on the development of the hair follicle and its stem cell reservoir; by contrast, the follicular product, the hair, which is interesting not only in terms of cosmetics, was neglected. This review highlights the current knowledge of the control of hair structure and shape with emphasis on mouse hair follicle biology and discusses continuing problems.

Paus et al in 2008 studied neuro-endocrinology of epithelial hair follicle stem cells [222]. The hair follicle is a repository of different types of somatic stem cells. However, even though the hair follicle is both a prominent target organ and a potent, non-classical site of production and/or metabolism of numerous polypeptide- and steroid hormones, neuropeptides, neurotransmitters and neurotrophins, the (neuro-) endocrine controls of hair follicle epithelial stem cell (HFeSC) biology remain to be systematically explored. Focussing on HFeSCs, it was attempted here to offer a “roadmap through terra incognita” by listing key open questions, by exploring endocrinologically relevant HFeSC gene profiling and mouse genomics data, and by sketching several clinically relevant pathways via which systemic and/or locally generated (neuro-)endocrine signals might impact on HFeSC. Furthermore, potential role of nerve growth factor (NGF) and substance P-dependent neurogenic inflammation in HFeSC damage was elaborated here, and how neuroendocrine signals may influence the balance between maintenance and destruction of hair follicle immune privilege was explored, which protects these stem cells and their progeny. These considerations call for a concerted research effort to dissect the (neuro-) endocrinology of HFeSCs much more systematically than before.

Talpur et al in 2009 determined the safety, including the dose-limiting toxicities with adverse events, and efficacy, i.e., response rate, of bexarotene in alopecia areata [223]. Alopecia areata, hair loss caused by perifollicular T-cell infiltrates, is refractory to therapy. Bexarotene, a retinoid X receptor is a selective retinoid, induces T-cell apoptosis. This design could not differentiate between drug-induced and spontaneous regrowth.
Literature Review

Arslan et al in 2009 observed the effects of biotin supplementation on serum and liver tissue biotinidase enzyme activity and alopecia in rats which were administrated to valproic acid [224]. Valproic acid (VPA) is a widely used and well-tolerable antiepileptic drug in epileptic patients. However, VPA has many side effects dose-dependent and non-dose-dependent. It is reported that VPA treatment may lead to biotin deficiency and low serum and liver tissue biotinidase enzyme activity (BEA). Major clinical manifestations in biotin deficiency are seborrheic dermatitis, dry skin, fine and brittle hair, and alopecia.

The effects of biotin supplementation on serum and liver tissue BEA and alopecia during VPA therapy were investigated. There were significant decreases in the levels of serum and liver tissue BEA of the study groups compared with the control group. It was concluded that VPA usage reduced the serum and liver tissue BEA and impaired the biotin utilization by affecting the liver. Partial biotinidase deficiency may lead to alopecia. It might be prevented by biotin supplementation in the patients receiving VPA therapy.

Willemsen et al in 2009 studied increased history of childhood and lifetime traumatic events among adults with alopecia areata [225]. Whether adult alopecia areata is associated with childhood or total lifetime traumatic events is not known. Previous studies have investigated only the relationship with recent stressful events. It was determined that patients with AA experienced more emotionally and physically traumatic events. The study documents an increased history of childhood trauma in patients with AA compared with control subjects.

Humbert in 2009 noted that alopecia is one of the most dreaded side effects of chemotherapy, notably by women with breast cancer [226]. It entails a deterioration of body image resulting in a negative selfimage and woman's femininity suffers. Current methods to prevent chemically induced alopecia are insufficiently effective and inconvenient. It has recently been shown that the oral administration of L-cystine combined with pyridoxin could prevent alopecia induced by tobacco in mice. Alopecia induced by tobacco involves apoptosis mechanisms as in drug-induced alopecia. This study showed that the apoptosis induced by doxorubicin was significantly reduced in
presence of the cystine + pyridoxin and that the glutathione rate was significantly higher in presence of cystine.

Garg et al in 2009 described alopecia areata as a common condition causing non-scarring hair loss [227]. It may be patchy, involve the entire scalp (alopecia totalis) or whole body (alopecia universalis). Alopecia areata can cause great psychological distress, and the most important aspect of management is counseling the patient about the unpredictable nature and course of the condition as well as the available effective treatments, with details of their side effects. Although many treatments have been shown to stimulate hair growth in alopecia areata, there are limited data on their long-term efficacy and impact on quality of life. Here the evidence for the following commonly used treatments: corticosteroids (topical, intralesional, and systemic), topical sensitizers (Diphenyl cyclopropenone), psoralen and ultraviolet A phototherapy (PUVA), minoxidil and dithranol was reviewed.

Trüeb in 2009 studied the emotional distress caused by chemotherapy-induced alopecia (CIA) [228]. The incidence and severity of CIA are variable and related to the particular chemotherapeutic protocol. CIA is traditionally categorized as acute diffuse hair loss caused by dystrophic anagen effluvium; however, CIA presents with different clinical patterns of hair loss. When an arrest of mitotic activity occurs, obviously numerous and interacting factors influences the shedding pattern. The major approach to minimize CIA is by scalp cooling. Several experimental approaches to the development of pharmacologic agents are under evaluation and include drug-specific antibodies, hair growth cycle modifiers, cytokines and growth factors, antioxidants, inhibitors of apoptosis, and cell-cycle and proliferation modifiers. Among the few agents that have been evaluated so far in humans, AS101 and minoxidil were able to reduce the severity or shorten the duration of CIA, but could not prevent CIA.

Bíró et al in 2009 explored the endocannabinoid system of the skin in health and disease [229]. The newly discovered endocannabinoid system (ECS; comprising the endogenous lipid mediators’ endocannabinoids present in virtually all tissues, their G-protein-coupled
cannabinoid receptors, biosynthetic pathways and metabolizing enzymes) has been implicated in multiple regulatory functions both in health and disease. Recent studies have intriguingly suggested the existence of a functional ECS in the skin and implicated it in various biological processes (e.g. proliferation, growth, differentiation, apoptosis and cytokine, mediator or hormone production of various cell types of the skin and appendages, such as the hair follicle and sebaceous gland). It seems that the main physiological function of the cutaneous ECS is to constitutively control the proper and well-balanced proliferation, differentiation and survival, as well as immune competence and/or tolerance, of skin cells. The disruption of this delicate balance might facilitate the development of multiple pathological conditions and diseases of the skin (e.g. acne, seborrhea, allergic dermatitis, itch and pain, psoriasis, hair growth disorders, systemic sclerosis and cancer).

Datta et al in 2009 investigated the efficacy of methanol extract of Eclipta alba as hair growth promoter [230]. The methanol extract of whole plant when tested for hair growth promoting potential, exhibited dose dependent activity in C57BL6 mice. The activity was assessed by studying the melanogenesis in resected skin, follicle count in the subcutis, skin thickness and surrogate markers in vehicle control and extracts treated animals. These findings suggest that methanol extract of Eclipta alba may have potential as a hair growth promoter.

Barahmani et al in 2010 determined whether serum cytokine profiles define the severity of the AA phenotype or are affected by co-existent atopy [231]. Alopecia areata (AA) is an organ-specific autoimmune disease characterized by folliculotrophic T-cell infiltrates around anagen-stage hair follicles. In the experiment a total of 17 serum cytokines were measured and compared in 269 patients with AA of varying severity with and without atopy and 18 unrelated controls. Of the 269 patients, 96% had active disease and 54% were atopic. Levels of Th1, interleukin (IL)-1 receptor antagonist (ra) and IL-8 levels were higher in all patients with AA than in controls. IL-1α, IL-12 and tumour necrosis factor-α levels were higher in patients with AA and atopy than in patients with AA without atopy. It was thus concluded that increased Th1 serum cytokines (IL-2, IL-12 and
interferon-γ) and IL-1ra levels are associated with AA regardless of disease severity or the presence of atopy.

Petukhova et al in 2010 studied genome-wide association in alopecia areata and found that it implicates both innate and adaptive immunity [232]. Alopecia areata (AA) is among the most highly prevalent human autoimmune diseases, leading to disfiguring hair loss due to the collapse of immune privilege of the hair follicle and subsequent autoimmune attack. The genetic basis of AA is largely unknown. A genome-wide association study (GWAS) in a sample of 1,054 cases and 3,278 controls was conducted and 139 single nucleotide polymorphisms were identified that are significantly associated with AA ($P \leq 5 \times 10^{-7}$). Here an association with genomic regions containing several genes controlling the activation and proliferation of regulatory T cells ($T_{\text{reg}}$ cells), cytotoxic T lymphocyte-associated antigen 4 ($CTLA4$), interleukin ($IL$)-2/$IL$-21, IL-2 receptor A ($IL$-2RA; $CD25$) and Eos (also known as Ikaros family zinc finger 4; $IKZF4$), as well as the human leukocyte antigen (HLA) region was studied. This study provides evidence for the involvement of both innate and acquired immunity in the pathogenesis of AA. Here, the genetic underpinnings of AA have been succinctly defined, and placed within the context of shared pathways among autoimmune diseases, and a novel disease mechanism, the upregulation of ULBP ligands, in triggering autoimmunity has been uncovered.

Sundberg et al in 2011 stated that primary follicular dystrophy with scarring dermatitis in C57bl/6 mouse substrains resembles central centrifugal cicatricial alopecia in humans [233]. A number of C57BL/6 (B6) substrains are commonly used by scientists for basic biomedical research. One of several B6 strain-specific background diseases is focal alopecia that may resolve or progress to severe, ulcerative dermatitis. Clinical and progressive histologic changes of skin disease commonly observed in C57BL/6J and preliminary studies in other closely related substrains are presented. Lesions develop due to a primary follicular dystrophy with rupture of severely affected follicles leading to formation of secondary foreign body granulomas (trichogranulomas) in affected B6 substrains of mice. Histologically, these changes resemble the human disease called
central centrifugal cicatrical alopecia (CCCA). Four B6 substrains tested have a polymorphism in alcohol dehydrogenase 4 (Adh4) that reduces its activity and potentially affects removal of excess retinol. Using immunohistochemistry, differential expression of epithelial retinol dehydrogenase (DHRS9) was detected, which may partially explain anecdotal reports of frequency differences between B6 substrains. The combination of these 2 defects has the potential to make high dietary vitamin A levels toxic in some B6 substrains while not affecting most other commonly used inbred strains.
Literature on Leeches.

Leeches are segmented, eucocelomate worms of the phylum Annelida, distinguished from other annelids by anterior and posterior suckers used for locomotion and feeding — on blood or soft body parts of other animals. Leeches are mainly aquatic, but the Asian land leeches are also well known. Potent anticoagulants are secreted to assist in feeding.

Weisblat in 2003 observed that leeches also prey on invertebrates, including crustaceans, snails and other annelids [234].

Whitaker et al in 2004 in a historical article on Hirudo medicinalis, wrote about its ancient origins, and trends in the use of medicinal leeches throughout history [235]. Exploring the relationship between Hirudo Medicinalis and the plastic surgeon, he stated that medicinal leech therapy such as bloodletting is an ancient craft that dates back to ancient Egypt and the beginnings of civilization. Their popularity has varied over the years, reaching such a peak in Europe between 1825 and 1850 that supplies were exhausted. Towards the end of the century they fell out of favor and, during this period, the leech, once used by the physicians of emperors and influential academic surgeons, became associated with lay therapists and quackery. Leeches have enjoyed a renaissance in reconstructive microsurgery during the last 15 years, having been used by maxillofacial [236] and other reconstructive surgeries to aid salvage of compromised microvascular for tissue transfers [237], replanted digits [238], lips [239, 240] and nasal tips [241]. Peer-reviewed evidence suggests that the survival of compromised, venous-congested tissues is improved by early application of a leech [242, 243, 244]. Leeches have also recently been used to treat a wide range of conditions, including periorbital haematomas [245], severe macroglossia [246, 247] and purpura fulminans [248]. Use of leech was first reported in the British Journal of Plastic Surgery in the year 1960.

Leeching is considered by many to be a discredited medical relic of the past. This view is not justified as leeches still play an important part in modern medicine, in microsurgery and in the treatment of patients with post-phlebitic syndrome.
In the search for other antihaemostatic factors in *Hirudo medicinalis* saliva, inhibitors of platelet aggregation induced by thrombin, collagen, adenosine 5'-diphosphate, epinephrine, platelet-activating factor and arachidonic acid were found. Apyrase (adenosine 5'-diphosphate diphosphohydrolase) was purified and analysed, which is a non-specific inhibitor of platelet aggregation by virtue of its action on adenosine 5'-diphosphate [Fig 7]. An inhibitor of coagulation factor Xa from leech saliva was isolated and characterized by Eldor *et al* in 1996 [249].

The spreading properties of leech extracts and the formation of lymph were initially explored by Claude in 1937 [250]. It was concluded that the injection of leech extracts into the skin increases its permeability, as shown both by the spread of fluid and of foreign particles through the dermis. The spread is followed some hours after the injection by more or less edema of the subcutaneous tissue.

Dickinson in 1890 studied the effect of leech extract on blood [251]. It was established by Haycraft that a watery extract of the anterior part of the medicinal leech has, when mixed with shed blood or injected into the circulation, a strong delaying or preventive action upon clotting takes place. Leech-extract is equally efficacious whether injected into the circulation or mixed with the blood as it flows from the vessels. Hence there seems to be ground for concluding that cell-globulin may be deprived of fibrino-plastic power without alteration in its physico-chemical qualities.
Literature Review

Markwardt in 1955 isolated a protein, hirudin from *Hirudo medicinalis* and demonstrated its thrombin inhibitor effect [252-254]. As a heparin-like substance, it is the most potent known natural inhibitor of thrombin. Due to its high affinity for thrombin, hirudin (Fig 7) inhibits almost all the physiological actions of thrombin. It does not cross-react *in vitro* with antibodies from patients with heparin-induced thrombocytopenia. In fact, its administration has exhibited no side effects, including effects on platelets. Hirudin has been cloned and is used in the treatment of cardiological and hematological disorders.

A thrombin inhibitor similar to hirudin, known as bufrudin, has been isolated from *Hirudo manillensis*, which differs in its structural and immunological properties [253].

Another component of leech saliva, Destabilase possesses glycosidase activity. Destabilase lysozyme is the first invertebrate lysozyme with combined enzymatic and non-enzymatic antibacterial action [254], and it also dissolves blood clots [255].

Hyaluronidase is another important moiety in the leech saliva. It is a spreading or diffusing substance that modifies the permeability of connective tissue through the hydrolysis of endoglucuronidic linkages of hyaluronic acid – a polysaccharide found in the intercellular ground substance of connective tissue [256]. The leech enzyme, although a P-glucuronidase, does not act on chondroitin or chondroitin sulfates A and C, which contain glucuronic acid but differ in the amino sugar from hyaluronate. Hyaluronidase from the leech, therefore, is the most specific enzyme known for identification of hyaluronic acid [257]. It reduces the viscosity and renders the tissues more readily permeable to injected fluids [257-261], increasing the speed of absorption. This promotes absorption of excess fluids and extravasated blood in the tissues and increases the effectiveness of local anesthesia. Hence, hyaluronidase from leech saliva helps increase the spread of all salivary secretions. Currently, it is being examined *in ex vivo* studies for drug delivery through human skin [262]. It has also been investigated as an additive to chemotherapeutic drugs for augmentation of the anticancer effect [258-261].
Gelin is a potent thrombin inhibitor analogous to eglin, and is isolated from the saliva of the *Hirudinaria manillensis*, a leech belonging to the same family as *Hirudo medicinalis*. Like eglin, gelin inhibits elastase, cathepsin G, and chymotrypsin, but has little or no activity on plasmin, thrombin, pepsin, or trypsin [263].

Probably the most important use of leeches has been the discovery of Antistasin. Antistasin has been proven to possess marked antimetastatic properties. Metastatic tumour spread is correlated to abnormal blood coagulation, possibly due to direct activation of Factor X by tumour cells. Moreover, since the deposition of fibrin provides tumour cells with a protective covering, impermeable to the immune system, the effect of antistasin on tumour spread might be exerted via Factor Xa inhibition [264-266].

Antistasin and ghilanten are potent specific inhibitors of the blood coagulation Factor Xa. In this respect selective Factor Xa inhibition by recombinant antistasin: prevents vascular graft thrombosis in baboons and rabbits, accelerates reperfusion and prevents re-occlusion in a canine model of femoral arterial thrombosis, reduces restenosis after balloon angioplasty of atherosclerotic femoral arteries in rabbits and affects the mitosis of cultured aortic smooth muscle cells [264-266].

During 1984-1989, it was experimentally proven that intravenous application of a compound of medicinal leech salivary glands to rats with experimentally-induced atherosclerosis, reduced atherosclerosis in abdominal and lung arteries.

Chalisova et al in 2003 proved that Destabilase and bdellin B to have neurite stimulating effects on spinal ganglia [267]. Members of the serine protease family, such as plasminogen activator, are known to have neurite stimulating activity in nervous tissue cultures. Some serine inhibitors (serines) have neurotrophic properties while, hirudin increases neurite growth in neuroblastoma, hippocampus and sympathetic ganglion cultures. Recent studies showed that an extract from the cephalic region of lyophilized leeches has marked neurite-stimulating activity in organotypic cultures of sensory
neurons of chick embryo. This activity may occur because of the presence of proteases and their inhibitors in the medicinal leech salivary gland secretions.

Gasic et al. in 1984 assessed leech salivary gland extract (SGE) from *Haementeria officinalis* was found to be a potent inhibitor of cyclophosphamide- and radiation-induced artificial metastasis enhancement [268]. Studies were designed to determine whether salivary gland extract (SGE) from the leech *H. officinalis* could inhibit enhancement of lung tumor colonization induced by pretreatment of mice with cyclophosphamide (CY) or local thoracic irradiation (LTI). SGE was similarly effective in inhibiting the enhancement of lung colonization when given before or after cytotoxic agents. In normal mice, the SGE was active when given on the day or 1 day before but not when given 4 days before tumor cells. The antimetastatic effect of SGE was ascribed to its anti-platelet-aggregating, anticoagulant, and antiproteolytic enzyme activities.

Righbi et al. in 1987 reported that leech saliva inhibits superoxide production by neutrophils stimulated by tetradecanoyl phorbol acetate or polyhistidine [269]. It was also observed that leech saliva led to inhibition of platelet aggregation and of leukocyte activity induced by collagen, ADP and epinephrine.

Orevi et al. in 1992 reported prevention of protein platelet aggregation inhibitors and a range of selective low molecular weight (LMW) aggregation inhibitors by leech saliva[270]. Gel filtration on Bio-Gel P-2 (cut-off kDa) yielded a protein fraction (Fr. I) and three LMW fractions (Fr II, III, and IV). Fr. I inhibit aggregation induced by collagen, ADP, epinephrine and arachidonic acid. Of all the fractions, only one, Fr. II (LMW) specifically inhibits aggregation induced by platelet activating factor (PAF, 1-0-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine). Fr. II also inhibits thrombin-induced platelet aggregation. Fr. III inhibits aggregation induced by ADP, epinephrine and arachidonic acid, and Fr. IV induced by arachidonic acid. Fr. II also inhibits PAF- and thrombin-induced thromboxane generation in platelets, but does not inhibit arachidonic acid-induced thromboxane generation. The inhibition may be due to a single inhibitor, though it may also be due to several inhibitors. Fr. II also inhibits superoxide anion.
production in formyl Met-Leu-Phe (fMLP) - and ionophore 23187- stimulated neutrophils. This may be due to the inhibition of the effects of PAF generated within the cell.

Neely in 1993 explored the role of substrate and calcium in neurite retraction of leech neurons following depolarization [271]. These results demonstrate the importance of substrate molecules in the responses of growth cones to depolarization and, therefore, in the differentiation of neurons.

Stefano in 1997 identified and characterized the CNS cannabinoid receptor of the leech with coupling to nitric oxide release [272]. The present study demonstrated that stereoselective binding sites for anandamide, a naturally occurring cannabinoid substance, can be found in leech (Theromyzon tessulatum and Hirudo medicinalis) central nervous system. Thus, the leech cannabinoid receptor may be a G-protein coupled receptor with seven transmembrane domains as in CB1R.

Zipser et al in 1998 determined that cholesterol and its derivatives are the principal steroids isolated from the leech species Hirudo medicinalis. Steroids were isolated from the blood-sucking leech species Hirudo medicinalis and their structure was studied with one- and two-dimensional NMR spectroscopy (DQF-COSY and HMQC), GC-MS and ESI-MS spectrometry [273]. The fact that cholesterol is the most abundant lipid in leech, comprising of approx 5% of the total leech lipid, suggests that H. medicinalis, a blood sucking leech, has adapted itself fully to its mammalian host in terms of its steroid content.

Nadav et al. in 2002 studied heparinoids and integrated control of haemostasis and metastasis [274]. Components of the coagulation systems (e.g., platelets) associate with metastatic cells and enhance their invasiveness. These interactions may be perturbed by heparinoids. Heparinoids inhibit the activity of heparinase that promotes the trans-endothelial migration of metastatic cells. The development of novel cancer therapeutics based on the application of heparinoids may be possible in future.
Salzet in 2001 examined some anticoagulants and inhibitors of platelet aggregation derived from leeches [262]. Increased life expectancy is associated with aging populations in the developed countries, and an increased incidence of cardiovascular and inflammatory diseases and cancers can hence be expected. A priority for medical research is to reduce such morbidity. Leeches have been demonstrated to be a useful source of drugs to treat cardiovascular diseases, as they have evolved highly specific mechanisms to feed on their hosts by blocking blood coagulation. Powerful molecules acting at different points in the coagulation cascade or in the inhibition of platelet aggregation have been purified from leeches. Moreover, clinical trials confirm their potential to treat cardiovascular diseases.

Michalsen et al in 2008 studied osteoarthritis of the first carpometacarpal joint (thumb saddle joint) in a randomized controlled trial [275]. Results were not affected by outcome expectation or consumption of analgesics. A single course of leech therapy is effective in relieving pain, improving disability and quality of life for at least 2 months. Leech therapy has been shown to be effective for symptomatic treatment of osteoarthritis of the knee.

Zaidi et al in 2010 researched that the beneficial effects of leeching, in addition to decongestion, include injection of a cocktail of several medicinally useful bioactive molecules present in their saliva [255].

Graf and Silver in 2011 reviewed the innate and procured immunity inside the digestive tract of the medicinal leech [276]. Invertebrate animals provide important insights into innate immunity because the immune response is not complicated by adaptive immunity that vertebrates evolved.

Rizis et al in 2011 reviewed leech therapy in digital replantation and revascularization of 760 consecutive digits [277]. The use of leeches has fluctuated greatly gaining and losing favor over time. In recent years, reconstructive surgeons have used leeches in the...
management of venous congestion in microvascular free-tissue transfer and reimplantation. The study was conducted to describe the Québec Provincial Replantation Program's experience with leeches in salvage of upper extremity replantation/revascularization surgery. It was thus concluded that Leech therapy is an effective modality for the treatment of non-surgically manageable venous congestion in digital replantation/ revascularization. Leeches remain a useful tool in the plastic surgeon's armamentarium for the management of venous congestion.

Gröbe et al in 2011 used leech therapy in reconstructive maxillofacial surgery [278]. Corrective plastic surgery is indicated after accidents, burns, cancer surgery, or postoperative wound healing disorders with large tissue defects. Main complications such as arterial and venous insufficiency caused by a vessel collapse or a vascular spasm are reported regularly in the area of anastomosed vessels and are the concern of any surgeon. The study confirmed the excellent and predictable healing after medical leech therapy for local and microsurgical anastomosed flaps in the case of venous congestion. Leech therapy should be considered as a reliable additional procedure and an advantage in maxillofacial and plastic reconstructive surgery to remedy complications resulting from a hemodynamic imbalance or venous insufficiency in the immediate postoperative period.

Literature review for egg:

Eggs are one of nature's most complete foods, providing the human with high quality proteins, phospholipids, vitamins and various minerals. Many consumers, nevertheless, are reluctant to increase egg consumption due to concern about dietary cholesterol. Although the cholesterol content (210mg) of a large egg appears to be substantially lower today than that reported 15 years ago, mainly due to more accurate methods for cholesterol determination, it is still considered to be high when compared with other foods. Health organizations usually recommend a limited intake of this lipid since an increasing amount of literature has documented a strong relationship between serum levels of cholesterol and risk of cardiovascular disease.
Literature Review

Cholesterol is a complex organic substance which serves as an important structural and functional component in the body and as a starting material for the synthesis of vitamin-D, sex hormones and bile acids. It is found in high concentrations in the egg together with other nutrients since the embryo has not the ability to synthesize cholesterol during the early stages of its life. The human body normally produces about 800mg cholesterol per day and another 400mg are provided by the typical human diet. Under normal conditions, dietary cholesterol is not expected to affect the total blood cholesterol level since a decrease in the dietary cholesterol input is expected to be compensated by an increase in cholesterol production by the human body. Patients, however, with defects in cholesterol metabolism may respond favorably to dietary cholesterol reduction.

Egg lipids, including cholesterol, are solely found in the yolk and comprise about 30-36% by weight of this egg fraction. Triglycerides constitute approximately 65% of yolk lipids and the total amount of saturated fatty acids is about 40% of fatty acids. Full-fat egg, therefore, is considered as high in both cholesterol and saturated lipids.

Paraskevopoulou in 1995 studied emulsifying properties of low-in-cholesterol egg yolk prepared with the use of polysorbate-80 [279]. Dehydrated egg yolk was treated with an ethanol/water mixture containing 1.5% (w/v) Polyoxyethylene (20) sorbitan monooleate (Polysorbate-80) which brought about a marked reduction in its total lipid and cholesterol content. The resulting yolk protein concentrate was used for the preparation of o/w emulsions in order to evaluate its emulsifying properties. It was observed that this low-in-cholesterol yolk is an efficient emulsifier and exhibits satisfactory emulsion-stabilizing ability when compared to the natural dehydrated yolk.

Paraskevopoulou in 1995 examined the effect of cholesterol extraction from dried yolk with the aid of Polysorbate 80 on yolk's functional properties [280]. A yolk protein concentrate with a reduced total lipids and cholesterol content resulted when freeze-dried or spray-dried yolk was treated with an ethanol water mixture containing 1.5% (w/v) polyoxyethylene (20) sorbitan monooleate (polysorbate 80). Emulsions prepared with the yolk protein concentrate obtained from freeze-dried yolk were not as stable as those prepared with the freeze-dried yolk while in the case of spray-dried yolk the resulting
yolk protein concentrate exhibited the same emulsion-stabilizing ability as the spray-dried yolk. Both yolk protein concentrates gave mayonnaise-like emulsions of higher shearing stress-rate of shear values when compared to mayonnaises prepared with the dried yolks. Foaming activity of the yolk protein concentrate prepared from freeze-dried yolk was lower than that of the respective dehydrated yolk while the opposite was observed in the case of protein concentrate obtained from spray-dried yolk. Foam liquid stability of both concentrates was similar to that of the dried-yolks.

Aluko in 1998 characterized oil-in-water emulsions stabilized by hen's egg yolk granule [281]. Egg yolk granule proteins at pH 4.0, 7.0 and 9.0 were used to stabilize oil-in-water emulsions containing pure triolein. Average particle size of the emulsified droplets decreased with increase in protein concentration. Concentration of protein at the interface was greater for emulsions made at pH 4.0 than at pH 7.0 and 9.0, a result attributed to formation of lipoprotein dimers at pH 4.0. On the other hand, storage of the emulsions for 7 days at 4°C showed that stability was higher at pH 7.0 and 9.0 with smaller increases in particle size than at pH 4.0 which had large increase in particle size. Electrophoretic analysis of the emulsified droplets revealed formation of higher molecular weight proteins at the interface than were present in the granule. At pH 4.0, more protein was dissociated from the interface after treatment of the emulsion with urea than at pH 7.0 and 9.0. Increase in protein concentration resulted in a higher phosphatidyl-choline (PC) to phosphatidyl-ethanolamine (PE) ratio for emulsions made at pH 4.0 and pH 7.0. However, at pH 9.0 the PC to PE ratio was decreased as the protein concentration in the emulsions increased.

Anton in 1999 surveyed the effect of pH on interface composition and on quality of oil-in-water emulsions made with hen egg yolk [282]. Constituents of egg yolk are key ingredients of many food emulsions. They contribute to create an interfacial film between oil and water, which determines largely the characteristics of the emulsions. At pH 6, all the proteins of yolk, except phosvitin, were adsorbed at the interface and the interfacial tension at steady-state was lower (10 mN m⁻¹) than at pH 3 (15 mN m⁻¹) and pH 9 (30 mN m⁻¹). At pH 3, proteins at the interface are mainly phosvitin, and, at pH 9, some
apoproteins of LDL and HDL. The pH modulates the composition of yolk proteins at the interface, mainly by modifying the net charge of the proteins causing their repulsion or dimerisation.

Kiosseoglou in 2003 studied egg yolk protein gels and emulsions. Egg yolk remains a key ingredient of a number of food products [283]. Yet, its main functional properties, e.g. emulsifying ability and gel structure formation, upon heating, have not attracted the attention of too many researchers specializing in the area of food colloids. It is not surprising then that there have been only very few major advances in the field over the period of the last few years. These are discussed in the present review and include recent research findings on competitive adsorption between yolk protein constituents at emulsion oil-water interfaces, and also on the relationship between yolk particle supermolecular structure disorganization and the rheological properties of yolk-based emulsions and gel-network structures.

Nikiforidis in 2007 investigated the role of Tween in inhibiting heat-induced destabilization of yolk-based emulsions [284]. The process of heat-induced destabilization of yolk-based emulsions and the role of Tween addition in inhibiting droplet aggregation/coalescence in the thermally treated emulsions were investigated. The aim of the study was to understand the mechanism behind yolk emulsion destabilization during the application of processes such as pasteurization/sterilization and/or cooking. The presence of unadsorbed yolk protein in the emulsion continuous phase is crucial for the thermal destabilization of the system. Tween addition inhibits droplet flocculation/coalescence phenomena by shielding the reactive groups of protein molecules adsorbed at the droplet surfaces and those of unadsorbed proteins in the emulsion continuous phase which become available for interaction following heating and protein denaturation.
Literature Review

Literature survey of Unani medicine

Maseehi in 1936 derived a therapeutic composition containing leech and oil of *Olea Europa* in a definite proportion [285]. The animal is added to the oil and placed in a brass container for a number of days after which the oil is extracted. The resultant oil is added to vinegar oil and applied to hair as hair dye.

Unani literature describes alopecia and baldness as different diseases. According to the Unani literature, alopecia is associated with some infection whereas baldness is devoid of infection. Alopecia is called as Da-us-saalab whereas baldness is described as Ganj.

Kabiruddin in 1935, recommended that in initial stage of the disease, the head should be shaved and irritants like ajwain (*Trachyspermum ammi*), seeds of soya (*Glycine max*), pepper (*Piper nigrum*), sulphur, murdaarsang (lead oxide), henna (*Lawsonia inermis*), totiya (copper sulphate), kameela (*Mallotus philippensis*), suhaga (sodium biborate), haldi, fruit rind of pomegranate in mustard oil should be applied to initiate healthy hair growth [286].

Irritants are applied because they increase the blood supply to the part and helps in formation of hair roots, but before using these drugs it is better to wash the scalp with decoction of neem and soap. For the treatment of alopecia, it advises initial administration of blood purifiers then shaving of the head and application of irritants of the same category as mentioned above.

Khan in 1892 describes the hair growing properties of egg yolk oil [287]. This oil is hair tonic and grows hair and lengthens them as well. Also it mentions that sucking of blood by leech, or leech therapy is beneficial for baldness.