Chapter II

Literature Review

Non-steroidal anti-inflammatory drugs (NSAIDs) are the main pharmacological agents used in the management of acute painful conditions. They are also frequently used as the first-line therapy in major arthritic diseases of osteoarthritis (OA) and rheumatoid arthritis (RA) (Blagbrough et al, 1992). Diclofenac has become one of the first choice NSAIDs owing to its favorable pharmacodynamic properties and relative freedom from unwanted effects (Todol and Sorkin, 1988). Diclofenac has marked anti-inflammatory as well as analgesic effects and a high therapeutic index. It is used mainly as an oral preparation and less often by the intramuscular or rectal routes (Reiss et al, 1978). Systemic diclofenac has proved to be a potent and reliable agent, achieving complete or substantial pain relief and functional improvement within a relatively short period of time (Hadidi and Garf, 1991). However, gastrointestinal side effects are a major drawback. Savage et al (1993) reported that the risk of peptic ulceration caused by treatment with diclofenac was 2.9 times larger than control. Therefore, cutaneous application of the drug has become popular in Germany, Austria and Switzerland. Reiss et al (1986) has shown that topical administration resulted in absorption of about six percent of the dose. This was also associated with serum drug concentrations well below those observed after standard oral or i. m. administration and below the range of which side effects usually occur (Radermacher et al, 1991).

It has been claimed that topical application of diclofenac over an inflamed joint results in synovial fluid drug concentrations which exceed plasma drug concentrations, suggesting direct penetration of the drug into the joint (Reiss et al, 1986). Other pharmacokinetic, pharmacological and clinical studies have shown that Voltaren Emulgel® (1.16% diclofenac diethylammonium equivalence to 1% of diclofenac in an emulsion gel) can produce therapeutic local concentrations of diclofenac in the area of inflammation without substantial systemic absorption (El-Hadidi and Garf, 1991).

Assessing the bioavailability of topical formulations is a complex process since many factors are involved. The active drug first has to be released from the preparation and this process alone can involve various stages such as the thermodynamic activity of the drug and the microviscosity of the medium through which the drug diffuses (Meyer, 1988). Whether or not these are rate determining steps depends on the nature of the phases and the physicochemical properties of the drug. At the skin surface the drug will partition from the preparation into the primarily lipid-rich environment of the stratum corneum. It will diffuse slowly through this region of the epidermis and then partition from the stratum corneum into the viable tissues. Further diffusion occurs until the drug reaches the dermal vasculature where it is removed into the systemic circulation (Khaliani et al, 1987). Thus, the topical bioavailability of a drug depends, at least in part, on the rate of its release from the formulated product. Wu et al (1992) stated that differences in release rates between generically equivalent drug products may lead to differences in percutaneous absorption and therefore differences in therapeutic effects.

Furthermore, the bioavailability of topically administered drugs is also a function of their permeability across the stratum corneum. Scheuplein and Bland (1971) have reported that for optimum permeability it is desirable that the drug have both adequate solubility in the formulation vehicle as well as a favorable partition coefficient from the formulation into the stratum corneum.

The stratum corneum forms the major barrier to transdermal penetration of drugs due to its highly ordered and rigid structure (Wu et al, 1992). For more perception, the skin and its characteristics are detailed as follows:

Skin and Its Characteristics

Human skin provides a nearly impermeable barrier to the transport of most drug. Consequently, the skin has many characteristics which resist the penetration of drug to the skin.

Barrier Properties of the Skin

As shown in Figure 1, the uppermost layer of the skin or stratum corneum is 10-15 mcm. thick and is composed of keratinized, unnucleated cells. This layer is known at the stratum corneum. The lower layer is the viable epidermis, about 50-100 μm thick, and consisted of proliferating cells. The permeability of the stratum corneum to most materials is generally 10-1,000 times less than that of the viable epidermis. It thus serves as the primary barrier to the absorption of materials into the skin. The next layer of the skin is the dermis, which contains many capillaries

so that it readily absorbed and systemically dilutes most chemicals which penetrate pass the stratum corneum and the viable epidermis that are involved in the removal of any drug that may have diffused down through the upper layers of the skin. The dermis consists essentially of a matrix of connective tissue woven from fibrous proteins (approximate composition : collagen 75% ; elastin 4% ; and reticulin 0.4%) which embed in an amorphous ground substance of mucopolysaccharide, blood vessel, nerves, and lymphatics cross this matrix and skin appendages(eccrine sweat glands, and apocrine glands, an pilocebaceus units) permeate it. Below the dermis is subcutaneous tissue with subcutaneous fat spreads all over the body as a fibrofatty layer. The subcutis provides a thermal barrier and a mechanical cushion (Barry , 1983).

As previously stated, a compound may permeate through the skin by one of the three pathways, i.e. a transappendageal route , an intercellular route and an transcellular route. The first pathway involves the passage of drug through hair follicles and sweat glands. This may be sometimes called shunt pathway because the drug can penetrate directly into the viable tissues without initial diffusion through the stratum corneum. As a result, diffusion thru the shunt pathway occurs much more rapidly than the inter or intracellular pathway. However, the area available for such absorption is rather limited particularly in humans, constituting less than 0.1% of the total skin area (Chien , 1992). Therefore, the most important pathway of drug penetration are inter and intracellular routes which provide passage of drugs through stratum corneum, the major component of the skin barrier.
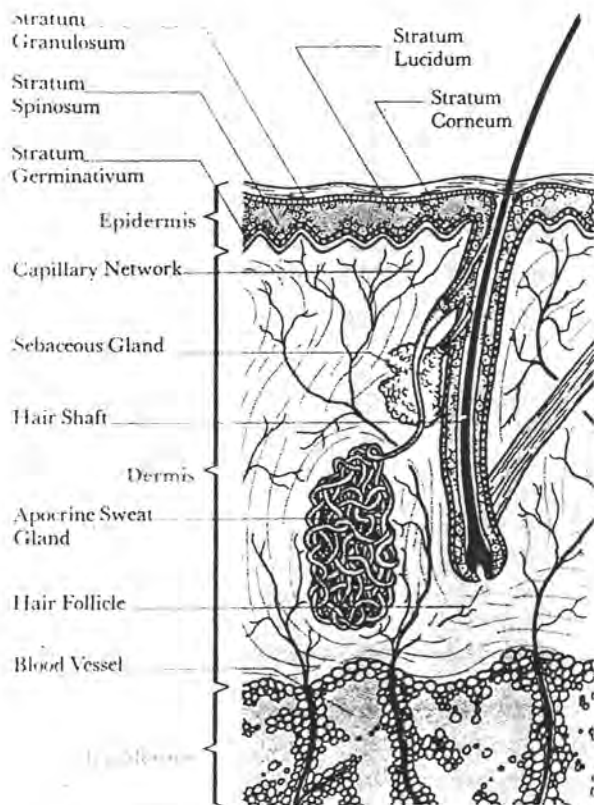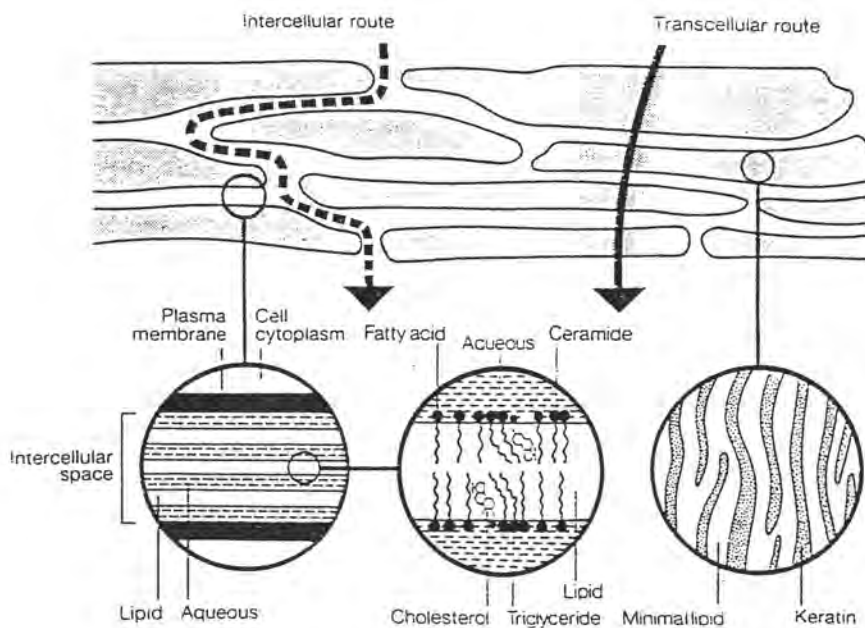
Figure 1: Component of skin structure

Figure 2: The three potential routes of penetration of a diffusant into the subepidermal tissue of skin

Some concept of the complexity of percutaneous absorption may be gained by considering Figure 2. This diagram represents a simple idealization of the drug flux which may arise clinically following the common treatment in which we apply a drug to the skin as a solid suspension in a topical vehicle.

The medicament may undergo any or all of the following events. The drug particles must first dissolve so that molecules may diffuse within the vehicle to reach the vehicle-stratum corneum interface. Interfacial effects are not usually considered important, but for the drug to move through the skin, it must partition into the stratum corneum or horny layer and diffuse within this very impermeable barrier. Some of the drug may bind at a so-called depot site whereas the remainder diffuses in the horny layer, meets a second interfacial barrier, and partitions into the viable epidermis. Although the initial partition process may have a favored and increased flux, the second partitioning will be unfavorable as the viable epidermis provides a more hydrophilic milieu as opposed to the stratum corneum. Any substance with a high affinity for the horny layer and a very low water solubility may not be absorbed percutaneously through the viable epidermis even though it may have penetrated the barrier layer, particularly when it is applied in low concentrations.

The thermodynamic activity of the diffusant in the viable epidermis immediately below the barrier may approach that in the vehicle and in the top layer of the stratum corneum. Now the rate determining step will not be the penetration of the barrier but rather the clearance rate from the barrier. Metabolism may alter diffusion in the viable epidermis.

The epidermis-to-dermis partition coefficient may usually be assumed to be

close to 1 and may be neglected as both tissues have a high water content. Within the dermis, additional depot regions and metabolic sites may intervene in the progress of the drug to a blood capillary, its partitioning into the capillary wall, thence out into the blood, and its subsequent removal by the systemic circulation. Very little is known about equilibration in the subepidermal environment and the pharmacokinetic factors which operate the dermis. Such the knowledge would be particularly important for prodrugs designed to operate in the dermis. A fraction of the diffusant may partition into the subcutaneous fat to form a further depot (Barry, 1983).

Factors Affecting Skin Permeability Evaluation

The permeability of the skin may change as a function of various factors that must be controlled or accounted for as part of the development of an evaluation protocol. Followings are some of the major factors that must be considerd in assessing skin permeabilities.

Hydration : The presence of moisture enhances the penetration of almost all agents into the skin by opening the compact structure of the stratum corneum. Hydration of the skin can be increased by the hydrating component of the vehicle such as sodium PCA or by the use of an occlusive dressing or hydrophobic ointment (Lindenbaum, 1973).

Age and race : Premature infants may not have a fully developed barrier to percutaneous absorption, resulting in an increased

bioavailability. The stratum corneum of the elderly is also more permeable than that of the young adult.

Skin condition : The condition of the skin can play an important role in percutaneous absorption. Studies in patients with psoriasis, a disease characterized by abnormal keratinization and increased blood flow, have shown greater penetration of corticosteroids into the skin, and increased excretion of the drugs in the urine (Shaw et al, 1973). Thus, considerations may be given to conducting the studies, both in the normal and in the diseased or damaged skin.

Site of application : The permeation of a given compound through the skin may or may not be affected by the site of topical application. There were many studies have demonstrated that there was a significant difference in the permeability of skin to certain steroids applied to different locations of the body (Aoyagi et al, 1992). Thus, the site of application should be standardized and controlled during any study.

Despite of the above complicating factors, evaluation of percutaneous absorption of drugs from topical semisolid preparations can often be simplified by monitoring two consecutive steps. The first is the release of the drug from the vehicle and the second is its penetration through the skin barrier. In most cases, absorption is controlled by the second parameter as it is the slower event. However, in some cases where the drug solubility and its diffusion coefficient in the vehicle are very small, the release rate of the drug can become more influential (Abdon et al, 1989).

Methods to Evaluate Percutaneous Drug Absorption and Topical Bioavailability

Main methods for measuring the percutaneous absorption of drug are the *in vitro* diffusion/permeation studies and *in vivo* permeation studies in animal and humans.

1. *In Vitro* Permeation Studies.

Percutaneus absorption studies are routinely conducted by *in vitro* methods utilizing skin membranes mounted in a diffusion cell. The purpose of those studies is to evaluate the *in vitro* evaluation characteristics of a topically applied drug through an excised skin specimen instead of using live animals or human subjects. The results from the *in vitro* permeation studies are expected to reflect the percutaneous absorption of drug that actually occurs *in vivo*. The main advantage of this technique over the *in vivo* method is the simplicity of the experiments. The data obtained are usually more reproducible. However, their predictive value of the *in vivo* results may not always be reliable, depending on the skin model and experimental conditions employed.

In general, an excised skin from common laboratory animals is often used in *in vitro* permeation experiments and the results then extrapolated to human (Regnier et al, 1993). A variety of model membranes have been used for transdermal research such as human cadaver skin, hairless mouse skin, and synthetic membranes. Although human skin is the best model membrane, the cost and limited availability put a limitation on its use. Also, the permeability through human skin varies up to ten folds

depending on the body site. On the other hand, it is easy to obtain animal skins of the same species with the same line and age. However, the time for using in the experiments of some animal skins could be limited because of deterioration of membrane integrity after prolonged use. Moreover, most animal skins of mammalian type such as pig, rat and monkey skins were more permeable than human skin because of a large number of hair follicles (Itoh et al, 1990).

Another interestng type of animal skin is the shed snake skin, which is a nonliving pure stratum corneum with no hair follicles. Snakes shed their skins periodically, leaving their old stratum corneum behind, which makes it possible to obtain multiple shed skins from the same individual snake. Unlike human stratum corneum, which consists of 10-20 layers of an α - keratin-rich intracellular layer and a lipid-rich intercellular layer, shed snake skin consists of three distinctive layers(Landman et al, 1981). As shown in Figure 3 ; these are the β - keratin-rich outermost beta layer, α - keratin and lipid-rich intermediate mesos layer and α - keratin-rich innermost alpha layer. Further, the mesos layer shows three to five layers of multilayer structure with cornified cells surrounded by intercellular lipids, which is similar to human stratum corneum. The mesos layer is also a major depot of lipids and both the mesos and the alpha layers are considered to be the main barrier to water penetration through the skin (Itoh et al, 1990).

Shed snake skins of *Elaphe obsoleta* (Black rat snake) have been used in this study. The similarities between the shed snake skin of this species and the human stratum corneum are their thickness, lipid contents, water evaporation rates, and water permeability, suggesting the possibility
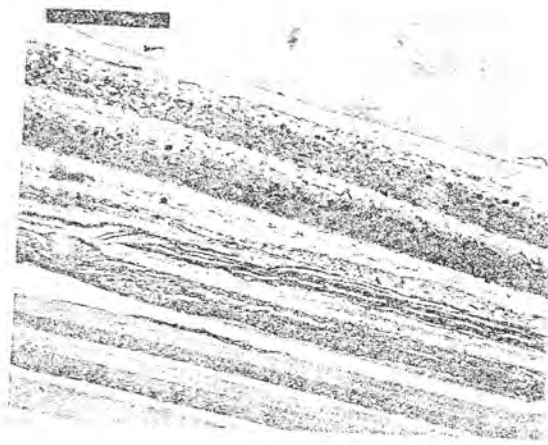
Figure 3: Component of shed snake skin structure (*Elaphe obsoleta)*

that the shed snake skin may offer a good model membrane as an alternate to the conventional animal skins commonly used in transdermal drug delivery research.

Another type of membrane using in *in vitro* permeation studies is synthetic membrane. There are many reasons for using synthetic membranes instead of biological membranes. For reducing the number of variables due to the result of biological membranes and to overcome the difficulty to obtain and storage.

Polydimethylsiloxane membranes(Silastic® membrane) have been much used for drug permeation studies because they are hydrophobic, relativily highly permeable to unionized species and are easily to prepare (Bottari et al ,1977 ; Hadgraft et al , 1992). The permeate dissolves in the barrier matrix and diffuses across silicone rubber is highly permeable to many drugs because the high segmental chain mobility readily forms passages for diffusion.

Cellulose acetate membranes have also been used to probe physicochemical effects (Barry et al, 1976). A series of steroids was examined and it was found that the least polar compound permeated the fastest.

The skin is a multilaminate system and need the model for permeation process across both lipophilic and hydrophilic regions. It is possible to combind polymer types to produce composites which posses serial lipophilic and hydrophilic phase. Young et al (1978) have developed a trilaminate in which hydrophobic (dimethylpolysiloxane) is

sandwiched between cellulose acetate. It ,therefore represents a serial combination of the two previously discussed membranes. In the study of three permeant molecules : water, salicylic acid and hydrocortisone. There is a good correlation between permeability coefficient and skin permeability for this limited number of compounds.

Membranes may also be obtained from biological materials, such as collagen (Nakano et al, 1976). A promising approach uses egg shell membranes, since like the stratum corneum which consisted mainly of keratin. This membrane behaves as a dialysis medium similar to cellulose acetate ; treated with isopropyl myristate to simulate the lipid phase of the horny layer. Permeability through this membrane was significantly greater than that from human stratum corneum (Hadgraft et al, 1992).

2. *In Vitro* Release Studies.

The *in vitro* experiments carried out with samples obtained from animal skins have shown that the stratum corneum represents a limiting step for the percutaneus absorption of drugs. Despite recently published papers demonstrating good correlation between data obtained from the *in vitro* permeation studies utilizing natural membranes, problems are still present including intrinsic biological variability, availability and the need to pretreat the skin before use(Young et al, 1987).

2.1 Types of Synthetic Membranes

With the aim of finding alternative experimental membrane models to overcome the disadvantage associated with the skin usage,

different synthetic membranes have been utilized to study the percutaneous absorption of topically applied drugs (Shah et al, 1991 ; Hatanaka et al, 1992). Apart from substituting the animal membranes in the permeation studies, the synthetic membranes can be used to evaluate the release characteristics of the drug from a topical formulation. For this purpose, a suitable synthetic membrane must be without significant diffusion barrier effects on the transport of a test compound from a formulation into the receptor fluid. The use of synthetic membranes for the support of topical products in diffusion cells is a simple and convenient technique for evaluating the release of drugs from topical formulation (Wu et al, 1992) and the relatively large amount of drug delivered across the membrane from the vehicle could be explained by the good solubility of the drug in the vehicle as well as the fatty nature of the membrane (Niazy, 1990).

2.1.1 Cellulose Acetate Membrane

Cellulose acetate membrane has been used in the drug release profile studies from creams. Celluose acetate membrane actually consists of cellulose acetate and cellulose nitrate. It was not pure cellulose acetate because EPA has banned some of the chemicals used in the production, therefore in this experiment, we use a MF-Millipore® membrane which is a combination of cellulose acetate and cellulose nitrate. This membrane is biologically inert and resistant to high temperature (about $121°C$). It is incompatible with ketone, strong acid or strong base. This membrane has been used in the drug releasing study from creams, lotions and ointment such as the release of hydrocortisone,

dexamethasone, testosterone, progesterone, benzocaine (Barry et al, 1983 ; Wu et al, 1992) from the ointment bases or hydrocortisone release from the cream formulations.

2.1.2 Durapore® Membrane

Durapore® membrane is a polytetrafluoroethylene membrane. It has 0.45 μm- pore size and hydrophobic property. The long protein-binding properties of Durapore® make them ideal for many biological applications. It is compatible with most chemicals including concentrated ketones, amines and ester.

3. *In Vivo* Permeation Studies

The *in vivo* permeation studies of diclofenac gels are usually determined directly by measuring the diclofenac concentrations in synovial fluids and plasma samples after cutaneous application in healthy subjects or in patients with joint diseases. In human studies however, the plasma levels of diclofenac are very low and often need a special analytical technique to detect this very low level (usually in nanogram per milliliter range), such as a gas chromatographic method with electron capture detection following formation of the pentafluorobenzyl ester and using the tracer (^{14}C-diclofenac) as an internal standard (Radermacher et al, 1991).

3.1 *In vivo* Pharmacological Response

The other *in vivo* studies include the use of pharmacological signs or clinical parameters such as joint circumference and joint mobility determined by the Neutral Zero Method (Macloid and Munro, 1986) as an indicator of percutaneous absorption. However, these

techniques are meaningful only when products can elicit adequate difference in the sign or response.

Clinical evaluation of topical NSAIDs has proceeded for several years. The value of these agents has been very limited in clinical practice. However, the attempt to develop effective topical NSAIDs continue because of the increased knowledge about the importance of prostaglandins and other derivatives. There are existing methods for discovery and definition of tradition activity of NSAIDs. The methodology that could lead to NSAIDs with an improved an usual activity spectrum remains undefined. Good NSAIDs activity couple with an improve activity profile in animal models enhances the odds that biologically and structurally novel NSAIDs will be tested clinically, and therefore improves the odds discovery in human advantageous therapeutic activity (Oterness et al, 1985).

3.1.1 Carragenan Foot or Paw Edema Test

One of the cardinal sign of inflammation is the pressent of edema. The edema tests are among the most prominant models used to assess the efficacy of drugs for treating inflammatory. The choice of irritant was at one time problematical. Egg white, formalin, dextran, kaolin, cantharidine and yeast were once commonly used agents (Winter et al , 1965; Boris et al ,1977). Each of these irritants failed to provided an adequate assay of anti-inflammatory drugs for one or more of the following reason: failure of the edema to be inhibited by NSAIDs in a dose-responsive manner, sensitivity of the model to classes of drugs other than NSAIDs (Oterness et al, 1985).

3.1.2 Ultraviolet Light-Induced Erythema Test.

One of the major consequences of inflammation is increased blood flow through the inflamed tissue. In the skin, increased blood flow causes a local reddening of the tissue and an increase in skin temperature (local hyperthermia). Erythema can simply mean a shift of blood closer to the surface of the skin rather than a true increase in blood content (Argenbright et al, 1982). A number of drug-testing paradigms have been based on the induction of erythema by irradiation with ultraviolet light. Other modes of induction have also been utilized: the tuberculin reaction in sensitized guinea pigs, the reaction to certain chemicals such as tetrahydrofurfuryl alcohol and retinoic acid (Heilmayer et al,1965 ; Haining, 1963 ; Ziboh et al, 1975). The erythrema induced by UV light has been extensively used for drug testing in animal. Most of the experimental studies have examined the ability to suppress UV-induced reactions. It has been shown that a single application of indomethacin either immediately before or after UVB radiation reduced significantly the degree of erythema but the delayed erythema following ultraviolet radiation is not suppressed (Snyder, 1975).

3.1.3 Exudative Models of Inflammation.

Animal models of acute inflammation such as the carragenan foot edema test and UV light-induced erythema test are useful for identifying and characterizing NSAIDs. However, these assay monitor only gross change such as edema or erythema. More specific measurements of the changes at an inflamed site are needed. The subcutaneous implantation of sponges or the intrapleural injection of carragenan in rodents provices inflammatory exudates that are easily samples. The mediators of the inflammatory response can be identified and measured conveniently in the exudate fluid. In general results, it

appears that conventional NSAIDs inhibit the synthesis of Pgs *in vivo* but fail to inhibit cell accumulation in the sponge model, except at doses higher than those found to inhibit the PG biosynthesis.

3.1.4 Adjuvant-induced arthritis

There are two types of arthritis studies : The preventive and the curative studies. These procedures use an emulsion of *Mycobacterium Butyricum* in mineral oil inoculated subplantarly into the hindpaw of rats. In the preventive studies, the topical preparation was applied to the inoculated paw at day 1 to day 21 after innoculated the emulsion and the curative studies, the topical NSAID was applied from day 15 to 36 after innoculate. The arthritis score was determined in both types of studies and it wes rate on a 6-point scale, the results of the treatment by using 1.16 % diclofenac diethylammonium in emulgel and 1 % of indomethacin in solution prepration can effectively prevent swelling in both acute and chronic phase of arthritis. And in the curative studies, both drugs can reduced paw volume about 40-50 % during the first seven days of the treatment , and decrease paw volume about 20 % during the entire treatment periods (Hiramatsu et al , 1990).

3.2 *In Vivo* Permeation Study by Studying Drug Content in Skin

Another *in vivo* permeation study is measuring the bioavailabi -lity of topical preparation using drug contents in skin (Pershing et al, 1992). This *in vivo* technique has been developed by determining the drug content in human stratum corneum following topical application. The stratum corneum is removed from the skin surface by means of an adhesive tape strip. Using this technique, correlation was found between the amount of corticosteroid remaining in stratum corneum and the

degree of skin blanching produced by the drug ($r^2 = 0.9935$) (Pershing et al, 1992).

The skin stripping technique is the method for identifying the distribution of the penetrant within the skin during the course of penetration, during which successive layers of the stratum corneum are removed using a series of adhesive tape strips. The consecutive strips are then analyzed for the amount of penetrant to produce a gradient of penetration versus number of strips. It must be assumed that the uppermost, drier cell layers will show good adhesion to the tape, where as the lower, more moist layers will show less adhesion, resulting in lesser amount of the deeper tissue being removed. Osamura et al (1984) illustrated this point by showing that half of the stratum corneum was removed by the first tape strip. Almost all of the stratum corneum had been removed by the third strip. Yet it took approximately ten tape strips to remove the lower layers of the stratum corneum. Simple gravimetric studies may well show equal weights of tissue per strip as increasing water content with depth compensates for the reduction in tissue removed. Tape stripping show qualitative gradients through the uppermost layer of the skin.

Rougier et al(1983) studied the *in vivo* relationship between stratum corneum concentration and percutaneous absorption in rats by using radioactivly-labeled benzoic acid, acetylsalicylic acid, dehydroepiandro- sterone, sodium salicylate, testosterone, hydrocortisone, and dexamethasone as model drugs. Twelve female, hairless Sprague-Dawley rats were used for each drug. Each animal was applied with the drugs (200 nmol/cm^2) on the 1 cm^2 area of the dorsal skin during 30 min.

At the end of application, the excess product on the treated area was rapidly removed by two washings with ethonol/water (95:5), followed by two rinsings with distilled water, and light drying with cotton wool. The twelve animals were then devided into two groups for each drug (Figure 4). The animals of group 1 were individually placed in cages for four days. Urinary excretion and feces were colleced daily, pooled and the amount of drug was analysed during this period. Four days later, the animals were sacrified by cervical dislocation. Series of 6 strippings using 3M adhesive tape were performed on the treated area in order to determine the amount of product not passed through the stratum corneum barrier within four days. The remaining skin of the treated area (epidermis and dermis)was sampled and counted by liquid scintillation after digestion. The animals were lyophilized, homogenized, and the sample were counted in liquid scintillation after combustion. The total amount of chemical penetrating through stratum corneum within four days was then determined by adding the amounts found in the excreta, in the epidermis and dermis of the application area, and in the whole animal body. The second group of rats, after the application and washing, the stratum corneum of the treated area of the animals was removed by six stripping, using 3M adhesive tape. The radioactivity on each strip was analyzed after digestion of keratinic material by liquid scintillation counting. The percutaneous absorption results show that after four days, the drugs are classified according to a decreasing order of penetration rate, the order, which was observed is similar to that found in the studies in human (Feldmann and Maibach, 1969, 1970). In addition, there was a strong correlation between the amount of chemical penetration within 4 days and the amount found in the stratum corneum after 30 minutes application time ($r = 0.98$, $p < 0.001$).

Determination of the four days
penetration and the stratum corneum
reservoir on thirty minutes application,
for each molecule.

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                    │   RATS AT THE TERM       │
                    │ OF APPLICATION PROCEDURE │
                    └─────────────────────────┘
                       ↙                    ↘
              GROUP ①                         GROUP ②
               n = 6                            n = 6
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   │ URINARY AND  │  │  SACRIFICE:  │   │  6 SUCCESSIVE    │
   │FECAL EXCRETION│ │ AMOUNTS IN THE│  │  STRIPPINGS ON   │
   │ DURING 4 DAYS│  │  WHOLE BODY  │   │ EACH RAT TREATED │
   └──────────────┘  │(S.C. EXCEPTED)│  │      AREA        │
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              │   TOTAL PENETRATED   │  │ TOTAL AMOUNTS PRESENT │
              │ AMOUNTS WITHIN 4 DAYS│  │ IN THE S.C. RESERVOIR │
              └──────────────────────┘  │ AT 30 MIN APPLICATION │
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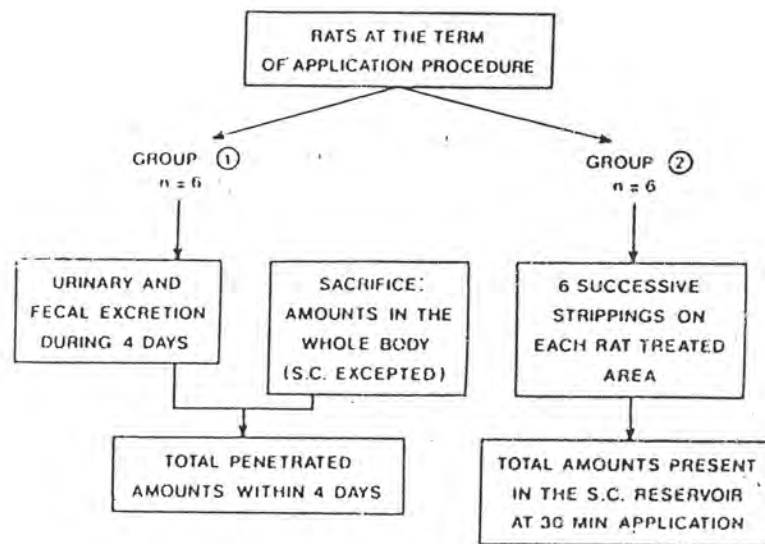
Figure 4: Procedures for determining total percutaneous absorption
with four drugs ,and the stratum corneum reservoir at the
end of application

3.2.1 <u>Factors relating to application conditions which may affect the relationship between stratum corneum concentration and percutaneous absorption</u> (Rougier et al, 1983).

3.2.1.1. <u>Application time</u>

The duration of application of a compound may influence the total amount absorbed. In a study of percutaneous absorption of four radiolabelled compounds : theophylline, nicotinic acid, acetylsalicylic acid and benzoic acid in hairless rats, 1,000 nmol of each compound was applied onto 1 cm^2 of dorsal skin during 0.5, 2, 4, 6 hours. As a result, the penetration amount of the tested compounds is strictly proportional to the duration of application (r = 0.98,p < .001) i.e. the longer the application, the greater the amount absorbed (Rougier et al, 1985).

3.2.1.2. <u>Dose Applied</u>.

It is well known that increasing the concentration of an applied chemical on the skin increases percutaneous penetration (Maibach and Feldmann, 1969; Scheuplein and Ross, 1974; Water and Maibach, 1976).

Percutaneous absorption of four radiolabeled compounds ; theophylline, nicotinic acid, acetylsalicylic acid and benzoic acid, dissolved in ethylene glycol / Triton X-100 (90:10) was studied in hairless rats. For each compound, increasing doses from 125 to 1,000 nmol were applied on 1 cm^2 area of dorsal skin for 30 minutes. The total percutaneous absorption was assessed for each drug at each dose within 7 days as well as the amount of drug in the stratum corneum reservoir at the

end of the 30 minute application time. As a result, within the limits of the concentrations used, there is generally a linear dose-penetration relationship ($r = 0.98$, $p < 0.001$) existing between the dose applied and the percutaneous absorption level and the amount of drug in the stratum corneum (Rougier et al, 1985).

3.2.1.3. Vehicle

It is now well established that substances added to formulations as excipients and other factor such as the physical form of the drug not only affect its release and absorption, but also its pharmacological action. However, very few techniques are available for routine use in elucidating the role that a vehicle or a component in a vehicle may have on the overall absorption of a drug *in vivo*. Applied vehicles have the mechanism of either increasing or decreasing the quantity of water in the horny layer and, thereby, increasing or decreasing the percutaneous absorption. So, the influence of various vehicle components on the *in vivo* penetration level of a chemical can be easily predicted by simply stripping the treated area and measuring the amount engaged in the stratum corneum at the end of application.

3.2.1.4. Anatomical site.

The anatomical location is of great importance for both the *in vitro* and *in vivo* studies. There is connection among the observed differences in the structure of the skin and the physicochemical nature of the penetrant. In the percutaneous absorption study of four radiolabeled compounds (acetylsalicylic acid, benzoic acid, caffeine and sodium benzoate), each drug was measured in human subjects on four body sites, i.e. arm, abdomen, post-auricular and forehead, using skin

stripping method (Rougier et al, 1987). 1,000 nmol of each compound was applied to an area of 1 cm^2, in 20 µl of ethylene glycol/water/Triton X-100 mixtures. Appearent skin permeability was found to be as follows : arm $\leq$ abdomen $<$ post-auricular $<$ forehead. A possible explanation of the higher penetration in areas where there are sebaceous glands, such as forehead, could be that absorption occurs through the follicles, rather than through the epidermis. In another human study of regional variation in percutaneous penetration using ^{14}C cortisol as a model drug (Feldmann et al, 1967), it was found that the absorption was increased in areas where follicles were larger or more numerous and decreased where the stratum corneum was thicker.

The stripping technique has a lot of benefits. For example, it would be easier to screen new drugs in animals and predict their toxicological or pharmacological implications. With this technique, it would also circumvent some ethical difficulties of human experiments using plasma data. It is self-evident that the *in vivo* investigations in humans are preferable to the *in vitro* methods. However, the experimentor has a much higher degree of responsibility when performing the *in vivo* percutaneous absorption studies in human subjects. Moreover, the blood and urine analyses generally used in the *in vivo* methods involve several technical problems due to the low concentrations that must be assayed. Radiolabeled compounds are detected with high sensitivity but imply ethical problems when applied to humans. On the other hand, because of the relatively large amount of substance present in the stratum corneum at the end of application, it should be possible to measure percutaneous absorption of topically applied drug in both animals and humans by a

simple skin stripping technique without seeking resource to the use of radioactive compounds or sophisticated plasma drug analysis.