CHAPTER II

REVIEW OF LITERATURE

Permeation Studies.

An ideal way to determine the transdermal delivery potential of a compound in man is to do an actual study in man of which the result is most relevant to a clinical situation. However, the *in vivo* studies in man encounter many problems such as cost, toxicity and complexity of such studies. Therefore, preclinical studies are needed. This involves either the *in vivo* studies in animals or the *in vitro* studies using animal skins.

1. *In Vivo* Percutaneous Absorption Studies in Man (Wester and Maibach, 1987).

The *in vivo* percutaneous absorption in man is usually determined by an indirect method of measuring radioactivity in excreta following a topical application of a labeled compound. Because plasma levels of a compound are extremely low and usually below an assay detection level, it is necessary to use the tracer methodology. The labeled compound, usually carbon-14 or tritium, is applied to the skin. Then the amount of radioactivity excreted in urine is determined. A tracer dose was also given parenterally and the percent radioactivity in the urine is used to correct for the

compound which may be excreted by some other routes and for the compound which may be retained within the body.

An equation used to determine the relative bioavailability of the percutaneous absorption is

$$\text{Percent radioactivity} = \frac{\text{Total radioactivity following topical administration}}{\text{Total radioactivity following parenteral administration}} \times 100 \quad (1)$$

The only way to determine the absolute bioavailability of a topically applied compound is to measure the compound by a specific assay in blood or urine following topical and intravenous administrations. This may be extremely difficult to do because plasma concentrations after the topical administration are often very low. However, as analytical methodology brings more sensitive assays, estimates of the absolute topical bioavailability will become a reality.

Another approach taken to determine the *in vivo* percutaneous absorption is to determine the loss of material from skin surface as it penetrates into the skin. However, the skin recovery from an ointment or solution application is difficult because total recovery of compound from the skin is never assured.

Another *in vivo* method of estimating absorption is to use a biological/ pharmacological response. An obvious disadvantage of the use of biological responses is that they are limited to compounds which elicit the response that can be measured easily and accurately.

หอสมุดกลาง สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

Other qualitative methods of estimating the *in vivo* percutaneous absorption include a wholebody autoradiography and fluorescence.

2. *In Vitro* Percutaneous Absorption Studies.

Kligman (1983) stated that *in vitro* data are more credible than *in vivo* data and that if differences do exist, then the *in vivo* data are suspected. Percutaneous absorption can be studied by the *in vitro* technique when it was discovered that a sheet of skin could be excised without loss of its essential membrane qualities. This *in vitro* technique is usually performed by placing a skin section between a donor and a receptor phase of a kind of diffusion cell. The permeation of a chemical from the donor phase to the receptor phase is followed carefully and the permeation rate (flux) is determined either from the depletion of the concentration of the phase containing the chemical (donor phase) or from the accumulating concentration of the chemical in the opposing phase in which the diffusing chemical is received (receiver phase).

There have been a wide variety of diffusion cell designs for *in vitro* measurement of skin permeation. These cells have generally been designed in one of the following two types: side-by-side (bichambers) and vertical *in vivo* mimic diffusion cells.

The side-by-side diffusion cells are useful in delineating mechanisms of permeation under controlled conditions but their usefulness are of more limited in predicting skin permeation *in vivo*. The vertical cells are more versatile because a

wide variety of experimental conditions can be set to gain information useful for the evaluation of formulations ultimately destined for clinical use (Friend, 1992).

An upright-type diffusion cell was first designed by Franz (Franz, 1975). The Franz diffusion cell (Figure 1) is one of the most widely used systems for *in vitro* skin permeation studies. It consists of a small upper (donor) compartment which has a wide opening at the top exposing to the air without any temperature control and a lower (receptor) compartment. The receptor solution in the inner chamber is thermostatted at a desired temperature by circulating water in the outer jacket and maintained at a constant hydrodynamic condition by a tiny magnetic bar rotating at a specific stirring rate. It was designed to simulated the clinical conditions. A skin sample is also sandwiched between the donor and the receptor compartments. The drug permeation through skin is followed by sampling the receptor solution via an open sampling port at a scheduled time interval.

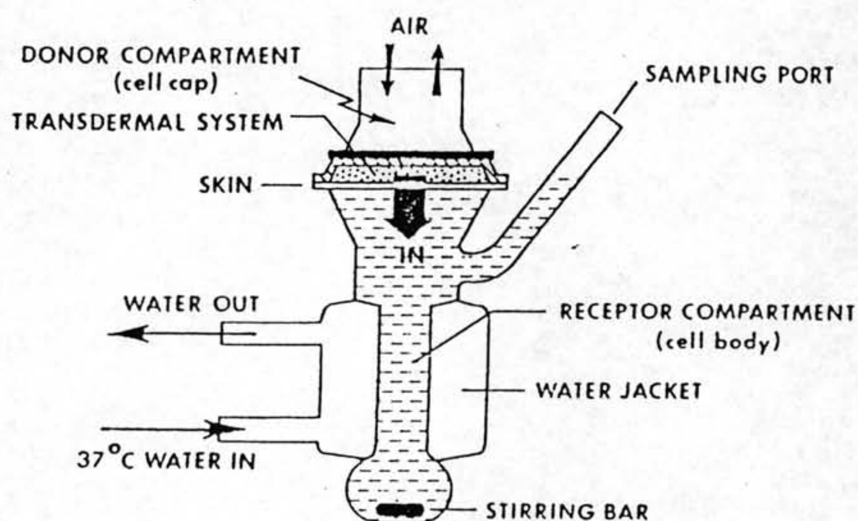


Figure 1: Diagrammatic illustration of the Franz diffusion cell.

2.1 Membranes Used in the *In Vitro* Permeation Studies.

Human skin provides an excellent barrier between external environments and the body. The human skin may be subdivided into three mutually dependent layers (Figure 2): the epidermis which is the outermost layer of the skin, the overlying dermis and the subcutaneous fatty layer (hypodermis).

The stratum corneum (or horny layer), which is the outermost layer of the epidermis, typically comprises 10 to 15 cell layers and is approximately 10 μm thick when it is dry. This membrane, which consists of dead, anucleate and keratinized cells embedded in a lipid matrix, is essential for controlling the percutaneous absorption of most drugs and other chemicals. The stratum corneum is the major rate limiting barrier to molecular diffusion through epidermis (Bartex et al., 1972). The architecture of horny layer may be modeled as a brick-and-mortar structure. In this model, the keratinized corneocytes function as a protein "brick" embedded in a lipid "mortar" (Figure 3).

A molecule may use two diffusional routes to penetrate normal intact skin: the skin appendages (sweat glands and hair follicles) and the intact epidermis. However, the appendages provide a small fraction of surface area which is approximately 0.1% of total skin area and are widely believed to provide an insignificant pathway for most drug permeation. This route may be important for electrolytes and for large polar molecules with low diffusion coefficient such as the polar steroids and antibiotics. The appendages are also important in drug absorption just after application to the skin and prior to the establishment of steady state diffusion (Scheuplein, 1967).

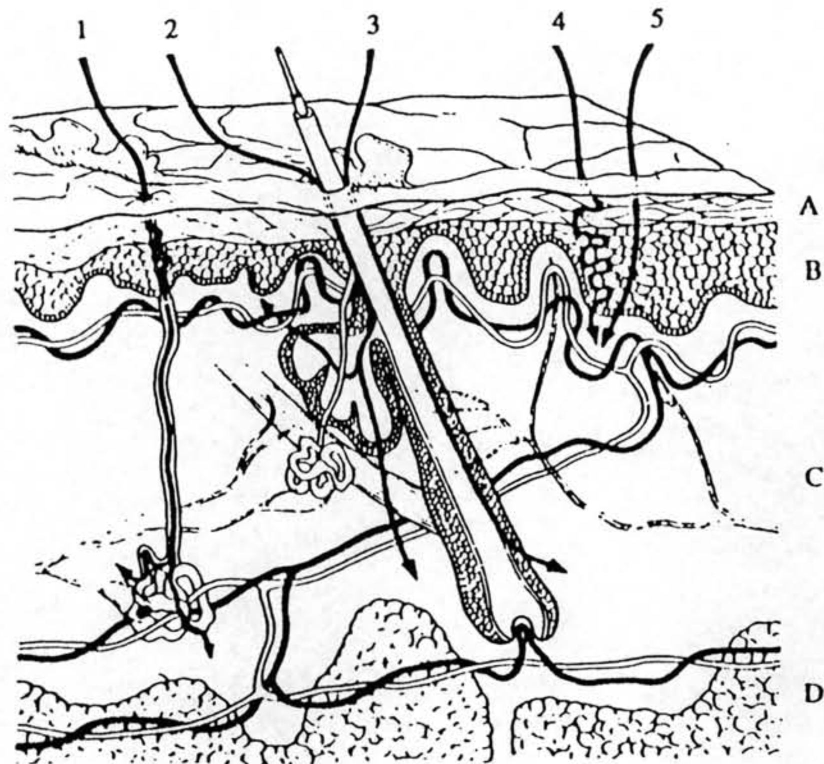


Figure 2: Cross section through skin. A, stratum corneum; B, viable epidermis; C, dermis; D, subcutaneous fat, 1, transeccrine route; 2, transebaceous route; 3, transfollicular route; 4, intercellular route; and 5, transcellular route.

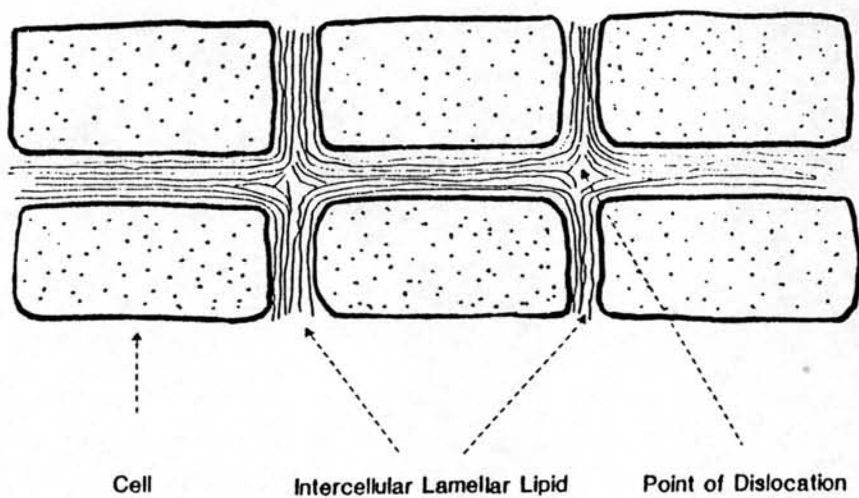


Figure 3: The current concept of the stratum corneum as a two-compartment system in which cells can be analogized to bricks and intercellular lamellar to mortar.

The transepidermal route is the singularly most important route of entry for most drugs. Figure 4 illustrates two potential routes of drug permeation: between the cells (intercellular route) or through the protein filled cells and across lipid-rich regions in tandem (transcellular route). Overall, at least for polar drugs, it is likely that the transcellular route provides the main pathway during percutaneous absorption. As penetrants become more non-polar, the intercellular route probably becomes more significant.

Since tissue is dead, the diffusion is a passive process governed by physicochemical laws in which an active transport mechanism plays no part. Once molecules pass the stratum corneum, they permeate rapidly through the living tissues of the epidermis and dermis and are swept readily into the systemic circulation.

Several investigators (Mc Greesh, 1965; Tregear, 1966; Campbell, 1976; Wester and Maibach, 1987) have determined the permeability through excised skins of different species. The studies generally showed that the skin of common laboratory animals (rabbit, rat and guinea pig) were more permeable than the skin of man. Generally, the skins of pig and monkey approximated the permeability of human skin more closely than the skins of other animals which is in close agreement with the *in vivo* data (Wester and Maibatch, 1987).

Except from the biological membranes mentioned, there have also been synthetic membranes used for percutaneous absorption studies such as Silastic® sheet which has been the one that is frequently used.

Since there is a significant advance in the area of tissue culture, a reliable model of human skin using this technique may be achievable. For example, human keratinocyte cultures grown at the air-liquid interface have been found to develop substantial barrier properties to water diffusion (Mak et al., 1991). Also, constructed human epidermis has been used to examine the nitroglycerin and sucrose permeability (Ponec et al., 1990).

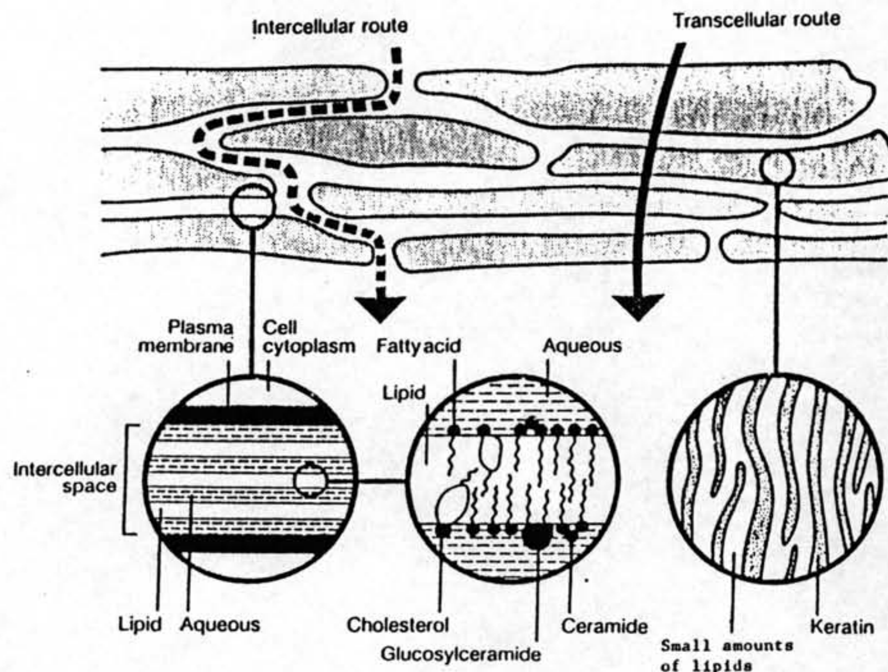


Figure 4: Diagram of possible permeation routes through the stratum corneum.

Membranes used in this study include newborn pig skin, human amnion and human placental membrane. Therefore, they will be mentioned in more details.

2.1.1 Newborn Pig Skin.

Newborn pig skin can be used to model many human skin properties (Bisset and Bride, 1983). Hawkins and Reifenrath (1986) found a good correlation between the diffusion of testosterone through pig skin *in vitro* and that through human skin *in vivo.*

A study of the anatomy of the pig (Sus scrofa) skin is very valuable since the pig skin is more closely related to human skin than skins of other laboratory animals commonly studied. Pigs and men are all backboned animals which places them in the subphylum Vertebrata of the phylum Chordata. Within the vertebrates, pigs and men belong to the same class, the Mamalia, since they have a high level of metabolic activity (warm-bloodedness) to control body temperature and they nurse their young with milk secreted by mammary glands.

The pigs have a gestation age of 112 to 115 days and they are about 12 to 14 inches long at birth. The fetal pigs studied usually range from 10 to 14 inches in length (Walker, 1981).

Frandson (1986) and Warran (1974) found that the skin composition of pig and man are remarkably similar. The pig skin (Figure 6) is divided into three major region: the stratum corneum, the viable epidermis and the dermis. It also has skin appendages like human skin. However, the human and pig skins may have some differences in tissue thickness, hair density, gland density, lipid composition, metabolism etc.

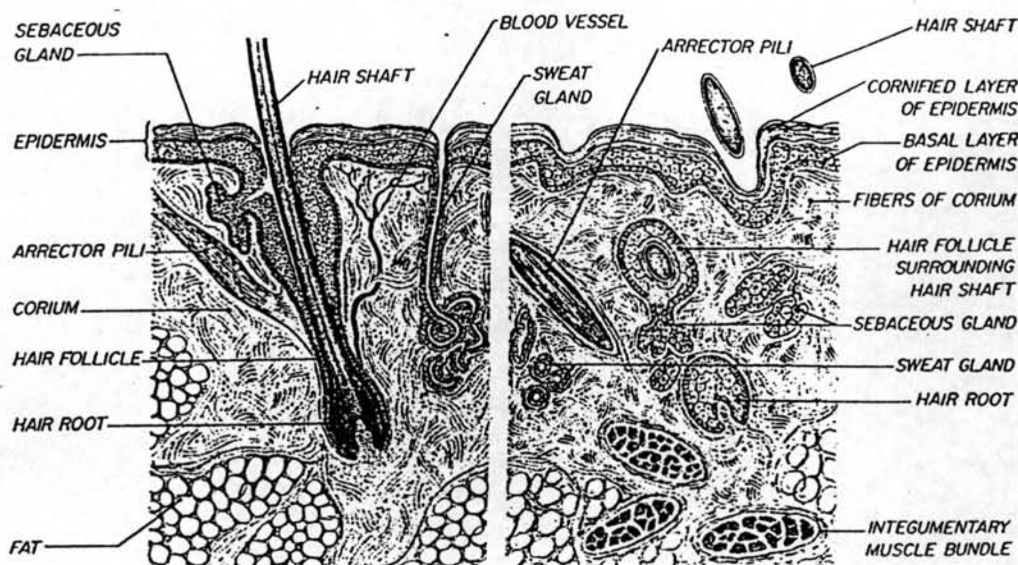


Figure 5: Microscopic vertical section through pig skin: *left,* diagrammatic section: *right,* typical appearance of a slide.

2.1.2 Human Amnion.

Human amnion is a tough, thin, transparent membrane that envelops the embryo (Pansky, 1982; Carola, Harley and Noback, 1990). Amniocentesis is the transabdominal aspiration of fluid from the amniotic sac (the innermost of the membranes developing the embryo in uterus). The amnion's epithelial cells have microvilli which may play a role in fluid transfer. Its interior space, the amniotic cavity, is filled with a watery amniotic fluid which is a suspension consisting of desquamated fetal epithelial cells and equal parts of organic and inorganic salts in 98-99% water. The fluid may initially be secreted by amniotic cells but most comes from the maternal blood. The fetus also contributes the fluid by excreting urine into the amniotic fluid.

The amniotic fluid is very important because the embryo can float freely in the fluid. It permits a symmetric external growth of the embryo, prevents adherence of the amnion to the embryo, cushions the embryo against some trauma, helps controlling the embryonic body temperature, helps the fetus move freely, and aids the skeletomuscular development of the fetus.

The epithelial membrane of human amnion has been used for the treatment of pediatric burns as burn-wound covering. It provides an ideal dressing for partial-thickness burns as temporary skin covering (Thomson et al., 1988).

Since human amnion, which consists of epithelial cells with microvilli, resembles the single layer periderm of human embryo of 2-4 weeks (West et al., 1991), it is very interesting to study the possibility of using the amnion as diffusion barrier of drugs.

2.1.3 Human Placental Membrane (Pansky, 1982; Witherspoon, 1984; Carola, Harley and Noback, 1990)

Human placenta is the most important accessory of fetal structure. It brings the fetal and maternal circulations into close relationship. Morphologically, it is partly of fetal origin (from the trophoblast) and partly of maternal origin (arising from the transformation of the uterine mucosa). The placenta is a disk about 20 cm in diameter, 3 cm in thickness, and about 500 g in weight (about one-sixth of the fetal weight).

The placenta (Figure 6 and 7) has two sides: maternal surface and fetal surface. The maternal surface is the side of placenta that faces the mother. It has grooves and protuberances which are caused by 10 to 30 cotyledons. The irregular surface increases the area for the interchange between fetal and maternal circulation. This rough side has a surface area of about 13 m^2 which is three times greater than the smooth side. This side of placenta grows from decidua basalis which is a part of uterine mucosa.

The fetal surface is relatively smooth with the umbilical cord usually attached somewhere near the center. This side of placenta grows from chorion frondosum of blastocyst cells. The connection between the fetal part of the placenta and the maternal part is closed by thousands of microvilli on the fetal part embedded in the maternal part making an enormous contact surface. The fetal capillaries come closely to maternal capillaries for exchange of substance. There is no actual blood flow between the fetal and maternal circulations and there is no nervous connections.

The placenta has three main functions. Firstly, it transports materials between the mother and the embryo via simple diffusion, facilitated diffusion, active transport and pinocytosis. The transported materials include gases (such as oxygen and carbon dioxide), nutrients (such as water, vitamins and glucose), hormones (especially steroids such as testosterone), antibodies (such as gamma globulin), wastes (such as carbon dioxides, urea, uric acid and bilirubin), drugs and infectious agents (such as rubella, measles, encephalitis, poliomyelitis, and AIDS viruses). Most drugs pass easily especially alcohol. Secondly, it synthesizes glycogen and fatty acids. It probably contributes nutrient and energy to the embryo, especially

during the early stages of pregnancy. Thirdly, it secretes hormones, especially the protein hormones, human chorionic gonadotropin (HCG) and human chorionic somato mammotropin (HCS).

During pregnancy, the placental membrane becomes progressively thinner and by the fourth month, the exchange is favorable. The placental membrane facing the embryo was used in this permeation experiment. The membrane consists of three layers: the syncytiotrophoblast, the fetal vascular endothelium, and a thin sheet of connective tissue.

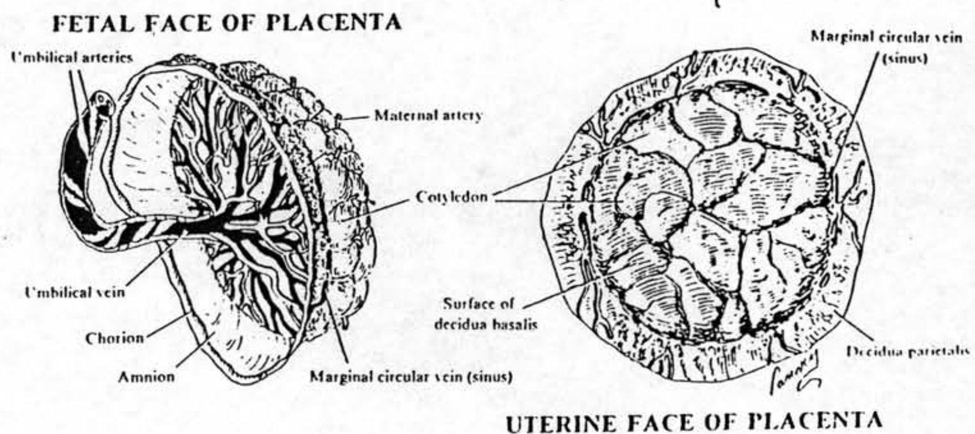


Figure 6: Fetal and uterine faces of placenta.

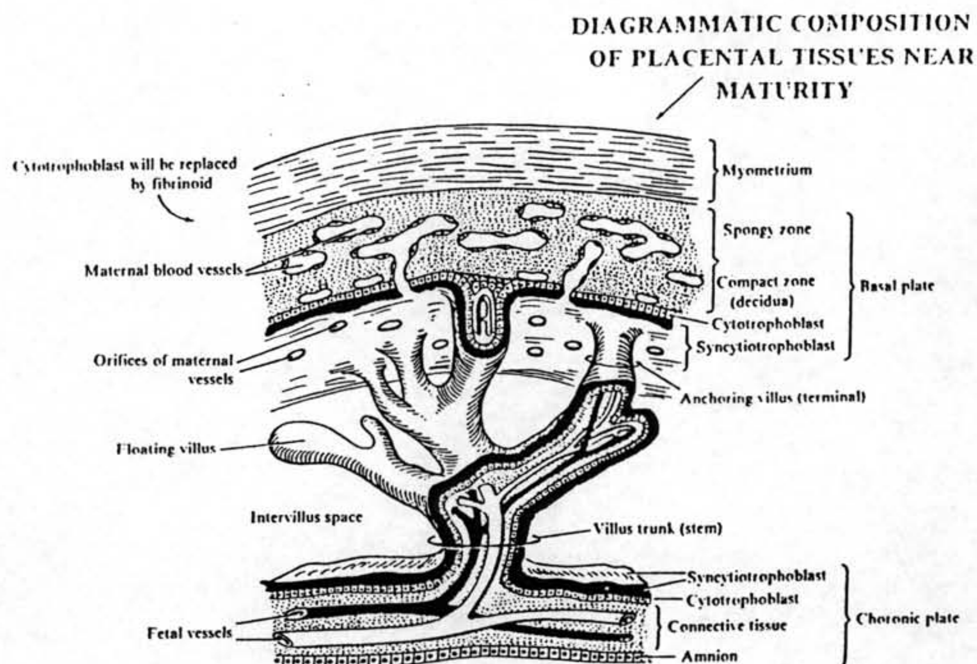


Figure 7: Diagrammatic composition of placental tissue near maturity.

2.2 Factors Influencing the *In Vitro* Percutaneous Absorption Studies.

Some important factors must be considered in an *in vitro* study (Bronaugh and Collier, 1990). These factors are:

1. Maintainance of physiological temperature.
2. Provision for adequate mixing of receptor fluid.
3. Choice of receptor fluid.
4. Preparation of skin.

2.2.1 Maintainance of Physiological Temperature.

The percutaneous absorption of a molecule is temperature dependent. A reasonable guideline is that a doubling in absorption would be expected with a 10 $^{\circ}$C increase in temperature. Although the average body temperature is 37 $^{\circ}$C,

the skin surface temperature is lower, i.e., 34 °C. A physiological temperature desired to be studied will determine the temperature setting. Diffusion cells submerged in a water bath should be heated to the exact temperature required. The circulating water of cells that are jacketed or mounted in heated blocks must be heated to a higher temperature to allow for the loss of heat during the process.

2.2.2 Provision for Adequate Mixing of Receptor Fluid.

A sufficient agitation must be controlled to prevent local concentration of drug and to minimize static diffusion boundary layers, thereby minimize diffusional resistance. In most diffusion systems, this step can be readily accomplished by using some kinds of automatic stirring device, e.g., a magnetic stirring bar. In a flow-through cell with a small receptor volume, the agitation by fluid flowing through the cell can be sufficient to provide adequate mixing.

2.2.3 Choice of Receptor Fluid.

The selection of receptor fluid has become an important factor. The receptor fluid is ideally isotonic saline buffered to pH 7.4. A different pH value can be used if it is justified in terms of physicochemical properties of drugs. Concentrations of penetrant that build up in the receptor phase should not exceed 5% of its concentration in donor phase to maintain the situation of sink condition. It may be necessary, for hydrophobic drugs, to use nonphysiological receptor media. These should be chosen so that they have no effect on the barrier function of the skin.

2.2.4 Preparation of skin.

A technique suitable for preparation of both human and animal skins is the separation of skin by use of a dermatome (Bronaugh and Stewart, 1984). Any hair shafts are remained in the follicles so no leaks are created at the appendageal openings. The hair on animal skins must first be lightly shaved with an electric clipper before the dermatome is used. Care must be taken to avoid cutting too closely and damaging the skin. A depilatory should not be used for hair removing as this procedure is known to damage the barrier properties of skin. Investigators should measure the thickness of skin prior to performing the permeation experiments. Dermatome slices of 200 to 350 μm thickness from surface of the skin have been used routinely in drug permeation studies. A thinner section of skin (200 μm) can be prepared without damaging the barrier properties when a hairless skin is used and for a hairy skin, a thick section (350 μm) should be prepared.

It was found that there was no significant difference between the permeability of human skin stored frozen for over one year and that of fresh skin (Harrison et al., 1984). Franz (1975) also concluded that freezing the skin for up to three months did not damage the barrier properties of the excised skin. It has also been recomended that 10% aqueous glycerin be used for the storage of frozen skin to inhibit the formation of ice crystals which can disrupt cell envelope (Cooper, 1985). Pramod (1986) found that there was no significant difference in steady state rates between fresh and frozen rat skins.

3. Enhancers Currently Studied.

Human skin is a multilayered organ which is complex in structure and function. The outermost layer of epidermis is the stratum corneum which is highly

impermeable. Anatomically, the stratum corneum is a coherent multilaminated membrane consisting of lipids and proteins arranged in a complex interlocking structure similar to brick and mortar. It is breached by hair follicles and sweat ducts which provide parallel diffusion pathway through the stratum corneum. The inner epidermis or viable epidermis, on the other hand, has been characterized as an aqueous gel and is thought not to present a significant barrier to penetration in most circumstances (Scheuplein, 1967).

Chemicals that promote the penetration of a topically applied drug are commonly referred to as accelerants, absorption promoters or penetration enhancers. An approach to increase the drug flux is to incorporate the penetration enhancer. The enhancers incorporated should be ideally safe, nontoxic, pharmacologically inert, nonirritating, and non allergic (Barry, 1983). In addition, the skin tissue should revert to its normal integrity and barrier properties upon removal of the chemical. The ability of the enhancer to increase drug penetration is important but it is critical that this task be accomplished without skin irritation or sensitization. The goal is to find an enhancer that will disrupt the impermeable stratum corneum barrier membrane without destroying the fragile living tissue underneath (Cooper and Berner, 1987).

There are three pathways suggested for drug penetration through the skin: polar, nonpolar, and polar/nonpolar. The enhancers act by altering one of these pathways. A key to altering the polar pathway is to cause protein conformational change or solvent swelling. A key to altering the nonpolar pathway is to alter the rigidity of the lipid structure and fluidize the crystalline pathway that substantially

increases the percutaneous absorption. Some enhancers, binary vehicles, act on both polar and nonpolar pathways by altering the multilaminate pathway (Potts, 1989).

Chemicals used to enhance the skin permeability are as follows:

3.1 Solvents

3.1.1 Water.

In a majority of cases, the hydration of stratum corneum results in a decrease in barrier function. Collective data indicate that the action is mediated by aqueous solvation of polar regions of glycosphingolipids and ceramides. This is supported by the observation that hydration effects are much less in the nail plate which contains less than 1% lipid compared to the 10% lipid content of stratum corneum (Walters et al., 1981).

3.1.2 Alcohols.

Some of low molecular weight alcohols have been shown to possess the ability to enhance permeation across the skin, such as methanol and ethanol (De Noble et. al, 1987).

Several published studies strongly suggest that the enhanced partition of solutes into the stratum corneum is the primary mode of action of dilute

หอสมุดกลาง สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ethanol as a permeation enhancer (Berner et al., 1989; and Pershing et al., 1990). Observations common to the results of many of these studies were

1. The solubility of solutes appeared to be an exponential function of ethanol concentration in the vehicle.

2. The transdermal flux of solutes and ethanol concentration in the donor vehicle appeared to have the same relationship as the solubility of solutes and ethanol concentration.

3. The increased flux of solute across the epidermis or stratum corneum seemed to be directly proportional to its concentration in the tissues. The solubility of which was also increased by ethanol.

4. These linearities between ethanol-induced solubilities of solutes and ethanol concentrations ceased to exist when ethanol concentrations exceed approximately 50 vol% beyond which solute and concomitant ethanol fluxes started to decrease, probably due to ethanol dehydrating effect on skin tissues.

5. The calculated diffusivities of solutes in the stratum corneum did not change appreciably in the presence of dilute ethanolic solution.

3.1.3 Alkyl Methyl Sulfoxides.

The use of alkyl methyl sulfoxides as penetration enhancers begins from early observations on the effects of dimethyl sulfoxide (DMSO). DMSO is a dipolar aprotic solvent which is miscible with both water and organic solvents. The mode of action of DMSO includes extraction of stratum corneum lipids, lipoproteins, and nucleoproteins (Emberry and Dugard, 1971); and osmotically inducing

delamination of the stratum corneum (Chandrasekaran et al., 1977). This type of irreversible alteration of horny layer is one reason curtailing the usefulness of DMSO. Furthermore, a significant permeability enhancement is only obtained when it is present at concentration of more than 70%. This raises the possibility of toxicological complications.

It has been subsequently demonstrated that decyl methyl sulfoxide (C_{10}MSO) is an effective enhancer for polar and ionized molecules. C_{10}MSO is approved for use by the U.S. Food and Drug Administration with ethanol and tetracyline in the treatment of acne (Weber et al., 1985).

Other aprotic solvents which have been shown to affect skin in a similar fashion to DMSO include dimethyl acetamide (DMA) and dimethyl formamide (DMF). However, they are not as effective as DMSO (Monro and Stoughton, 1965).

3.1.4 Pyrrolidones.

The need for skin penetration enhancers with minimal toxic potential has prompted the investigation of naturally occurring compound. A group of compounds collectively known as natural moisturizing factor (NMF) consist mainly of free fatty acids, urea, and pyrrolidone carboxylic acid and its sodium salt. The principal humectant appears to be sodium pyrrolidone carboxylate and this compound has been shown to increase the water-binding capacity of stratum corneum (Barry, 1983). The collective data make it very difficult to assign a specific mode of action of the pyrrolidones. A primary site of action is most likely to be the polar route. And there

is little doubt that the hydration of skin, owing to their intrinsic humectant activities, is a significant factor in their effectiveness concerning the percutaneous absorption.

3.1.5 Laurocapram.

Laurocapram (1-dodecylazacycloheptan-2-one or Azone®) has been shown to be a very effective enhancer for both hydrophilic and hydrophobic drugs (Stoughton and Mc Clure, 1983). The mechanism of action whereby Azone® enhances the skin permeability is that an ionpairing-type mechanism may be operative for some permeants and probably acts by disrupting lipid structure (Hadgraft et al., 1985). A single dose of Azone® is capable of enhancing the permeation of subsequent dose of drug for at least 5 days (Wotton et al., 1985). This indicates that the enhancer preferentially partitions into the stratum corneum and has little tendency to diffuse into the more queous viable epidermis.

3.1.6 Acetone.

Acetone is widely used as a solvent for depositing permeants on the skin during *in vitro* experiments. It can produce a transient acceleration in permeation (Behl et al., 1980).

3.1.7 Tetrahydrofurfuryl Alcohol.

Tetrahydrofurfuryl alcohol can enhance the skin permeability of steroids, presumably because of its ability to solubilize skin lipid. It is, however, an irritant to skin and mucous membranes which restricts its usefulness (Barry, 1983).

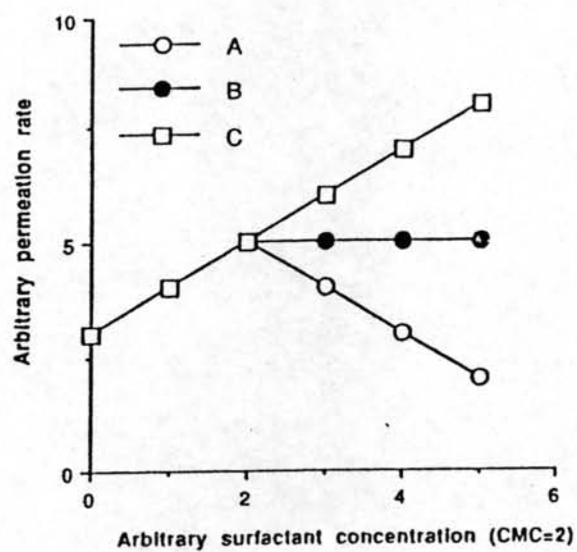
3.1.8 Propylene Glycol.

Propylene glycol enhances the skin permeability of estradiol and metronidazole. It is likely that this liquid functions simply as a cosolvent and produces saturated or nearly saturated solution of the permeant (Wotton et al., 1985).

3.2 Surfactants.

Surfactants are major components of pharmaceutical, cosmetic and food formulations. The classification of these compounds is based on the charge carried by their hydrophilic "head" groups that can be anionic, cationic, or nonionic. Reduction of transport of a permant in a surfactant system is due to the ability of surfactant to form micelles, and is normally observed if an interaction between micelle and permant occurs. It can be considered that an overall effect of the surfactant is the result of two opposing effects: an interaction of the surfactant with the membrane and an interaction of the permeant with the micelle. Thus, there are five possible profiles of surfactant-induced alterations in the permeability of biological membranes (Figure 8). In this figure, profile A is typical of many systems where permeation is increased at premicellar concentrations but a high level of complexation or solubilization of permeant occurs at a micellar concentration resulting in a reduced thermodynamic activity of the permeant in donor medium. Profiles B and C are obtained when the surfactant increases permeability but has little or no affinity for the permeant. For a surfactant with no membrane permeability effect, profile D would be obtained if the micellar interaction occurs, or profile E if the permeant has no affinity for the micelles.

3.2.1 Anionic Surfactants.



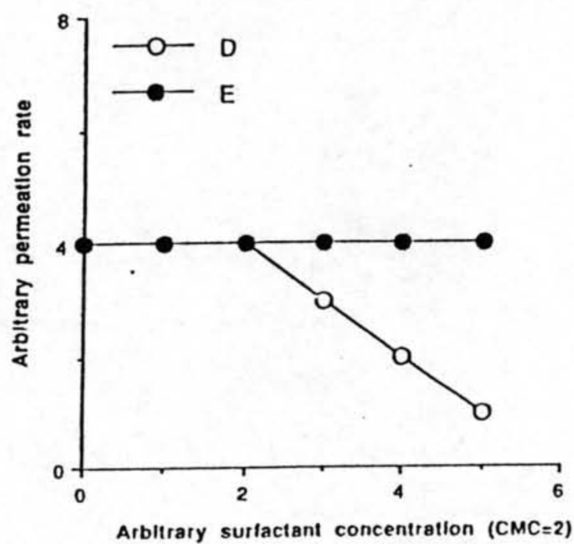


Figure 8: Possible profiles of surfactant-induced alterations in the permeability of biological membranes.

Anionic surfactants can penetrate and interact strongly with skin. The most widely studied surfactants in this group are the alkyl sulfates. Most anionic surfactants can induce swelling of the stratum corneum and viable epidermis (Gibson and Teall, 1983). This is not the case for cationic and nonionic surfactants suggesting that anionic effects play a primary role in swelling mechanism. The hydrophobic interaction of alkyl chain with the substrate leaves the negative end group of surfactant exposed. This results in the development of repulsive forces that separate protein matrix that would also result in a disruption of the long-range order within the keratinocyte, possibly leading to an increased intercellular diffusivity and enhanced percutaneous absorption.

3.2.2 Cationic Surfactants.

Cationic surfactants are reputedly more irritating than the anionics. They have not been widely studied as skin penetration enhancers. The cationic surfactants have been shown to bind more readily to epidermal protein than sodium dodecyl sulfate but do not appear to cause swelling (Wood and Bettley, 1971).

3.2.3 Nonionic Surfactants.

Nonionic surfactants have long been recognized as those with the least potential for irritancy. Although there are many different types of nonionic surfactants, the majority of studies concerning their effects on biological systems are limited to four principal series. These include the polysorbates, polyethoxylated alkyl ethers and esters, polyethoxylated alkyl phenols, and poloxamers.

The collective data of nonionic surfactants suggest that their mode of actions on skin are related to their ability to partition into the intercellular lipid phase of stratum corneum. This results in an increase in fluidity in this region which reduces diffusional resistance. There is an evidence that the lipid monolayer fluidity can be increased when polyoxyethylene alkyl ethers are incorporated (Walters et. al., 1982). The degree of interaction is dependent on alkyl chain length such that those surfactants based on a dodecyl chain cause a greater increase of fluidity. The dodecyl compounds have generally proved to be the most active in biological systems when a series of surfactants with similar hydrophilic portions were studied. A lack of skin permeability-enhancing activity of those surfactants with branched chains or aromatic group in their hydrophobic portions suggests that these compounds are not readily incorporated into the lipid structure of the stratum corneum (Walters et al., 1988).

3.3 Fatty Acids and Alcohols.

Long-chain fatty acids have been shown to be effective penetration enhancers for a variety of drugs (Cooper, 1985). Aungst et al. (1986) examined the effects of a series of saturated fatty acids ranging from heptanoic (C_7) through stearic (C_{18}) acids. A maximum enhancement of flux was obtained by the C_{12} analog, the lauric acid. This was also true for long chain alcohols, but the degree of enhancement was somewhat less.

3.4 Phospholipids.

Liposomes are microscopic vesicles composed of membrane-like lipid bilayers surrounding aqueous compartments. The lipid bilayers are made up of

mainly phospholipids which are amphiphilic with a hydrophilic head and a lipophilic tail. The fatty acid tails of the lipid bilayers are nonpolar part located in the interior of membrane. Whereas, their polar heads point outward. Liposomes penetrate the skin and serve as "drug carriers" even for drugs that would not penetrate the barrier layers of the skin. While the drug is encapsulated in the lipid vesicles, it is not available for absorption into the blood circulation since the lipid vesicles do not penetrate into the blood vessels. The liposome, therefore, can be served as a depot or as a slow-release vesicle within the dermis which is highly vascularized (Mazei, 1994).

3.5 Terpenes.

The term "terpene" usually describes a compound that is a constituent of an essential oil. Terpenoid molecules are designated by their chemical structure, being based on their isoprene (C_5H_8) units.

Effects of a variety of terpenes on percutaneous absorption of indomethacin in rats have been studied (Nagai et al., 1989). For a lipophilic drug like indomethacin, limonene was an effective enhancer at a concentration of 1% in gel. The effectiveness was comparable to the laurocapram (Azone®). The terpenes act in part by modifying intercellular lipids, therefore, disrupting their highly ordered structure to increase diffusivity.

3.6 Miscellaneous Chemicals.

3.6.1 Urea.

Urea is capable of increasing hydration of the stratum corneum and also has keratolytic effect. However, it does not appear to enhance its own permeability rate, suggesting that its principal mode of action is related to its hygroscopic nature. It has been proposed that urea may lower the phase transition temperature of the stratum corneum, causing an increase in its fluidity at ambient temperature (Beastall et al., 1986).

3.6.2 N, N-Dimethyl-m-Toluamide.

N, N-Dimethyl-m-toluamide has been reported to enhance the permeation of a number of drugs across hairless mouse skin. But evidences for its usefulness on human skin are sparse (Windheuser and Haslen, 1982).

3.6.3 Calcium Thioglycolate.

A mechanism of this compound probably involves a reduction of cysteine links, leading to a disruption of keratin matrix. In view of its action on keratin, however, further toxicological evaluation is necessary (Kushida et al., 1984).

4. Theory of Diffusion.

Although mechanisms of drug transport across the skin have yet to be fully elucidated, it is clear that the process is essentially a passive diffusion. This is a phenomenon by which a diffusant moves down a concentration gradient (or more accurately, a chemical potential gradient) by a random molecular motion.

In the situation of a permeant entering the skin, its diffusion is usually considered as a unidirectional process, i.e., the concentration gradient is directed only into the skin. This unidirectional diffusion in an isotropic medium may be explained by Fick's second law of diffusion and expressed mathematically as:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad (2)$$

where C is the concentration of the diffusing substance in the donor solution at time t, x is a position normal to the effective area of diffusion for one-dimensional diffusional process, and D is the diffusion coefficient in membrane. With skin permeation studies *in vitro*, investigators often use a membrane clamped between two compartments, one containing a drug formulation (the donor) and the other containing a receptor solution providing sink conditions (essentially zero concentration). After a period of time, the steady state diffusion across the membrane prevails. Under these conditions Eq. (2) may be simplified to Eq. (3):

$$\frac{dM}{dt} = \frac{D\,Co}{h} \qquad (3)$$

where M is the cumulative mass of permeant that passes per unit area through the membrane in time t, Co is the concentration of diffusant in the first layer of the membrane at the skin surface contacting the donor phase and h is the membrane thickness.

In most diffusion experiments, it is difficult to measure Co. However, the concentration of diffusant in the donor phase bathing the membrane, Co', may be easily determined. Also, Co and Co' are related as shown by Eq. (4):

$$Co = P\,Co' \qquad (4)$$

where P is the partition coefficient of the diffusant between the membrane and the bathing solution. Substitution of Eq. (4) into Eq. (3) yields Eq. (5):

$$\frac{dM}{dt} = \frac{DPCo'}{h} \qquad (5)$$

This is a classic and most important equation used in skin permeation studies. A graph of M, the cumulative amount of drug crossing a unit area of skin, against time yields a profile of drug penetrating the membrane (Figure 9). An extrapolation of the pseudo-steady state portion of the graph to the intercept on the time axis provides the lag time (t_L). This is the period during which the rate of diffusion across the membrane is not constant. Steady state conditions prevail after approximately 2.7 times the lag time (Barry, 1983). The lag time is related to the diffusion coefficient by Eq. (6).

$$t_L = \frac{h^2}{6D} \qquad (6)$$

Thus, in theory, D may be obtained by measuring t_L, providing the membrane thickness, h, is known. In practice, however, this method for evaluating D has several disadvantages as the exact thickness of the stratum corneum is difficult to measure and may vary with penetration enhancer treatment. The measured thickness of membrane does not allow for a tortuous pathway of diffusion and the value obtained for D is therefore an apparent one. Additionally, lag times obtained from permeation experiments using human skin tend to be varied widely and include a component arising from penetrant-horny layer binding.

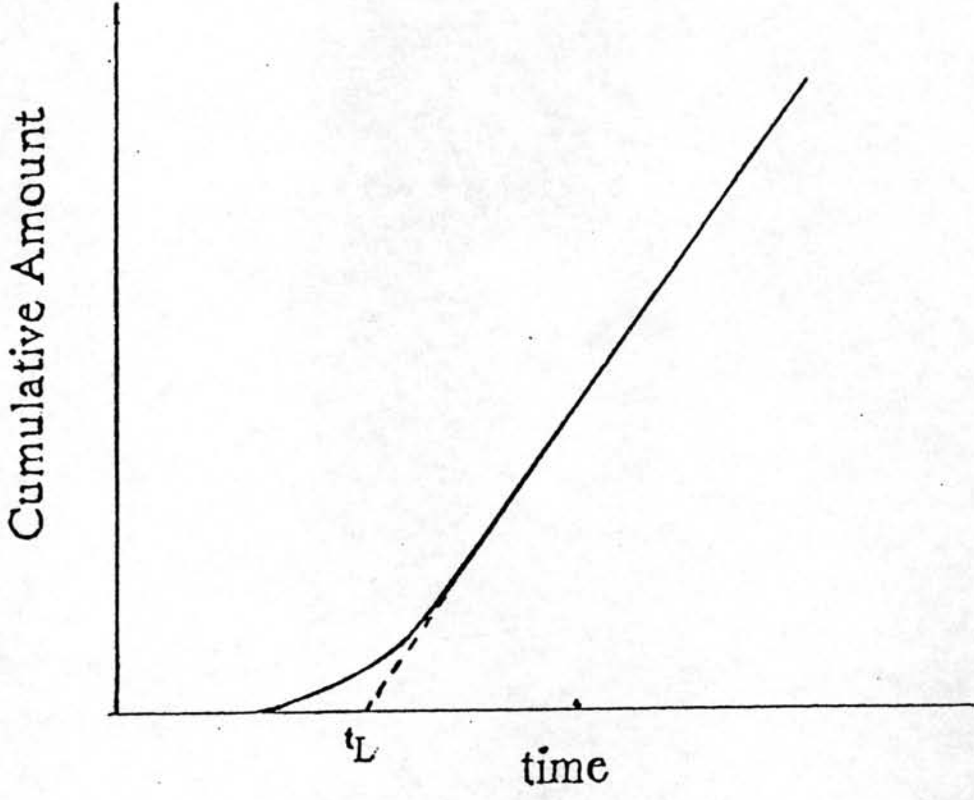


Figure 9: Typical cumulative amount versus time profile.

The permeability coefficient of a diffusant through a membrane, Kp, may be defined by Eq. (7):

$$Kp = \frac{PD}{h} \quad (7)$$

which may be substituted into Eq. (5) to give Eq. (8):

$$\frac{dM}{dt} = J = Kp\, Co' \quad (8)$$

The rate of change of cumulative mass of diffusant that passes per unit area through the membrane, dM/dt, is termed the flux of diffusant, J, and may be evaluated from the steady state portion of the drug permeation profile.

Thus, if the donor concentration and the flux of permeant are known, the permeability coefficient can be determined. The permeability coefficient is widely used to characterize the percutaneous absorption of drugs as it represents the flux of drug per unit skin area per unit concentration.