# CHAPTER II

# LITERATURE SURVEY

## 2.1 LIPOSOMES

A liposome can be defined as a closed, highly ordered, lamellar lipidic structure enclosing an aqueous phase in balance with the latter. It is formed spontaneously when lipids are added to a saline aqueous solution(1). Thus liposomes are artificial microscopic spheres constituted by the same phospholipids which integrate cellular membranes and which are capable of accomodating water soluble material in the aqueous space whereas lipophilic substances get associated in the lipid part. Discovered by Alec D. Bangham in 1965(2), they are considered to be efficient and specific therapeutic carriers of drugs.

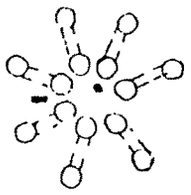
Depending on size, number of bilayers and multimembrane structure, there are 3 classes of liposomes (Fig 2.1) (3a) :

(i) Multilamellar vesicles (MLVs) :

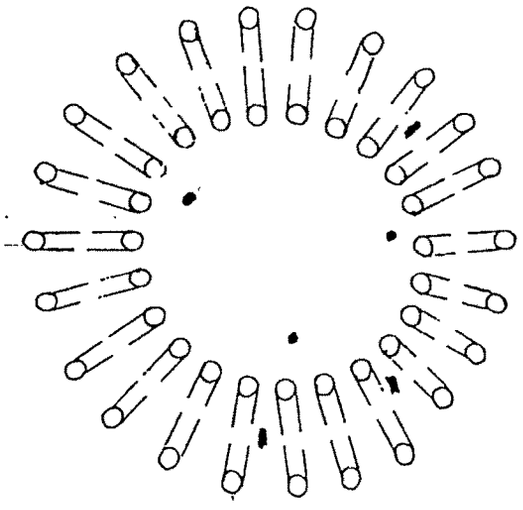
These usually consist of a population of vesicles covering a wide range of sizes (0.5 - 20 μm), each vesicle generally consisting of five or more concentric lamellae. Vesicles composed of just a few concentric lamellae are sometimes called oligo-lamellar liposomes or paucilamellar vesicles.

(ii) Small unilamellar vesicles (SUVs) :

Liposomes with a single bilayer and of the size 20-100μm are called small unilamellar vesicles.

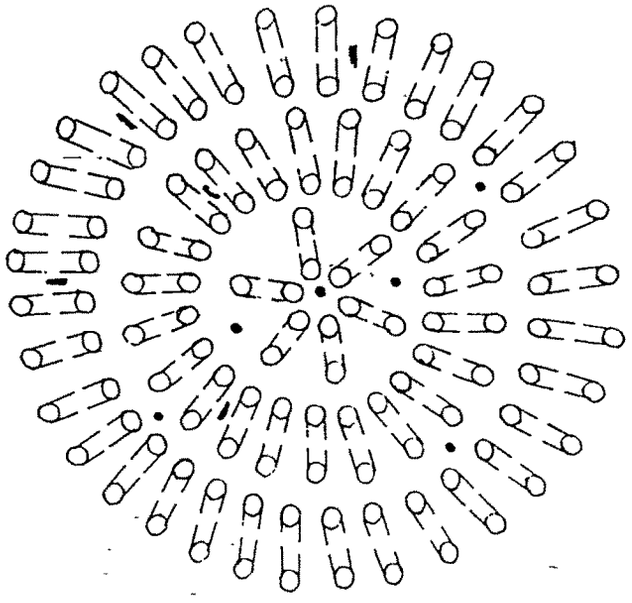


Small unilamellar vesicle
(SUV)



Large unilamellar vesicle

(LUV)



Multilamellar vesicle
(MLV)

FIG. 2.1: SCHEMATIC REPRESENTATION OF DIFFERENT TYPES OF LIPOSOMES.

( Polar drug; Non polar drug)

(iii) Large unilamellar vesicles (LUVs) :

Liposomes with a single bilayer and of the size of 100-1000 nm are called large unilamellar vesicles.

**2.11 Materials used in liposome preparation (4a)**

(a) Phospholipids :

Glycerol containing phospholipids are by far, the most commonly used component of liposome formulations and represent more than 50% of the weight of lipid present in biological membranes. Some naturally occuring phospholipids include phosphatidyl choline (PC), phosphatidyl inositol (PI) and phosphatidyl glycerol(PG) while dipalmitoyl phosphatidyl choline (DPPC), dipalmitoyl phosphatidyl serine (DPPS), dipalmitoyl phosphatidyl ethanolamine (DPPE), dipalmitoyl phosphatidic acid (DPPA), dipalmitoyl phosphatidyl glycerol (DPPG), dioleoyl phosphatidyl choline (DOPC) and dioleoyl phosphatidyl glycerol (DOPG) are some synthetic phospholipids.

(b) Steroids :

Cholesterol and its derivatives are often included as components of liposomal membranes. Cholesterol has been called the "mortar" of bilayers because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the phospholipid molecules, anchoring them more strongly into the structure. Its inclusion in liposomal membranes has 3 effects (i) increasing the fluidity or microviscosity of the bilayer (ii) reducing the

permeability of the membrane to water soluble molecules and (iii) stabilizing the membrane in the presence of biological fluids such as plasma.

(c) Other Substances :

Diacyl glycerol, stearylamine and dicetylphosphate have been incorporated into liposomes so as to impart either a negative or a positive surface charge to these structures.

(d) Antioxidant :

All the liposomes undergo auto-oxidation even in the presence of trace amounts of oxygen and this process is accelerated by elevated temperature, light, metal ions and some solutes. As a result, there is a dramatic, often abrupt, change in liposome permeability. Incorporation of $\alpha$-tocopherol into liposomes has been reported(5) to prolong the characteristic induction phase of auto oxidation. Addition of 0.1 mole % of $\alpha$-tocopherol roughly doubles the induction time relative to liposomes containing no $\alpha$-tocopherol. Addition of cholesterol to liposomes enhances the effect of $\alpha$-tocopherol, even though cholesterol itself is subject to peroxidation, presumably as a result of the decreased membrane fluidity. It has been reported(6) that the release of entrapped carboxy fluorescein from liposomes was markedly retarded by the presence of $\alpha$-tocopherol in the bilayer of liposomal PC membrane as compared to cholesterol-containing liposomes and pure PC liposomes. In yet another study(7) it was established that $\alpha$-tocopherol suppresses the oxidation of PC liposomes by scavenging both, the aqueous radicals

attacking from outside of the membrane and lipophilic radicals within the membranes. It was suggested that laterally, $\alpha$-tocopherol moves fairly rapidly but it gets less efficient for it to scavenge radicals as they go deeper into the interior of the membranes.

## 2.12 Why liposomes are formed (4a)

Lipids capable of forming liposomes exhibit a dual chemical nature. Their head groups are hydrophilic and fatty acyl chains are hydrophobic. The head group of PC has an overwhelming preference for water phase while the hydrocarbon fatty acid chains prefer each other's company to that of water. The large free energy change between a water and a hydrophobic environment explains the overwhelming preference of typical lipids to assemble as in bilayer structures, excluding water as much as possible from the hydrophobic core in order to achieve the lowest free energy level and hence the highest stability for the aggregate structure.

## 2.13 Methods for preparing liposomes

There are atleast 14 major published methods for making liposomes (8,9). The 7, most commanly employed methods are listed below :

(i) Lipid film hydration method (2)

(ii) Ethanol injection method (10)

(iii) Ether infusion method (11)

(iv) Detergent dialysis method (12)

(v) French press method (13)

(vi) Rehydration-dehydration techniques (14)

(vii) Reverse phase evaporation method (15)

## 2.14 Characterization of liposomes

Liposomes are characterized with respect to the following parameters (3b, 4a, 16) :

(i) Size and size distribution :

This is determined using light scattering, light microscopy, negative stain electron microscopy and freeze fracture electron microscopy.

(ii) Lamellarity :

The average number of bilayers present in liposomes can be found by freeze fracture electron microscopy and $^{31}$P-NMR spectroscopy.

(iii) Percentage capture :

This refers to the quantity of material entrapped inside liposomes. Minicolumn centrifugation method and protamine aggregation method are commonly used to determine this parameter.

(iv) Internal volume :

This refers to the aqueous entrapped volume per unit quantity of lipid (µg/ml)$^{-1}$. NMR spectroscopy is used for such measurements.

## 2.15 Liposomes as durg delivery systems

The properties of liposomes offering potential for drug delivery are as follows (17) :

(i) Ampiphilic nature (ii) Range of sizes and structural types (iii) Targetability (iv) Controllability of drug release rate (v) Stability in vivo (vi) Direct interaction with cells (vii) Sterilizability (viii) Ability to protect body from drug and drug from body. (ix) Non toxicity, non immunogenicity and biodegradability.

The problems associated with liposomes as drug delivery systems are as follows(18) :

(i) Difficulty in procuring pure phospholipids
(ii) Difficulty in scale-up
(iii) Poor stability over a long shelf life
(iv) Expensive
(v) Batch to batch variation in performance in vivo
(vi) Low drug loading
(vii) Difficulty in avoiding the reticulo-endothelial system
(viii) Possibility of unwanted vascular obstruction caused by large liposomes.

Extensive research has been carried out on liposomal drug delivery of the following classes of drugs (17,19) :

(i) Antineoplastic agents (ii) Antiviral agents
(iii) Chelating agents (iv) Vaccines
(v) Antifungal agents (vi) Enzymes

(vii) Diagnostics (viii) Antiinflammatory agents

(ix) Protiens and peptides (x) Drugs used in glycogen storage disease

(xi) topical preparations for dermal, transdermal, opthalmic, mucosal and pulmonary delivery.

An obvious major consideration in the use of liposomes in drug delivery is that a given liposomal drug formulation should exhibit clear benefits over and above those seen with the conventional formulation of the drug. The major modes of liposomal action, in mediating effective drug delivery are listed in Table 2.1.

Since a decade, several companies, specifically investigating on liposomes have been founded and as a result, work on liposome research and technology has been chanelled into more realistic goals. Some 17 years after liposomes were first injected into humans, the first injectable liposome based product (Vestar's AmBisome, a formulation of Amphotericin B) has been licensed for marketing in a number of countries. Other products undergoing clinical trials and expected to be licensed in near future are listed in Table 2.2.

Research into the use of liposomes in drug delivery has led to vastly improved technology in terms of drug capture, vesicle stability on storage, scaled-up production and the design of formulations for specialized tasks. The future of liposomes in drug delivery appears to be secure. They will

**TABLE 2.1**

MAJOR MODES OF LIPOSOMAL ACTION AND RELATED APPLICATIONS(20).

| Mode of action | Application |
|---|---|
| Intracellular uptake (lysosomes, endosomes/ cytoplasam) | Microbial disease, Metal storage disease, Gene manipulation, uptake by some tumour, cells, macrophage activation to a tumouricidal/microcidal state, efficient antigen presentation by antigen presenting cells (vaccines) |
| Slow release of drugs near the target area | Tumours near fixed macrophages |
| Avoidance of tissue, sensitive to drugs | Cardiotoxicity of doxorubicin |
| Circulating reservoirs | Blood surrogates |
| Facilitation of drug uptake by certain routes | Drug delivery to skin, lungs, eyes, mucosal tissues. |

**TABLE 2.2**

LIPOSOMAL PRODUCTS UNDERGOING CLINICAL TRIALS (20).

| Drug | Indication | Company |
|---|---|---|
| Nystatin | Fungal infection | Argus |
| Muramyl tripeptide | Activation of tumour-icidal macrophages | Ciba Geigy |
| Salbutamol aerosol | Asthma | Liposome-Technology Inc. |
| Stealth Doxorubicin | Kaposi's sarcoma | Liposome-Technology Inc. |
| Amphotericin B | Fungal infection | The Liposome Company |
| Doxorubicin | Cancer | The Liposome Company |
| Gentamicin | Intracellular mycoplasma | The Liposome Company |
| Doxorubicin | Kaposi's sarcoma | Vestar |
| $^{111}$Indium | Imaging | Vestar |

no doubt continue to contribute significantly to more efficient use of 'old' drugs and also find applications with agents now produced by recombinant DNA technology.

## 2.2 LIPOSOMES IN DERMAL DRUG DELIVERY

The skin is the most easily accessible organ of the body and therefore, direct application of drugs to the skin for treatment of its disease and disorders can, in theory, be targeted precisely. However, the topical route is often found to be cumbersome and inefficient consequently compromising efficacy in many instances; the main reasons for this inefficiency being either the inability of the drug to penetrate through the stratum corneum and reach an effective concentration in the viable epidermis (eg. methotrexate) or due to rapid penetration of drug reaching systemic circulation which results in short duration of local effect and undesirable systemic toxicity (eg corticosteroids). As a general rule, the above complications, limit the treatment of a number of dermatological diseases and disorders such as cutaneous tumors, eczema, infections and psoriasis by topical application of therapeutic agents. Over the past decade, a number of attempts have been made to produce topical preparations that (i) are easy to apply (ii) enhance the local therapeutic index and (iii) minimize unwanted systemic toxic effects. Since one decade, **liposomes** have staked their claim as potential drug carriers and reservoirs for controlled release of drugs within various layers of the skin

(19a, 21-28). Although dermal liposomal products have been exploited by the cosmetic industry since 1987, with currently over 100 liposomal and niosomal products in the market, only one therapeutic liposomal gel (Pevaryl, Cilag A G), containing econazole, an antifungal agent, has been introduced recently in Switzerland.

## 2.21 Drugs encapsulated in liposomes for dermatological purposes.

(a) Androgens and sex steroids :

The effect of topical application of the androgen, 5$\alpha$-dihydro testosterone (DHT), both, encapsulated in liposomes and dissolved in acetone, was evaluated(29) using the female hamster flank organ as a model system. Systemic absorption of DHT was significant from the acetone solution but negligible from the liposomal system. Since this study involved only the determination of DHT in the systemic side, the workers reported no advantage of the liposome system over the acetone solution in achieving the desired biological effect.

Topically applied liposomal progesterone, reduced the rate of hair growth in idiopathic hirsutism(30).

(b) Local Anesthetics :

The superiority of liposomal 0.5% tetracaine products over conventional tetracaine cream was confirmed in experiments involving normal human volunteers(31,32). A detailed study facilitating topical liposomal product

development, in general, using tetracaine as a model drug has been undertaken(33).

The superiority of liposomal lidocaine, as a topical spasmolytic agent, over aqueous lidocaine was confirmed in a study(34) conducted using rabbits. When applied to the femoral and iliac vessels, the serum levels of lidocaine were significantly lower and the peaks in concentration appeared later, for liposomal lidocaine than after the use of aqueous lidocaine solution. This was attributed to local accumulation of liposomal lidocaine followed by the slow release of the drug from the liposomes.

Lignocaine in negatively charged liposomes was found to get localized in the skin and exhibited a prolonged anesthetic effect when tested in vivo by the pin prick method(35).

(c) Topical Antifungal Agents :

Econazole base and econazole nitrate were encapsulated in various types of MLVs. Comprehensive in vivo biodisposition studies indicated that most of the liposomal products produced higher drug concentration in the skin and lower drug concentration in the internal organs of guinea pigs than those of conventional cream, gel and lotion dosage forms(19,21,22). Clinical studies indicated that the liposomal econazole was as or more active in lower concentration (0.2% or 0.5%) than the 1% econazole in conventional cream form. The liposomal form required less

frequent application and had better patient acceptance due to lack of irritation than the cream form.

(d) Antipsoriatic agents :

A clinical study involving free and liposomal methotrexate (MTX) in soft paraffin wax in treating psoriatic lesions showed a total disappearance of psoriatic lesions in all the patients applying the liposomal preparation within 2 weeks while no marked improvement was observed in the patients receiving free MTX with empty liposomes(36,37). It was concluded that liposomes could deliver and release MTX in a pharmacologically active form in psoriasis, where, the disease has root in epidermal cells. Subsequent studies on nude mice demonstrated a 4-fold more in the epidermis and a 2 fold reduction in the subcutaneous absorption of MTX following application of liposomal MTX as compared to MTX with empty liposomes.

From the results of <u>in vitro</u> studies using hairless mouse skin, liposomal dyphylline showed a lower permeation flux and partition coefficient as compared to free dyphylline when the bases used for both the products were PEG and carbopol(38). It was concluded that dyphylline liposomes may be superior to free dyphylline for local delivery of the drug to the skin in the treatment of psoriasis.

(d) Minoxidil :

Minoxidil in liposomes delivered more drug to hair follicles and less to internal organs as compared to free minoxidil in solution and suspension(22).

(e) Interferons :

Using a cutaneous herpes guinea pig model it was proved that the topical delivery of interferon - $\alpha$ was more efficient in liposomal form than in solution, emulsion and gel forms. The topically applied liposomal product caused a reduction of lesion scores while the solution and emulsion forms were ineffective(39). A 70-80% of the dose of liposomally encapsulated gamma interferon was found to be associated with the skin over a period of 24-hours in yet another study involving skin of different species. Since the order of deposition in deeper skin layers decreased with decreasing number of hair follicles, it was speculated that the transfollicular route may be an important pathway for the deposition of drugs in deeper strata of the skin(28). Herpes simplex genitalis infection in guinea pigs was successfully treated with liposomes of recombinant glycoprotien D antigen of herpes simplex virus(27).

(f) Non steroidal Anti-inflammatory agents :

Topical formulations containing anthranilic acid derivatives in liposomes showed a 50.4% control of UV induced irritation on guinea pig skin as compared to 35.4% by the free drug in olive oil(40). Increased concentration of diclofenac in the subcutaneous tissue as well as increased permeation through the skin was reported for liposomally entrapped diclofenac as compared to the free drug(41).

(g) Agents used in the treatment of Acne :

In hairless mice, liposomes containing tretinoin gave 10 times more comedolytic activity than the same concentrations in alcohol. Effective amounts of encapsulated tretinoin were less irritating to rabbits than effective amounts of the drug in alcohol(42).

When topical bioavailability of tretinoin in dipalmitoyl phosphatidyl choline liposomes was compared with that of free drug in gel, the % of tretinoin in epidermis and dermis of hairless rat skin was significantly higher with the liposomal form. Drug retention in cutaneous structures with liposomal drug was associated with reduced systemic absorption. A 0.005% of tretinoin in liposomes had a comedolytic activity almost as effective as 0.05% tretinoin in gel in hairless rhino mice alongwith improvement in tolerance(43).

Clinical treatment of acne with a lotion of liposomal clindamycin hydrochloride showed better efficacy than non-liposomal lotion forms especially in the treatment of pustules where clinical improvement was 77% of the initial number. Application of a conventional lotion solution, a non-liposomal emulsion lotion and a liposome emulsion lotion resulted in decreases of 42.9, 48.3 and 62.8% respectively in the total number of lesions after a 4 week treatment indicating the superiority of the liposomal dosage form(44).

(h) Antibiotics :

A single dose of liposomally entrapped tobramicin and silver sulphadiazine decreased bacterial counts as compared to untreated controls and to a similar extent as compared to multiple applications of free drug in adult rats. It was concluded that such liposomal formulations might offer potential clinical advantage(45).

(i) Miscellaneous Agents :

The decreased skin superoxide dismutase (SOD) activity in mice after exposure to UV radiation was lessened by pretreatment of skin with liposome entrapped SOD. This protective effect of the encapsulated SOD may have potential clinical application for photodermatological reactions(46).

A single dose of liposomally incorporated biosynthetic human epidermal growth factors produced a 200% increase in tensile strength of an experimentally induced wound as compared to a single dose of the factors in saline, within 7-14 days, indicating that the liposomal formulation may provide prolonged local delivery of the factors to the wound(47).

(j) Topical corticosteroids :

The effect of liposomal incorporaton of topically applied hydrocortisone on penetration into human skin, its potential to produce skin blanching, its serum concentration and urinary excretion has been reported (48,49). Hydrocortisone in liposomes had an improved concentration

time profile in different layers of human skin after external application, as compared with a conventional ointment. This was accompanied by increased degree of blanching in the vasoconstrictor test. A decreased serum concentration and urinary excretion in guinea pigs following topical application suggested of the system acting as a drug localizer with a possible increase in therapeutic efficacy and decrease in adverse systemic effects.

Percutaneous absorption of hydrocortisone octanoate encapsulated in DPPC liposomes was studied using rabbits with aqueous application to the skin surface. The availability, as determined by measuring total area under the plasma drug concentration time curve between 0 and $\infty$ time, was in the order of liposome < free drug(50).

An increased activity and tolerability of betamethasone dipropionate in liposomal form as compared to conventional gel has been reported(51). Yet another study(52) reports of liposomal encapsulation as being suitable for a trans-cutaneous effect.

It has been reported that liposomal encapsulation of triamcinolone acetonide (TRMA) favourably changes the drug biodisposition. When a liposomal lotion was applied in rabbits and the various internal organs analysed for drug content, it was seen that the percutaneous absorption of TRMA was greatly reduced while the drug concentration was increased by 4-5 folds in the epidermis and dermis. Besides,

a 3 fold decrease in the drug concentration was seen in the thalamic region-a site for side effects(53). When the study was repeated using a gel dosage form (54), the liposomal gel delivered 4.9 times more drug to the epidermis and 2-3 times less drug to the thalamic region as compared to the gel form containing free drug.

Results of in vitro diffusion experiments, with hairless mouse skin and guinea pig skin, conducted to study the uptake and distribution of liposomes of hydrocortisone and flucinolone acetonide, showed a 4 times higher concentration of the drugs in the skin as compared to that from aqueous solutions of respective drugs(55).

## 2.22 Factors affecting dermal delivery by liposomal systems

(i) Liposome Composition :

Keeping the drug concentration constant and increasing the phospholipid cover in liposomes resulted in lower penetration rates of radio-labelled butyl paraben which was explained by a lower thermodynamic activity of the drug in the liposome bilayer(56).

The transdermal delivery from liposomes prepared with saturated phospholipids (DMPC and DPPC) was shown to be approximately 10 times smaller as compared to unencapsulated drug while that from liposomes made with unsaturated phospholipids (EPC and DOPC) was only reduced by about 50%(57). When either 1% unsaturated (oleic) or 1% saturated (stearic) free fatty acid was added to DPPC liposomes, the resulting transdermal delivery of progesterone was pointedly

different : delivery from stearic acid containing liposomes was practically identical to delivery from DPPC liposomes while delivery from oleic acid containing liposomes was almost identical to delivery from EPC or DOPC liposomes, being saturable over a range of 0.1-10% of oleic acid present. It was concluded that presence of free fatty acids in liposomes contributed to a fluidization of lipid domains within the stratum corneum which in turn facilitated transdermal flux of progesterone.

Liposomes of cyclosporin and interferon, prepared using bovine brain ceramides, cholesterol, palmitic acid and cholesteryl sulphate (skin lipids) penetrated into deeper strata of stratum corneum as compared to those prepared using PC, cholesterol and PS; the reason being the greater ease of mixing of 'skin lipid' liposomal bilayers with stratum corneum bilayers as compared to the phospholipid liposomes(55).

The effect of liposomal charge on the *in vitro* permeation into skin of hairless mouse was evaluated using positively (stearylamine) and negatively (PS) charged phospholipid multilayer liposomes(57). Results indicate that positively charged liposomes lead to almost twice the amount of lipids deposited in the deeper layers of the skin as compared to application of negatively charged liposomes suggesting that mixing and interaction of the liposomal bilayers with the stratum corneum bilayers was more extensive

with positively charged liposomes. However, application of formulations carrying a net positive charge to the skin often results in marked irritation.

The polar moiety of the amphiphilic molecules of the phospholipid used in the preparation of liposomes plays a dominant role with respect to interaction with human skin lipids. PC with its relatively small hydrophilic groups seems to be able to interact with skin lipids whereas PI with its very strong hydrophilic groups is not able to penetrate into the human stratum corneum(58).

Incorporation of 50 mole % of cholesterol into a liposomal formulation decreased the flux of drugs and the release rate showed little temperature dependence since cholesterol modulates the fluidity of PC bilayers(60).

(ii) Type of Liposomes :

Liposomes prepared by dehydration-rehydration technique were observed to penetrate deeper into skin strata than LUVs(58).

In a study involving caffeine liposomes, SUVs exhibited lower skin permeation, higher accumulation in skin and longer lag time before steady state permeation was reached while LUVs showed low skin permeation with low accumulation in the skin and shorter lag time than that for SUVs(61).

The plasma drug concentration-time curve between 0 to $\infty$ time was in the order MLV < SUV < free drug in a study

involving percutaneous absorption of liposomal hydrocortisone octanoate in rabbits. The in vitro release of drug from MLVs was more rapid than that from SUVs(50).

(iii) Nature of the drug :

Encapsulation of both, hydrophilic (inulin) and hydrophobic (hydrocortisone) drugs into liposomes altered their biodistribution such that more amount of drug remained in the skin and less entered into the systemic circulation(62).

(iv) Size and size distribution of liposomes :

When liposomes of cyclosporin A, of different particle sizes, were studied for their in vitro permeation using skins of different animals, intermediate sized liposomes gave both, the highest reservoir in the deeper skin strata as well as the highest drug concentration in the receiver, indicating that there might be an optimum particle size for optimal dermal drug delivery(63).

## 2.23 Mechanism of liposomal action in dermal drug delivery

Ever since the introduction of liposomes as dermal drug delivery systems, debates have continued as regards the mechanisms behind their effects. One of the most disputed questions is whether intact vesicles are able to penetrate into the stratum corneum(56,64-66) or even into the deeper layers of the skin(53,54); whether there is a partial transfer of liposomes into the deeper skin layers with their lipids protecting the entrapped drug from chemical

transformation(67,68) or mixing of phospholipids from applied liposomes takes place with the stratum corneum lipids via monomer exchange or by direct fusion(69,70). Some of the theories postulated regarding the mechanisms of lipsomal action in dermal drug delivery are described below :

(A) Theory which supports the passage of liposomes into the deeper skin layers(22) :

Fig.2.2 illustrates the sequence of events related to a drug applied topically on the skin in the conventional dosage forms, ointment, cream or lotion and in the liposome (LIP) encapsulated form. In the conventional dosage form the 'free' drug should be released, diffused to the surface of the skin, dissolved (if it is not in solution form) before absorption into the horny layer takes place. The drug in the liposomal form should not be released and, if diffusion to the keratin layer is required, that is less of a problem, since the nature of the lipid vesicles makes that easier : the vesicles are readily miscible with the skin surface lipid which can often serve as a barrier, especially to lipid insoluble drugs.

In the second step, the drug should get through the horny layer, which is the main barrier, and often serves as a reservoir because of protein binding. The vehicle may have an occlusive effect that enhances hydration of the keratin layer, this in turn increases its permeability. The liposomal form has an excellent potential for hydrating the horny

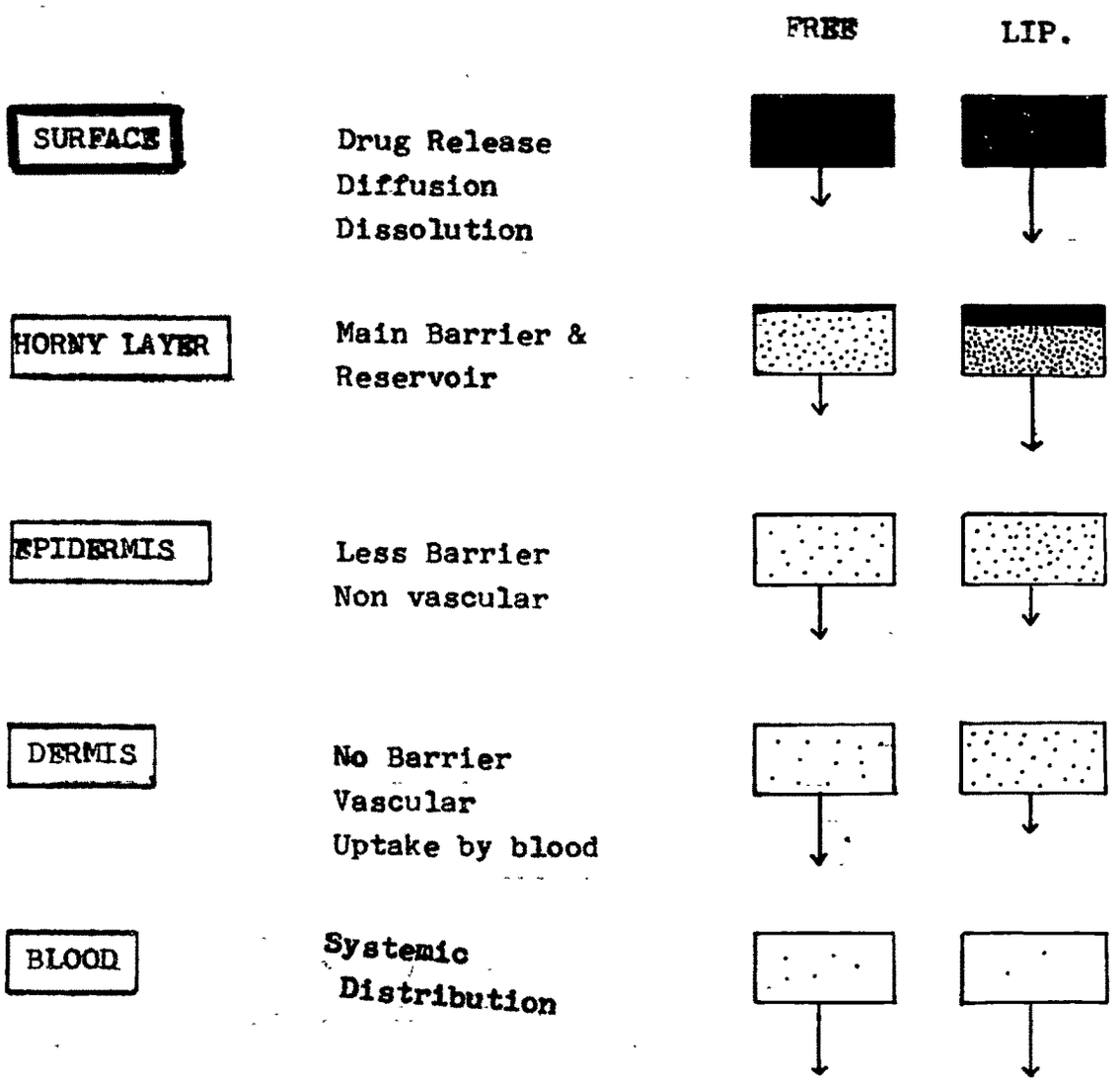



FIG. 2.2: SCHEMATIC REPRESENTATION OF THE MECHANISM & RATE OF PENETRATION OF FREE AND (LIP) LIPOSOMAL DRUG. THE DENSITY OF THE SPOTS INDICATE THE CONCENTRATION; THE LENGTH OF THE ARROWS INDICATE THE MAGNITUDE OF THE RATE OF DIFFUSION.

layer, since the lipid vesicles create a lipid film, supplementing the skin surface lipids. Consequently the chance of increased permeability of the main barrier layer is greater with the liposomal form than that with some of the conventional forms. The chance of protein binding is probably greater for the 'free' drug than for the liposome encapsulated drug.

In the third step, when the drug reaches the epidermis, the diffusion rate of the 'free' form is expected to be higher than the liposome encapsulated form because of the difference in size. Since the free form is in the molecular state, the size of the penetrating drug is equivalent to the size of its molecule. The slower diffusion of the lipid vesicles provides a longer residency time for the encapsulated drug.

In the fourth step, because the dermis is highly vascularized and because of a high concentration gradient, the 'free' drug is quickly removed by the blood circulation. The larger liposomes, because of their size are not able to penetrate the blood vessels, therefore, the cutaneous clearance of liposomal drug is less than the 'free' drug.

(B) Theory which supports that neither intact liposomes nor the phospholipids of which they are made of, diffuse across the skin(65) :

As per this approach, three probable mechanisms may exist for transfer of drug from liposomes to skin (Fig.2.3) :

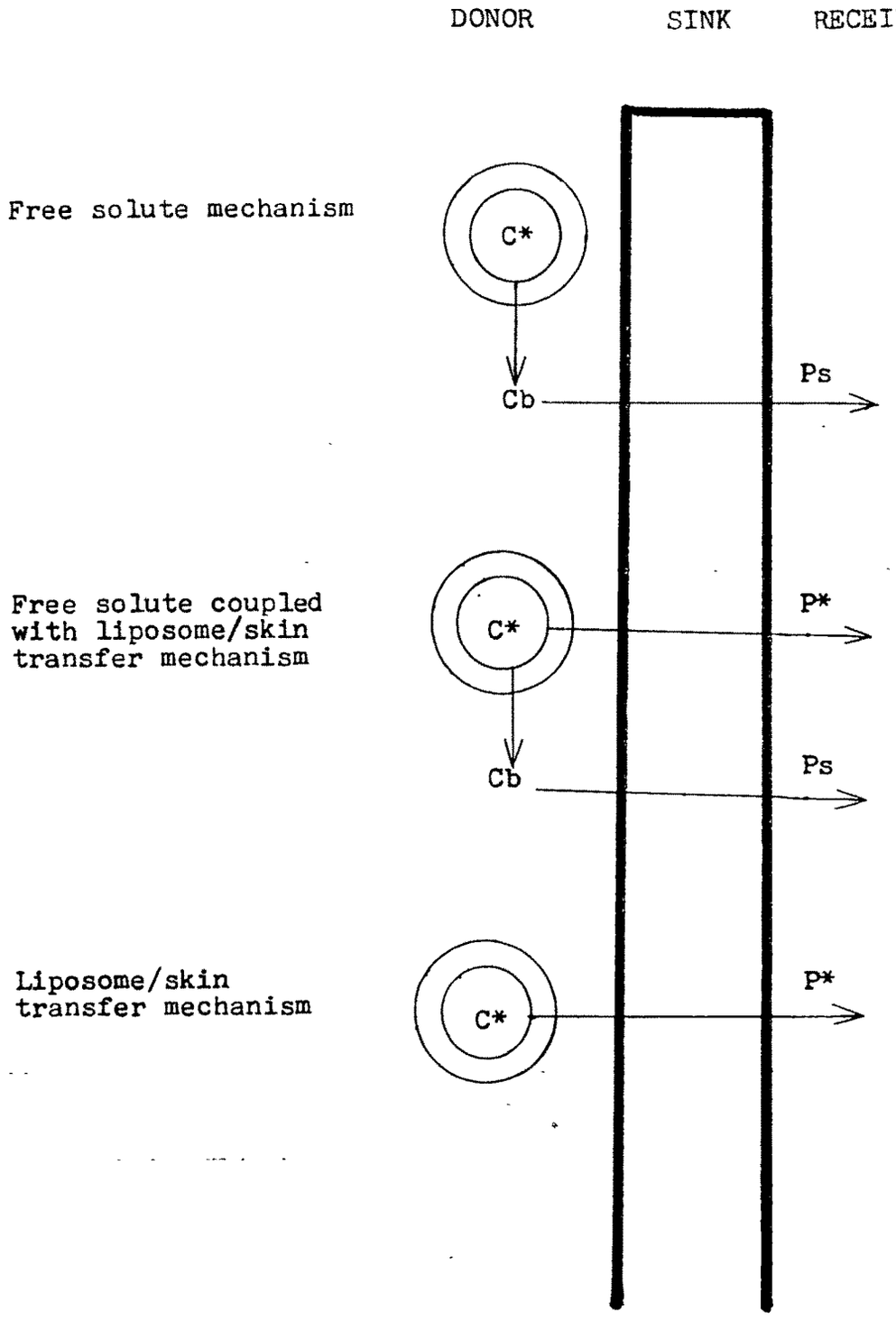



FIG. 2.3: SCHEMATIC DESCRIPTION OF VARIOUS MECHANISMS IN SKIN PERMEATION OF LIPOSOME ENTRAPPED SOLUTES. (Liposomes are not adsorbed intact nor fused with stratum corneum.)

C* - Liposome associated drug concentration

Cb - Bulk drug concentration

Ps - Intrinsic skin permeability constant

P* - Permeability constant resulting from interaction with the liposomes.

(a) release of liposome entrapped solute and percutaneous absorption of free solute

(b) release of liposome entrapped solute coupled with skin permeation of free solute and also direct liposome/ skin solute transfer.

(c) skin permeation involving liposome/skin solute transfer.

If fusion was a dominant mechanism, the effective permeability of entrapped hydrophilic drug should approach the values of the free solute. However, the fact that this does not happen, fusion, as a means of transport may be ruled out. Hydrophilic solutes in liposomes have significantly lower permeation rates than its aqueous counterpart. The skin uptake rate will depend largely on the free solute which is governed by the very slow leakage rates from liposomes. The interfacial transfer of the free solute involves stratum corneum/water partitioning i.e. mechanism 'a'.

As far as the hydrophobic solute goes, the permeation rates for liposomally entrapped solute is smaller than that for free solute in aqueous solution but one gains by the many fold higher solubility in liposomes. Hence for hydrophobic drugs in liposomes, interfacial transfer occurs by collison complex transfer between the drug intercalated in the liposomal bilayer and the surface phases of the stratum corneum i.e. mechanism 'c'.

(C) Theory which proposes that dehydration of liposome bilayers controls the extent and rate of drug transfer(61) :

The basis of this theory is that transfer of drug from lipid bilayers to the skin can occur as long as the bilayers are in a liquid crystalline state. Alteration from this state to the gel state, which results due to dehydration, ceases the transfer of drug from the bilayer to the skin. For a hydrophilic drug, dehydration will cease transfer from liposome to skin because the drug is no longer in a dissolved state. Dehydration also leads to enrichment of the drug concentration in the aqueous phase of the bilayers leading to an enhanced flux of drug across the skin. In liposomes of a hydrophobic drug, the major fraction of the encapsulated drug would be intercalcated within the lipid bilayer. Dehydration would lead to formation of a strong adhesive patch of the liposomal bilayer on the skin which maximizes the intimacy of contact between drug-laden bilayers and the skin.

Hence for liposomal systems that retain a constant amount of water within the bilayers following dehydration to an equilibrium state, the drug transport would continue over extended periods of time.

On dehydration, the follicular pathway is available - the bilayers partitioning and packing the follicular or hair ducts. This partitioning is favourable since follicular ducts contain lipids.

In conclusion, following application of liposomes to the skin atleast 2 types of interactions seem possible (i) some intact vesicles can penetrate through the stratum corneum perhaps through the intercellular and appendageal routes; hence they can act as reservoirs for drugs in the skin (ii) vesicles may fuse with lipid bilayers and as a result may increase penetration.

## 2.24 Advantages of topical liposomal formulations

The following are the advantages of topical liposomal formulations(58) :

(i) In a manner similar to that of biological cells, liposomes can store water-soluble substances in their interiors and lipophilic and amphiphilic substances in their membranes where they are positioned to be transferred to other membranes such as the skin.

(ii) Most conventional vehicles are inefficient in their ability to deliver their active ingredient into the skin because of their failure to penetrate the horny layer. The bilayers of liposomes, on the other hand, efficiently penetrate the skin.

(iii) Liposomal incorporation of drugs which readily penetrate the skin results in a decreased systemic absorption as compared to that resulting from topical application using conventional vehicles.

(iv) Liposomal deposition into the stratum corneum results in a substantial reservoir effect.

The concept of selective targeting of topically applied therapeutic agents by incorporating into liposomes to various cell types in the skin, shows promise and warrants further studies. The real potential of liposomes in this field can be evaluated only by careful analysis of the results of such investigations.

## 2.3 TOPICAL CORTICOSTEROIDS

The development of topical corticosteroid therapy has been a major therapeutic advance in dermatology. Attaching a long carbon side chain or removing a hydroxyl group from the systemically active anti-inflammatory steroids increases lipophilicity and hence improves their topical effectiveness greatly(71). Based on their potencies, topical anti inflammatory corticosteroids are classified as very potent (e.g. clobetasol proprionate 0.05%), potent (flucinolone acetonide 0.025%, triamcinolone acetonide 0.1%), moderately potent and mild.

### 2.31 Mechanism of action of glucocorticosteroids

A specific hormone receptor system in the cytoplasam of the target tissue appears to be necessary for initiation of glucocorticoid action. After interaction between drug and its receptor protein, the steroid receptor complex leaves the cytoplasam and binds to specific sites on chromatin of the cell nucleus. This results in stimulation by an unknown mechanism of new transcription of m-RNA and r-RNA which

results in translation of specific induced proteins and mediation of the physiological effects by these induced proteins(71).

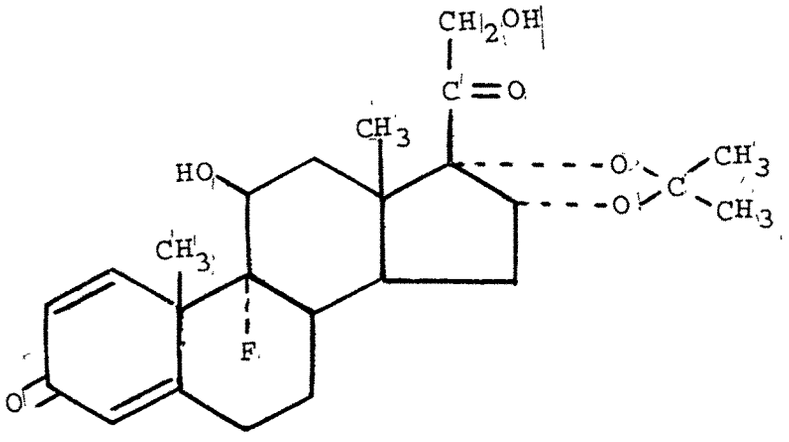
## 2.32 Analytical profiles of corticosteroids under study

### 2.321 Triamcinolone acetonide (TRMA)(72) :

TRMA is a 9∝-fluoro-11β-16∝,17,21-tetra-hydroxy-pregna-1,4 diene-3,20 dione cyclic, 16,17,-acetal with acetone.

Molecular formula : $C_{24}H_{31}FO_6$

Molecular Structure :




Molecular weight : 435.5

Description : White to off-white odourless, crystalline powder.

Melting range : 277-281°C

Solubility : 50mg/ml in 95% ethanol, 40 mg/ml in isopropyl alcohol, 90 mg/ml in acetone, 25ml/ml in chloroform 250, ug/ml in dimethyl formamide, 0.004 mg/ml in water.

Analytical methods :

(i) UV spectrophotometry : TRMA gives an absorption maxima at 238 nm in methanol.

(ii) Colorimetric Analysis : Reaction of TRMA with tetrazolium blue in alkaline medium gives a blue color (520nm) which can be quantified. Reaction of TRMA with isoniazid gives a yellow hydrazone (404nm).

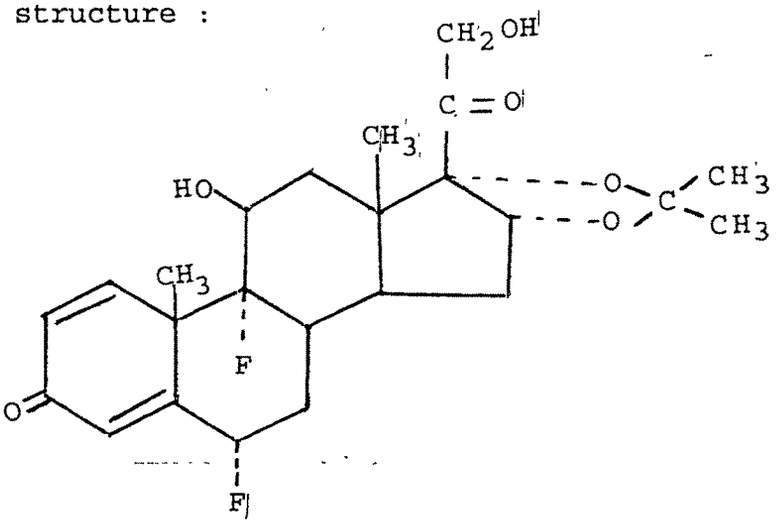
(iii) Other methods : These include fluorimetric and titrimetric methods and radioimmuno assay(72).

**2.322 Flucinolone acetonide (FLU)** (73a) :

FLU is a 6∝-9∝-difluoro-11β, 21 dihydroxy 16∝,17∝-isopropylidene dioxy-pregna-1,4-diene-3,20 dione.

Molecular formula : $C_{24}H_{30}F_2O_6$

Molecular structure :




Molecular weight : 452.5

Description : White to offwhite crystalline powder.

Melting point : About 270°C with decomposition.

Solubility : Practically insoluble in water, soluble 1 in 26 of dehydrated alcohol, 1 in 10 of acetone, 1 in 15 to 1 in 25 of chloroform, 1 in 350 of ether and soluble in methanol.

Analytical methods :

(i) UV spectrophotometry FLU gives an absorption maxima at 240nm in methanol.

(ii) Colorimetry : Same as that for TRMA.

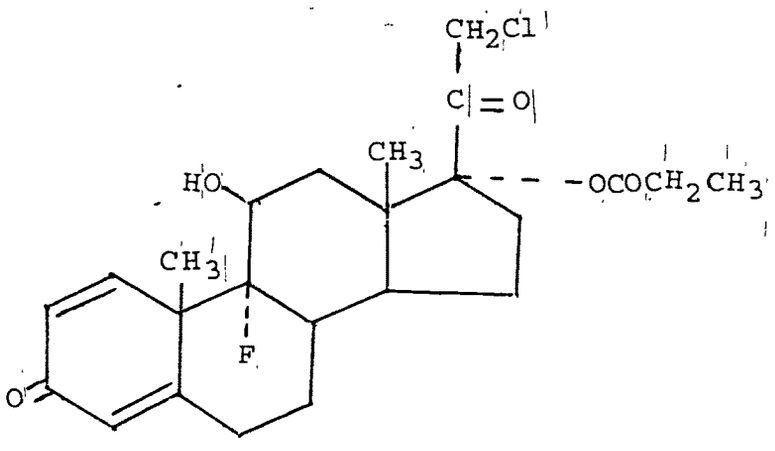
(iii) Other methods : These include differential pulse polarography(74) and mass spectroscopy(75).

**2.323 Clobetasol propionate (CLO)** (73b) :

CLO is a 21 chloro, 9∝ fluoro, 11ß, 17∝-dihydroxy-16ß methyl pregna -1,4-diene 3,20-dione 17-propionate.

Molecular formula : $C_{25}H_{32}ClFO_5$

Molecular structure :




Molecular weight : 467

Description : White to off white crystalline powder.

Melting point : 195.5-197°C

Solubility : Practically insoluble in water, soluble 1 in 100 of ethanol, 1 in 1000 of ether, soluble in acetone and chloroform.

Analytical Methods

(i) UV spectrophotometry : CLO gives an absorption maxima at 239nm in methanol and at 237 nm in ethanol.

(ii) Colorimetric methods : Reaction of CLO with isoniazid gives a yellow colored hydrazone (404nm).

(iii) Other methods : These include other colorimetric methods(76,78,79) and HPLC method(78).

## 2.4 SKIN BLANCHING ASSAY

A large number of well established techniques have been reported(80) for the bioassay of topical corticosteroids. Since the human skin blanching assay in used not only to evaluate the intrinsic activity of a topical steroid for correlation with possible clinical anti-inflammatory action but also as a test in fundamental biopharmaceutical studies, it is the most extensively studied technique(81-95).

Glucocorticoids when applied topically on human skin, undergo percutaneous absorption resulting in apparent vaso-constriction of superficial vasculature and skin blanching. While the precise mechanism of action is unknown, the observed pharmacodynamic response is the blanching effect. This blanching is used as a measure of percutaneous absorption of corticosteroids in the human skin blanching assay. In short, in the assay, 10-15 healthy male and female Caucasian volunteers are made to participate. Adhesive labels from which 7mm x 7mm squares have been punched are applied to the flexor aspect of both forearms of each volunteer. The preparations to be tested are applied in the punched holes and the holes are left either occulded or unoccluded for atleast 6 hours. After this, the tapes are removed, the hands

washed clean and the pallor assessed by observers or any other mechanical techniques. When human observers are used, a 0-4 scale rating is fixed, where 0 represents no blanching and 4 represents intense blanching over the whole application site with the values of 1,2 and 3 representing the respective grades of blanching between the two extremes. The most commonly used mechanical techniques to assess pallor are as follows :

(i) Reflectance spectrophotometry(96) which can provide a spectral plot in the visible range of 320-720nm-the comparison of the spectral plot between the treatment area and the treatment area after glucocorticoid administration may permit reproducible determination of blanching effect.

(ii) Doppler laser velocimetry(92) which monitors cutaneous blood flow by quantifying red blood cell flow in the cutaneous microvascular bed. Comparison of the blood flow between the pretreatment area and the treatment area after glucocorticoid therapy may give a direct estimate of vasoconstriction.

(iii) Thermography(92) which uses a thermocouple sensor or thermographic camera, sensitive enough to detect a temperature difference of $0.05^{o}C$.

(iv) Tristimulus color analysis(97) which makes use of a chromameter to record changes in skin color.

Inspite of the availability of so many mechanical means for measuring blanching, the only really satisfactory

technique, employs the trained human eye, which is adept at assessing subtle color differences and making automatic allowances for skin imperfections. This fact was further emphasized by a study(98) which found perfect correlation between the amount of corticosteroid in the stratum corneum (found by stripping) and the blanching score.

## 2.5 ECZEMA AND KELOID

### 2.51 Eczema(99):

Eczema or dermatitis is a reaction pattern manifested by variable clinical and histological findings. It is the final common expression for atopic dermatitis, allergic contact and irritant contact dermatitis, lichen simplex chronicus and seborrheic dermatitis. Primary lesions may include papules, erythematous macules and vesicles which can coalese to form patches and plaques. In severe eczema, secondary lesions such as weeping and crusting may dominate. Long-standing dermatitis is often dry and is characterized by thickened, scaling skin. The histological features of dermatitis have been divided into 3 patterns : acute, subacute and chronic. Chronic dermatitis demonstrates epidermal acanthosis, hyperkeratosis, upper dermal fibrosis and a predominantly perivascular, mononuclear cell infiltrate. Treatment involves the use of systemic topical corticosteroids alone in case of dry eczema and in combination with systemic antibiotics in case of weeping eczemas.

**2.52 Keloid** (99)

It is a non malignant fibroproliferative disorder with growth of firm tumors, anywhere but most commonly at a site of previous injury, which may be pink, purple or brown in color. Intralesional triamcinolone acetonide (10-40 mg/ml) is the treatment of choice in such conditions. However, unsatisfactory results are often obtained when these lesions are treated in advanced stages(100).

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