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pH Dependence of Morphine Absorption in Rabbit Oral Epithelia *in vitro*

by

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(Under the supervision of Professor Joseph R. Robinson)

Abstract

pH dependency of weak acids and weak bases on oral mucosal absorption is still a controversy. Traditional theory holds that the oral mucosa is a hydrophobic barrier. Therefore, the partition coefficient should play an important role in drug absorption. Since the partition coefficient of a weak acid or a weak base is highly pH dependent, the permeability coefficient should also be pH dependent. Many reports support this theory. However, after reviewing the way their experiments were conducted, their conclusions appear questionable. Other reports suggest that oral mucosal drug absorption is pH independent. Using careful experimental approaches, which monitor the drug concentration in the receiver chamber as a measure of drug absorption, the pH dependence of morphine absorption has been studied. The permeability coefficients for buccal tissue are 2.70±0.38, 2.58±0.57, 3.4±1.5 and 3.5±1.3x10^{-7} cm/sec; for sublingual tissue are 3.3±1.1, 4.0±1.6, 4.8±1.7 and 9.6±3.0x10^{-7} cm/sec at pH 6.5, 7.5, 8.5 and 9.5, respectively. The corresponding lag times for buccal tissue are 66.7±4.7, 70.3±3.2, 64.8±7.8 and 58.0±9.0 min; for sublingual tissue are 31.2±7.3, 42.5±3.4, 50±13 and 51.5±6.9 min at pH 6.5, 7.5, 8.5 and 9.5, respectively. Apparently, there is no pH dependency for both buccal and

sublingual tissue, or perhaps, the dependence is small and is obscured by the standard deviation. This result suggests that morphine transport is predominantly through the paracellular route, where the media is hydrophilic and has similar hydrophilicity to the applied solution. The preferential partition of morphine between paracellular media and the applied solution appears to be pH independent. A new model of oral mucosal absorption is proposed to evaluate the effect of stirring, penetrating routes, partition coefficient and diffusion coefficient.

To my parents and brothers

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I. INTRODUCTION

A. Oral Mucosa as a Route of Systemic Drug Delivery

The human body is a system that is separated from the environment by epithelial tissue. Thus, the main functions of epithelia are to maintain integrity of the body and to limit and control material exchange between the body and the outside environment. Epithelia act as a first line of defense to prevent invasion by foreign chemicals, including drugs. Therefore, it is fundamental to drug delivery that the drug must penetrate through epithelia. Drug delivery routes, each with somewhat different epithelia, can be divided into: oral administration (buccal, sublingual and gastrointestinal tract), parenteral injections (intravenous, intramuscular and subcutaneous), transdermal (skin), rectal, nasal, vaginal, etc. (Li, 1987).

The gastrointestinal route, because of its accessibility and ease of administration, is the most common route for drug delivery. The gastrointestinal epithelium has a large surface area and a relatively high permeability. Nevertheless, there are a number of potential problems for oral drug delivery. The gastrointestinal tract is a highly acidic environment and contains many hydrolytic enzymes. All drugs absorbed by this route are subject to hepatic first-pass metabolism. It is also difficult to remove drug from the gastrointestinal tract in an emergency. These properties can limit the use of the gastrointestinal tract as a delivery route for certain drugs, such as peptides.

Parenteral injection via the intravenous, intramuscular or subcutaneous routes is an alternative to mucosal drug delivery. These systems bypass the acidic environment of the stomach, and avoid first-pass

metabolism. Since it produces an immediate high blood level, especially for intravenous injection, it is the most common route for emergency treatment. It is also the most common route for peptide drug delivery. However, parenteral administration is poorly accepted by most patients, especially infants and small children, and is undesirable for chronic use. Parenteral administration is not practical or convenient for self-administration. Aseptic procedures are required during the preparation of drug solutions, and their parenteral administration, which leads to high costs. Drugs administrated parenterally usually have a relatively short blood level residence time, unless IV infusion is used.

The alternatives to parenteral and oral administration include oral mucosal, nasal, rectal, and vaginal administration. Among these alternative drug delivery routes, the oral mucosal administration has its own unique characteristics. The oral mucosa is usually more permeable than skin, and possesses a rich blood supply, which can result in a rapid onset and high drug-blood level. Oral mucosal administration is easily achieved, and patient compliance is better than that for nasal, rectal and vaginal administration. The limitations of oral mucosal administration are that the relatively small surface area may limit the amount of drug absorbed, and the general properties of the epithelia may also limit passage of large molecules.

The oral mucosa has been used for drug delivery for more than a hundred years. In 1847, Sobrero, who discovered nitroglycerin, noted the systemic effects of this drug following absorption of the compound via the oral mucosa. This route became firmly established in 1879 when William

Murrell introduced nitroglycerin drops under the tongue for the treatment of angina pectoris. Since then, many qualitative studies about the absorption of drugs through the oral mucosa have been published. Reviews of this subject have been written by Gibaldi and Kanig in 1965, Squier and Johnson in 1975, Wertz and Squier in 1991 and Harris and Robinson in 1992.

There are many drugs that are delivered clinically via absorption through the oral mucosa and some of these are listed in Table I-1. Many others are under study (Table I-2). Current research in this area has focused on the mechanism of drug penetration and development of suitable delivery systems.

B. Anatomy and Physiology of the Oral Mucosa

The oral cavity can be divided into two regions, the outer oral vestibule and oral cavity proper. The outer oral vestibule consists of the mucosal area between the lips and dental arches, including the lips, buccal mucosa and gingiva. The oral cavity proper consists of the mucosa inside the dental arches, including the hard palate, soft palate, tongue, gingiva, and the floor of the mouth. As shown in Fig. I-1, the various regions have different mucosa, because they have different functions. In regions subject to the mechanical force of mastication, such as the gingiva and hard palate,

Table I-1. Clinical Applications for Systemic Delivery via the Oral Mucosa. (From Drug Facts and Comparisons, 46th ed., 1992)

Compound	Trade Name	Dosage Form	Manufacturer
Nicotine	Nicorette	Chewing Gum	Merrell Dow
Nitroglycerin	Nitrostat	Sublingual Tablet	Parke-Davis
Isosorbide Dinitrate	Isosorbide Dinitrate	Sublingual Tablet	Barr, Geneva Marsam, Goldline, Major, Moore, Schein, URL, Rugby
	Isordil	Sublingual Tablet	Wyeth-Ayerst
	Sorbitrate	Sublingual Tablet	ICI Pharma.
	Sorbitrate	Chewable Tablet	ICI Pharma.
Methyltestosterone	Methyltestosterone	Buccal Tablet	Dixon-Shane, Major, Rugby, Schein
	Android-5	Buccal Tablet	ICI Pharma.
L-Hyoscyamine	Levsin/SL	Sublingual Tablet	Schwarz Pharma Kremers Urban
Isoproterenol	Isuprel Glossets	Sublingual Tablet	Winthrop Pharm.
Ergoloid Mesylates	Ergoloid Mesylates	Sublingual Tablet	Bioline, Bolar, Major, Dixon-Shane, Moore, Goldline, Parmed, Zenith, Qualitest, Geneva
	Gerimal	Sublingual Tablet	Rugby
	Hydergine	Sublingual Tablet	Sandoz
	Niloric	Sublingual Tablet	Ascher
Ergotamine	Ergomar	Sublingual Tablet	Fisons
	Ergostat	Sublingual Tablet	Parke-Davis
	Wigrettes	Sublingual Tablet	Organon
Erythrityl Tetranitrate	Cardilate	Sublingual Tablet	Burroughs Wellcome

Table I-2. Drugs are Under Investigation for Systemic Delivery via the Oral Mucosa

Compound	Region	Clinical Usage	Reference
Alprazolam	Sublingual*	Panic Disorder	Scavone, 1987
Buprenorphine	Sublingual*	Pain Relief	Carl, 1987
Captopril	Sublingual*	Hypertension	Coronel, 1988; Dessi-Fulgheri, 1987
Clindamycin	Buccal*	Infections	Taraszka, 1970
Clonidine	Sublingual*	Hypertension	Gilkeson, 1987
Desmopressin	Sublingual*	Central Diabetes Insipidus(CDI)	Kappy, 1987
Diclofenac	Buccal°	Infections	Ebert, 1987
Diltiazem	Buccal°	Angina Pectoris	Yamahara, 1990
Fentanyl	Sublingual* Buccal*	Anesthesia	Stanley, 1989
Flunarizine	Sublingual*	Magraine Attack	Amery, 1987; Bonuso, 1986 Takeshima, 1987
Flunitrazepam	Sublingual*	Anxiety Disorder	Huttel, 1986
Insulin	Sublingual* Buccal*	Diabetes	Aungst, 1987
Lorazepam	Sublingual*	Anxiety Disorder	Gram-Hansen, 1988; Yager, 1988
Medifoxamine	Buccal*	Depression	Randhawa, 1986
Midazolam	Sublingual*	Anesthesia	Fujii, 1988
Morphine	Buccal*	Pain Relief	Al-Sayed-Oman, 1987; Bell, 1985
Nifedipine	Sublingual*	Hypertension	Abraham, 1986; Brown, 1986
Prazepam	Sublingual*	Panic, Insomnia	Ansseau, 1987
Propranolol	Buccal*	Hypertension	Henry, 1980
Temazepan	Sublingual*	Insomnia	Russell, 1988
TRH	Buccal°	Dwarf	Dowty, 1991
Triazolam	Sublingual*	Insomnia	Scavone, 1986
Verapamil	Buccal*	Arrhythmia	Davis, 1979

* Regions of drug delivery are not controlled. ° Animal models.

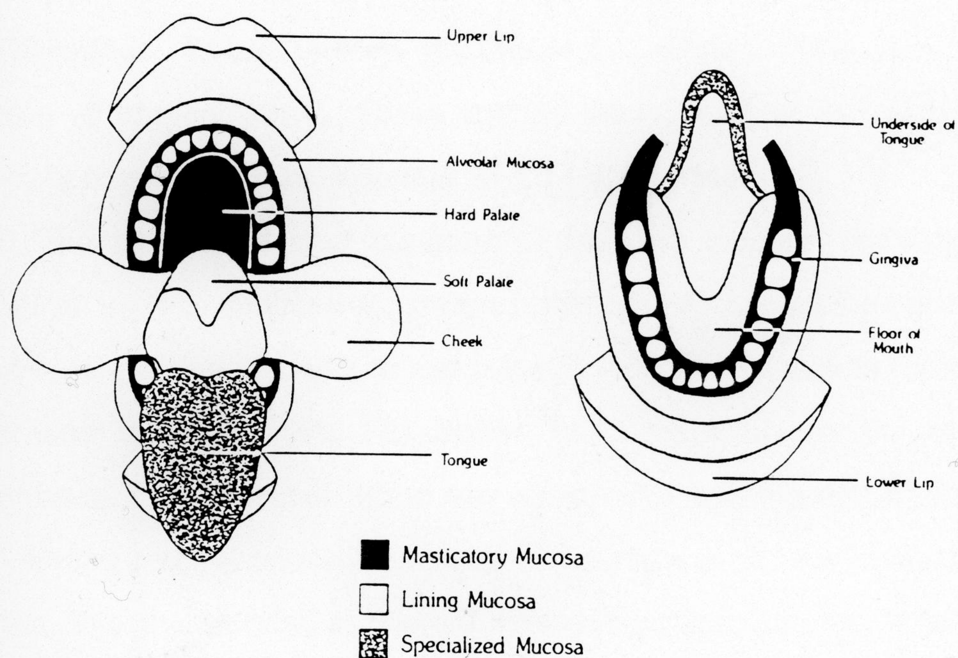


Figure I-1. The oral cavity showing the various regions and types of mucosa (Taken from Wertz, 1991).

the epithelial surface is keratinized. This type of mucosa is called masticatory mucosa, and represents about 24% of the total surface area of the oral mucosa. Regions of the oral mucosa that are stretched or compressed during speech and mastication, such as the cheeks (buccal mucosa), floor of the mouth, and ventral surface of the tongue (sublingual), have a nonkeratinized epithelium and are called lining mucosa occupying about 60% of the oral mucosa (Collins, 1987). The dorsal surface of the tongue, about 16% of the total oral mucosa, has many special structural configurations. These include the fungiform, circumvallate and foliate papillae (Farbman, 1984). These specialized mucosa of the tongue are generally considered less important for drug absorption.

The general cellular structure of the oral mucosa consists of a superficial layer of stratified squamous epithelium, an underlying connective tissue layer (lamina propria and submucosa), and a basal lamina (basement membrane) in between (Fig. I-2) (Squier, 1976). Depending on the region of the mucosa, the connective tissue may consist of the lamina propria alone or both lamina propria and submucosa. In the case of the submucosa, it is flexible, like the buccal, sublingual and soft palate mucosa. Where the submucosa is absent, as in the gingiva and hard palate, the mucosa is fixed in position. The main role of connective tissue is to support the epithelium. The mucosa also has a vascular and sensory nerve network to provide nutrition, eliminate metabolites and obtain outside signals (Squier, 1976).

The interface between the epithelium and connective tissue has an irregular formation, the upward projections of the connective tissue papillae interdigitate with the downward projecting epithelial ridges. This

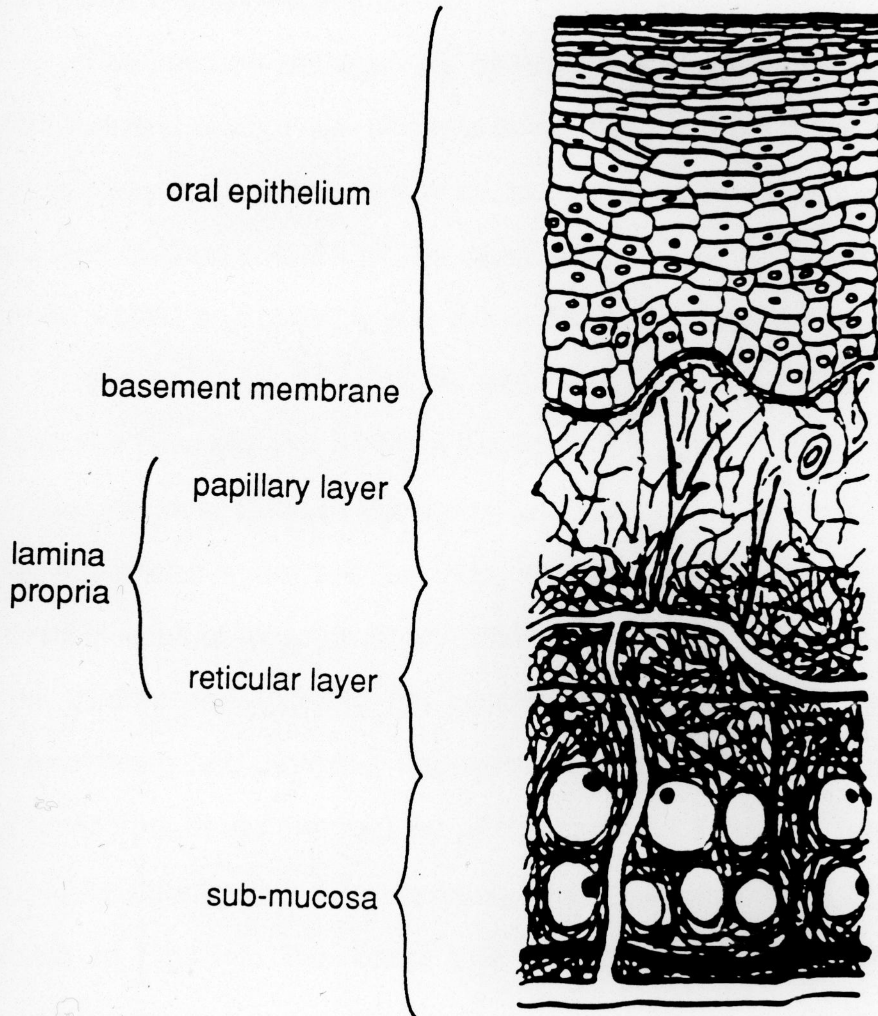


Figure I-2. General structure of the oral mucosa. All the main tissue components can be identified under light microscope (Taken from Squier, 1976).

configuration not only distributes the mechanical stress applied to the epithelium, but also provides better material exchange between the epithelium and connective tissue.

The junction between the epithelium and connective tissue is called the basement membrane, which is about 1-2 μm in thickness (Squier, 1976). It is actually not a membrane at all, but a complex of anchoring fibrils which interlock collagen fibrils of the connective tissue with lamina densa and lamina lucida, and possibly with the epithelium as well (Fig. I-3).

The epithelial lining of the oral mucosa forms a protective cover for the tissue beneath and hence a barrier to the entry of foreign materials and microorganisms. Cells in the epithelium are closely opposed and stratified. The superficial layers are the most differentiated and are constantly being lost as a result of wear and tear. Cells near the basement membrane are the most undifferentiated and are constantly moving upward while they are differentiating and providing replacement for the superficial layer. The tissue turnover time in various regions of the mouth is shown in Table I-3. Typical cell layer differentiation for keratinized and nonkeratinized oral mucosa is shown in Fig. I-4. The basal and prickle cell layer together constitute approximately half to two-thirds of the thickness of the epithelium.

To a large extent, keratinized oral mucosa shows the same structure as the skin. The outermost layer is the stratum corneum. It consists of an orderly array of flattened hexagonal cells completely filled with an aggregation of cytokeratin and surrounded by a complex mixture of lipids (Wertz, 1991). Although the cells in this layer lack most cell organelles and are not vital, they are the rate limiting barrier to diffusion of substance across

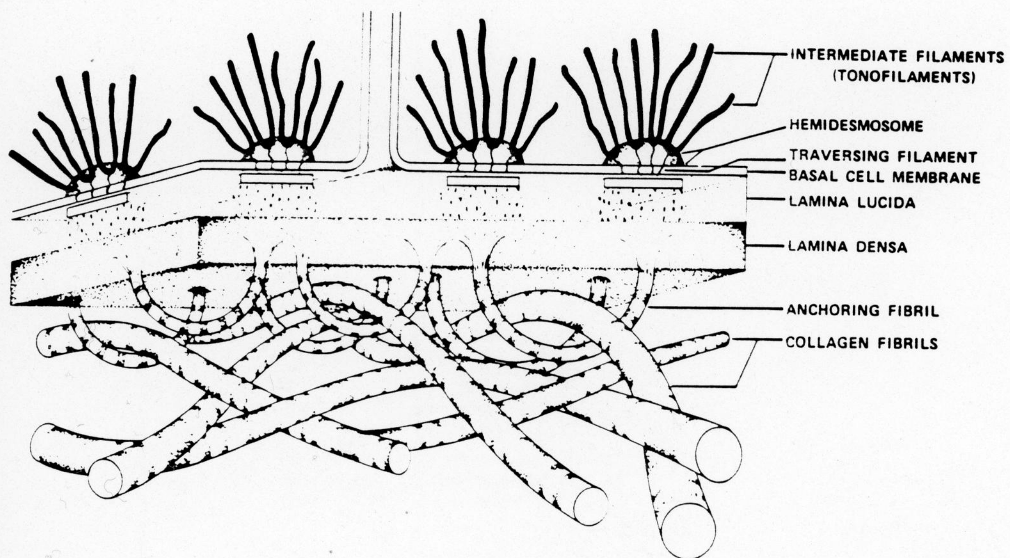
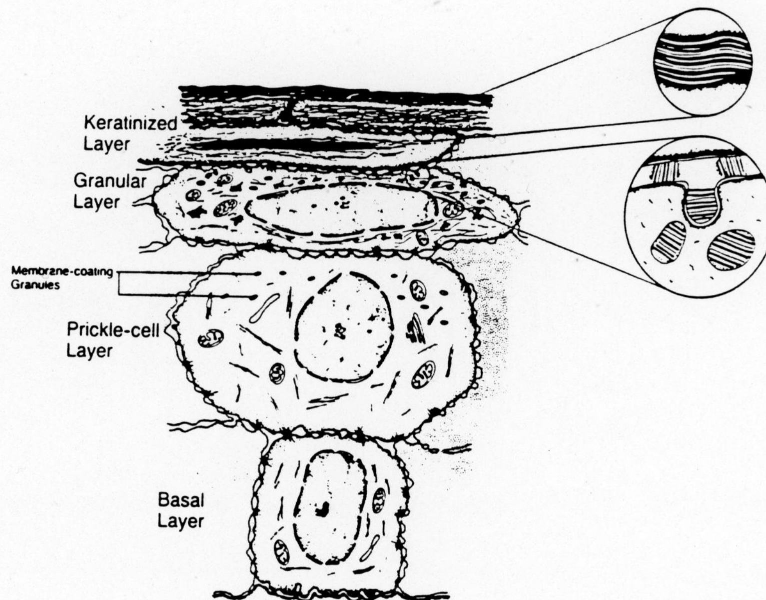


Figure I-3. The basal complex in oral mucosa (Taken from Chen, 1984).



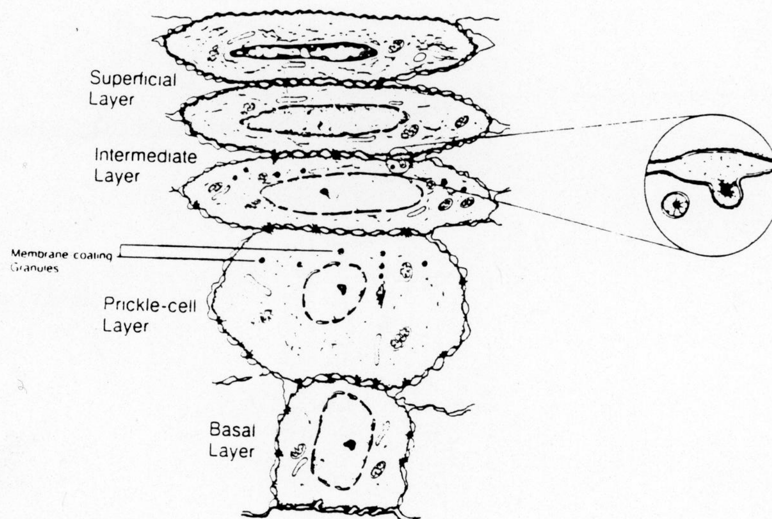


Figure I-4. Differentiation of stratified squamous oral epithelium: (A) keratinized, (B) nonkeratinized (Taken from Wertz, 1991).

Table I-3. Characteristics of Various Regions of the Oral Mucosa

Region	Turnover Time (Day)[1]	Epithelium Thickness(μm)[2]	Blood Low Rate* (ml/min/100cm^2)[3]	Keratinization[4]
Hard Palate	24	250	0.89	yes
Gingiva	68	200	1.47	yes
Buccal	14	500-600	2.40	no
Sublingual	20	100-200	0.97	no

* Rhesus monkeys were used as an animal model.

[1] Squier, 1975.

[2] Chen, 1984.

[3] Squier and Nanny, 1985.

[4] Squier, 1976.

the mucosa (Kligman, 1964).

The nonkeratinized oral mucosa has a similar cell arrangement and differentiation, except for the superficial layer, where there is no keratin covering. These cells do contain a variety of organelles and are considered viable. It is believed that the permeability barrier of the nonkeratinized oral mucosa is the superficial layer (Squier and Hall, 1985).

Other characteristics of the oral mucosa are given in Table I-3.

C. Permeability of the Oral Mucosa

One of the primary functions of the oral mucosa is to protect the underlying tissue, not only in terms of resistance to mechanical insult but also to restrict the entry of micro-organisms and toxic substances.

The stratified squamous epithelium forms the main barrier for transporting materials. However the oral epithelium is always hydrated by saliva and some regions (buccal and sublingual) are not covered by a keratin layer. Therefore the oral mucosa is *a priori* expected to be more permeable than skin, although it is less permeable than the gastrointestinal mucosa, because the surface of the gastrointestinal tract is lined with columnar epithelia and is highly specialized for nutritional absorption. Therefore, the permeability of the oral mucosa is expected to be between that of the skin and the gastrointestinal tract, and the permeability coefficients of many compounds agree with this expectation (Harris, 1992).

It is generally assumed that the nonkeratinized oral mucosa (buccal

and sublingual) is more permeable than keratinized mucosa (hard palate and gingiva) because it appears that the keratin layer in skin is an effective penetration barrier for water-soluble substances (Kligman, 1964). However, the difference between keratinized and nonkeratinized mucosa is not as large as the difference between the skin and oral mucosa. Kaaber (1974) studied the penetration rates of ions across keratinized and nonkeratinized oral mucosa and found that they are similar. Squier and co-workers (1973 and 1975) have also claimed that the keratin layer in keratinized oral mucosa does not play a major permeability barrier role for horseradish peroxidase and lanthanum. On the other hand, the permeability of fluorescent dyes is higher in nonkeratinized than in keratinized oral mucosa (Kaaber, 1974; Adams, 1974). Plasma isosorbide dinitrate levels in humans is significantly detectable if applied to buccal and sublingual area, but is undetectable if applied to the palate (Pimlott, 1985). Consequently, the permeability barrier role of the keratin layer may depend on the chemical nature of the penetrants and the applied solution.

Throughout the epithelium of the oral mucosa, the cells are undergoing constant differentiation. Therefore the barrier function is not unified. Using horseradish peroxidase and lanthanum, Squier and co-workers (1973 and 1974) demonstrated that, for both the keratinized and nonkeratinized oral mucosa, only the top quarter to one-third of the epithelium is the permeability barrier. These results coincide with the appearance of membrane coating granules at the cell margins and suggest that the barrier to horseradish peroxidase and lanthanum may be the result of intercellular material secreted by these granules.

D. Transport of Material Across the Oral Mucosa

There are many models concerning the mechanism of transporting materials across biological membranes, depending on the position, type, structure and function of the epithelia. Endocytosis is the process wherein cells take up solid particles (phagocytosis) or liquids (pinocytosis) from their external environment by engulfing the material in membranous vesicles. Exocytosis is the opposite of endocytosis, involving the expulsion of the contents of the membranous vesicles into the external environment. Material can move across a cell multilayer by endocytosis on one side of the cell followed by exocytosis on the opposite side, and repeating the same sequence on the next cell layer. This phenomenon has been shown to occur during the transfer of intravenously injected protein across the capillary endothelium (Karnovsky, 1967), the transport of horseradish peroxidase across intestinal epithelium (Cornell, 1971), and the penetration of macromolecules and small particles across mesothelial cells of mouse omentum (Fedorko, 1971). Oral mucosal cells are capable of taking up material by endocytosis, particularly in the basal and prickle layers (Wolff, 1969; Squier, 1973). However, it is not likely that this is the major route of transporting materials through the entire epithelium (Berridge, 1972).

Active transport of a material is a process requiring metabolic energy and moves molecules against their concentration and electrochemical gradients. Some sugars and amino acids are transported across intestinal and corneal epithelium by this process (Davis, 1972; Liaw, 1991). However this process may be specific for sugars, amino acids and certain ions for

nutritional purposes. It is unlikely to be a general process for transport across the oral mucosa. Diffusion is the process of molecules moving down a concentration gradient. Diffusional movement across biological membranes can be further divided into intracellular (transcellular) and intercellular (paracellular).

Transcellular diffusion involves the movement of material across the cell membrane (Fig. I-5). There are several mechanisms involved, depending on the diffusants. Hydrophobic compounds can diffuse directly through cell membranes, whereas hydrophilic compounds may diffuse directly or diffuse with the help of a specific carrier system. The later is called carrier-mediated diffusion (facilitated transport).

Paracellular diffusion, by its name, is the process where diffusants move through an intercellular space (Fig. I-5). Although epithelium consists of closely opposed cells, the hydrophilic intercellular space is sufficiently large to allow diffusion of some molecules and ions. The three types of intercellular junctions in oral epithelium are the desmosomes (macula adherens), the gap junctions (nexus), and the tight junctions (macula or zonula occludens) (Chen, 1984). The tight junction is very rare in oral epithelium (Barnett, 1973; Shimono, 1976; Squier, 1977). The gap junction and desmosomes predominate, and are seen above the basal layer. They appear frequently in the hard palate, and less frequently in the cheek (Chen, 1984). Consequently, the intercellular junction of oral epithelium is considered to be "leaky" and the major route for transport of large, hydrophilic molecules. Horseradish peroxidase and lanthanum trace studies clearly demonstrate this possibility (Squier, 1973; Squier and Rooney,

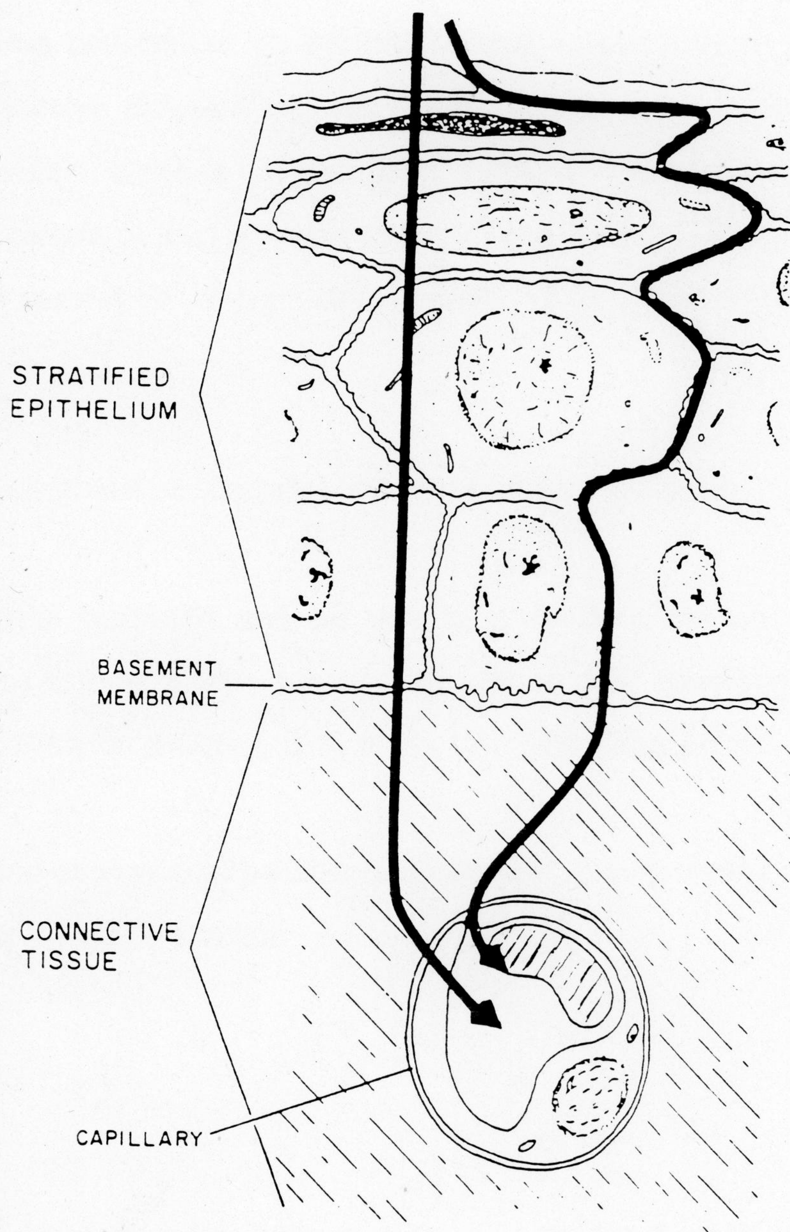


Figure I-5. Routes of transepithelial penetration: paracellular vs. transcellular route (Taken from Wertz, 1991).

1976).

Drug penetration via the oral mucosa is generally considered as a simple passive diffusion process. Facilitated diffusion requires a specific carrier protein, which is not commonly available. Many studies have shown that the pattern of drug diffusion through the oral mucosa is similar to that of a simple passive diffusion process (Gandhi, 1990; Dowty, 1991).

E. A Mathematical Consideration of Mass Transport

Mass transport can be due to a random motion of molecules (diffusion) or bulk motion (convection). Since drug absorption is a simple passive diffusion process, only mass transport diffusion will be discussed here.

One-dimensional passive diffusion in a homogeneous media can be described by Fick's first law,

$$J = D \frac{dC}{dx} \qquad (1)$$

where J, the flux, is the amount of solute crossing a plane of unit surface area, which is normal to the direction of transport, per unit time. D is the diffusion coefficient of solute in the media, C is concentration of the solute, and x is the distance of solute transport. If the membrane has a thickness of h and a solute concentration gradient of C_1 and C_2 (Fig. I-6), the flux can be

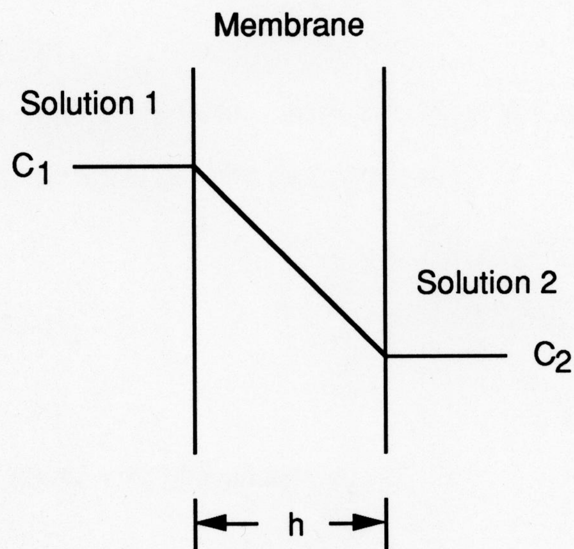


Figure I-6. A membrane of thickness h separating two solutions at concentration C_1 and C_2. The concentration gradient inside the membrane is linear assuming steady state conditions and an homogeneous membrane (Modified from Burnette, 1987).

expressed as:

$$J = \frac{D}{h}(C_1 - C_2) \qquad (2)$$

Note that the concentration gradient inside the membrane is linear because the membrane is assumed homogeneous and the transporting process is at a steady state condition.

For a solute with a partition coefficient K_p, between solution and membrane (Fig. I-7), equation (2) can be written as:

$$J = \frac{D\,Kp}{h}(C_1 - C_2) \qquad (3)$$

The permeability coefficient P, is defined as:

$$P = \frac{D\,Kp}{h} \qquad (4)$$

or:

$$P = \frac{J}{(C_1 - C_2)} \qquad (5)$$

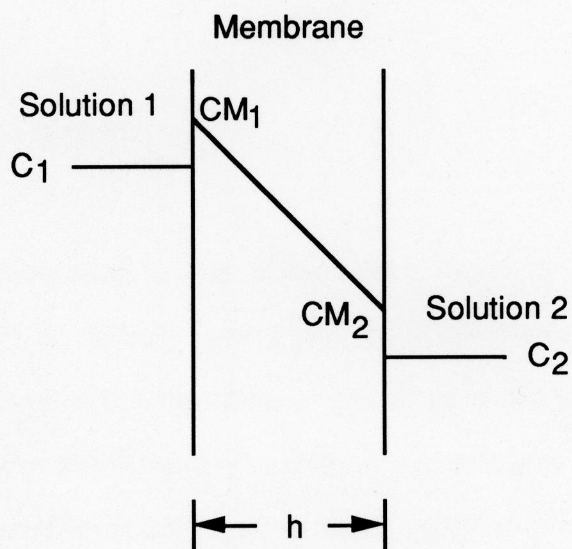


Figure I-7. A membrane of thickness h separating two solutions at concentration C1 and C2. CM_1 and CM_2 were the solute concentrations in the membrane at the membrane solution interface. The membrane was homogeneous and steady state conditions were assumed. Preferential partition of the solute into the membrane occurred (Modified from Burnette, 1987).

Experimentally, P is obtained from equation (5), since determining the diffusion coefficient, partition coefficient and membrane thickness separately can be a difficult task.

The drug concentration on the receiver side is usually less than 5% of that on the donor side. Thus, equation (5) can be further simplified to equation (6):

$$P = \frac{J}{C} \qquad (6)$$

where C is the concentration on the donor side.

Equation (6) is widely used for quantitatively measuring the permeability coefficient of the membrane to a drug, even without knowledge of the thickness of the membrane, the diffusion coefficient of the drug in the membrane, or the partition coefficient of the drug between the solution and membrane. However, equation (6) can only be used when steady state has been achieved. For non-steady state diffusion, Fick's second law is required. The equation for non-steady state flux is:

$$Amt(t)_{out} = (A\ Kp\ h\ C_1)\left[\frac{Dt}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\, e^{\frac{-Dn^2\pi^2 t}{h^2}}\right] \qquad (7)$$

Equation (7) is based on the assumption that the concentration of drug in the receiver chamber is zero at t = 0, and the concentration of C_1 is added to the donor chamber at t = 0.

A plot of $Amt(t)_{out}$ versus time obtained by equation (7) is shown in Fig. I-8. As time increases, the series term in the equation goes to zero, resulting in the steady state expression:

$$Amt(t)out = A\ Kp\ h\ C_1\left(\frac{D\,t}{h^2} - \frac{1}{6}\right) \qquad (8)$$

Equation (8) shows that at steady state, the $Amt(t)_{out}$ versus time is a straight line, where the slope is:

$$\frac{d\,(Amt(t)_{out})}{dt} = \frac{A\ Kp\ C\ D}{h} \qquad (9)$$

and the x-axis intercept is:

$$t = \frac{h^2}{6\,D} \qquad (10)$$

which is called the lag time.

Equation (9) is actually the same as equation (3). If C_2 is much smaller than C_1 in equation (3) and can be negligible, then:

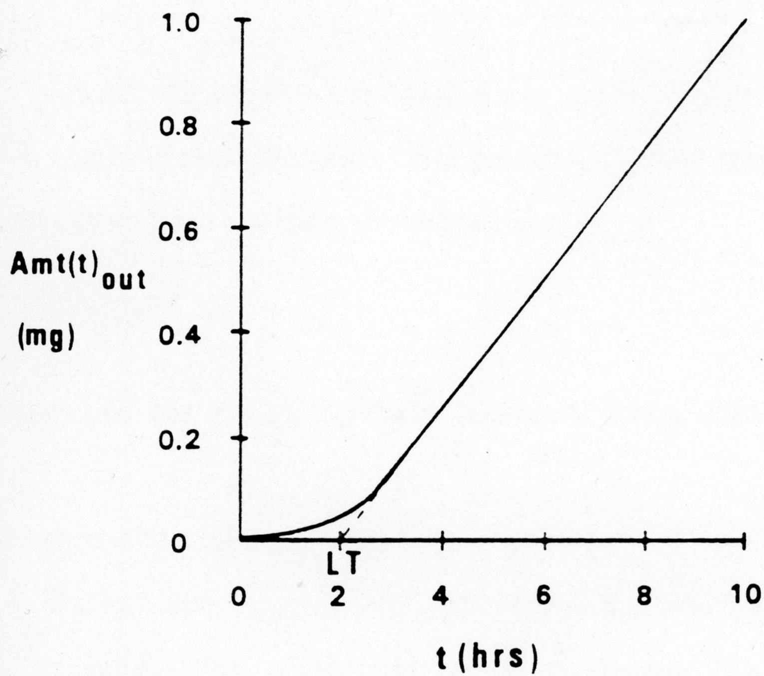


Figure I-8. A plot of the amount of drug passing through a membrane as a function of time using Fick's second law at non-steady-state conditions. LT is the lag time (From Burnette, 1987).

$$J = \frac{1}{A}\frac{d\,(Amt(t)_{out})}{dt} = \frac{Kp\,D\,C_1}{h} = P\,C$$

or

$$P = \frac{1}{C\,A}\frac{d\,(\,Amt(t)_{out}\,)}{dt} = \frac{1}{C\,A}\,\text{slope} \qquad (11)$$

Apparently, even if the initial stage is non-steady state, it will achieve steady state as time proceeds. The permeability coefficient and lag time can be calculated from the data at steady state.

F. Methods for Studying Oral Mucosal Drug Delivery

1. *In vivo* drug absorption test

Beckett and Triggs (1967) first introduced the *in vivo* drug absorption test, in which the absorption of a compound is derived from the concentration difference before and after rinsing the solution around the mouth. This is a very simple method to measure drug absorption. The oral mucosa, connective tissue and blood circulation are all in their natural condition, no alteration of the tissue is introduced. However the drug lost from the applied solution may not have been delivered into the blood circulation completely. The buccal tissue has a high storage capacity, and some investigators have shown that the drug lost from the delivery solution

can be partially recovered by rinsing the mouth. The recovery rate varies from 5% to 60% depending on the time of absorption, the pH of the washout solution, and the contact time (Davis, 1979; Henry, 1980). Therefore, the drug absorption determined by this method dose not accurately represent the amount of the drug delivered. In addition, the region and the surface area of the delivery site is uncontrolled. It is also impossible with this method to study the absorptions of drug through the various regions of the oral mucosa, or to calculate the permeability coefficient. This makes it difficult to compare results from this approach with other studies.

2. *In vitro* diffusion study

The basic set up of an *in vitro* diffusion study consists of donor and receiver chambers, and tissue mounted between them. The drug is placed in the donor chamber, and samples are taken periodically from the receiver chamber. The drug found in the receiver chamber has penetrated completely through the mucosa. Drugs stored in the tissue, or metabolized during the penetration are not detected. The type and surface area of the tissue is well-defined. Permeability coefficients can be easily obtained and compared with other studies. The pH, ionic strength, osmolarity and other physical/chemical properties of the solution in both donor and receiver chambers can be easily controlled and specified. The sensitivity of drug transport measurement can be high because there is no drug metabolism in the receiver chamber and the volume of the receiver chamber is small.

The major disadvantage is that the tissue is dissected and may no longer represent the original tissue. It is difficult to prove that the barrier

function remains the same, although it has been established that rabbit buccal tissue does not undergo major changes for six hours after dissection (Longer, 1988).

3. Diffusion study with cultured epithelium

This method is a modification of the *in vitro* diffusion study. The difference is that the tissue is not dissected from the animal but instead a buccal cell culture is used (Tavakoli-Saberi, 1989). The cultured buccal epithelium is characterized by electron microscopy as a stratified multilayer of epithelial cells, with well developed tonofibrillar-desmosome complexes. Only the superficial layer of the cultured cells exhibited evidence of terminal differentiation. The biochemical properties (selected enzyme activities) are similar to those of fresh buccal epithelium. This method could be a useful screening tool to select a particular formulation or dosage form, but is limited in its ability to study the mechanism of drug transport.

4. *In situ* perfusion study

This method is modified from the *in vivo* drug absorption test. A perfusion cell, with known area, is clamped on the region to be tested. The perfusion solution is circulated from a reservoir to the oral mucosa. Drug absorption is measured by disappearance of the drug from the reservoir (Barsuhn, 1988) or by the plasma concentration (Yamahara, 1990). The permeability coefficient of the drug can be calculated and compared with other studies. An *in situ* perfusion system coupled with plasma concentration measurement and an I.V. infusion model is the best combination for a

clinical study. A limitation of this method is that the drug plasma concentration is usually low and difficult to measure. Animal handling is also a difficult task if animals are used.

G. Factors Effect Permeability in *in vitro* Diffusion Study

Oral mucosal drug absorption is a process whereby the drug molecules diffuse through the oral epithelium. Fig. I-9 shows a model of the oral drug absorption process in *in vitro* diffusion study. Fick's first law is applied for each layer, therefore, the following relations can be obtained

$$C_D' = C_D - \frac{J_D}{D_D} q_D \qquad (12)$$

$$CM_R = CM_D - \frac{J_M}{D_M} h \qquad (13)$$

$$C_R = C_R' - \frac{J_R}{D_R} q_R \qquad (14)$$

where h, q_D and q_R are thicknesses of membrane, stagnant layer on donor and receiver side, respectively; J_M, J_D and J_R are fluxes on membrane and stagnant layers.

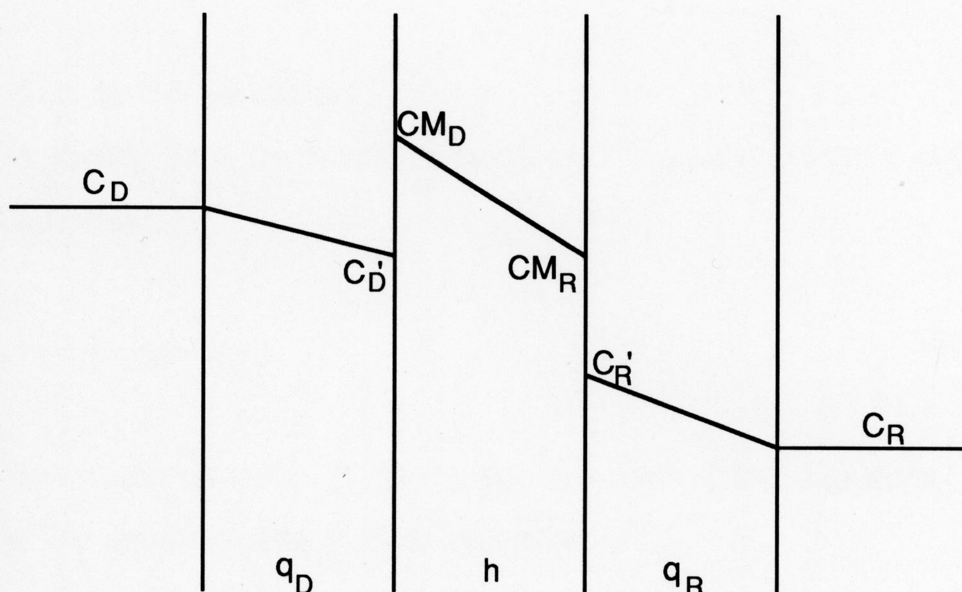


Figure I-9. A model of oral mucosal drug absorption process in *in vitro* diffusion study, where h is the thickness of the membrane; q_D and q_R are thicknesses of stagnant layers on donor and receiver side; C_D and C_R are the drug concentrations in donor and receiver chamber; C_D' and C_R' are the drug concentrations at solution-membrane interfaces; CM_D, and CM_R are drug concentrations inside the membrane. The membrane is assumed heterogeneous. Steady state conditions are also assumed.

The partition coefficients are the same on both donor solution-membrane and receiver solution-membrane interfaces, because the donor and receiver side have the same buffer solution. Thus:

$$K_p = \frac{CM_D}{C_D'} = \frac{CM_R}{C_R'} \qquad (15)$$

where K_p is partition coefficient.

At steady state, the fluxes are the same in the stagnant layer and the membrane, then:

$$J = J_D = J_R = J_M \qquad (16)$$

Substitute equation (12)-(15) into equation (16), rearrange and simplify. The equation of the flux can be obtained.

$$J = \frac{1}{\frac{h}{K_p D_M} + \frac{q_R}{D_R} + \frac{q_D}{D_D}} (C_D - C_R) \qquad (17)$$

Equation (17) clearly shows that the drug absorption process is effected by stirring, transporting route, partition coefficient and diffusion coefficient.

1. Stirring

Proper stirring can reduce the thickness of the stagnant layer, and therefore increase flux. However, no matter how efficient the stirring, the stagnant layer always exists. It has less significance if the membrane has much higher resistance, which, mathematically, can be stated as:

$$\frac{h}{K_p D_M} >> \frac{q_R}{D_R} + \frac{q_D}{D_D} \qquad (18)$$

then:

$$J = \frac{K_p D_M}{h} (C_D - C_R) \qquad (19)$$

On the other hand, if the membrane is very permeable and the stagnant layers become the major barrier, which is called diffusion controlled transport (Burnette, 1987), the flux is independent of membrane properties. Thus:

$$J = \frac{1}{\frac{q_R}{D_R} + \frac{q_D}{D_D}} (C_D - C_R) \qquad (20)$$

This is probably the reason that the upper level of the oral mucosal

permeability coefficient is around 1×10^{-4} to 1×10^{-5} cm/sec.

The effect of the stagnant layer is determined not only by stirring, but also by chemical properties of penetrants and membranes, e.g., the diffusion coefficient of the penetrants in solution and membrane, partition coefficient, etc., as shown in equation (18). Usually, if the overall permeability coefficient is low, around $10^{-6} \sim 10^{-7}$ cm/sec, the stagnant layer is not the limiting step. Therefore, its effect is negligible.

2. Transporting Route

The traditional theory of oral epithelial drug absorption is that the epithelium of the oral mucosa is a hydrophobic barrier. Thus, as shown in equation (19), the drug absorption permeability coefficient should be proportional to the partition coefficient. Many studies support this concept (Beckett, 1971; Moffat, 1971; Siegel, 1981). Beckett and Moffat were using an *in vivo* drug absorption test, and, as has already been discussed, the conclusions are questionable. Siegel, on the other hand, did use an *in vitro* diffusion model with rabbit frenula and measured absorption on the receiver side. However, the data is not entirely supportive of his conclusion. Fig. I-10 to Fig. I-12 are constructed by using data from his paper (Siegel, 1981).

A new theory about drug epithelial absorption is based on the structure of the epithelial tissue (Wertz, 1991; de Vries, 1991). As mentioned in the introductory section, two possible penetrating routes are proposed, the intracellular and paracellular route. Generally, hydrophobic compounds may diffuse directly through the cell membrane (intracellular). Hydrophilic

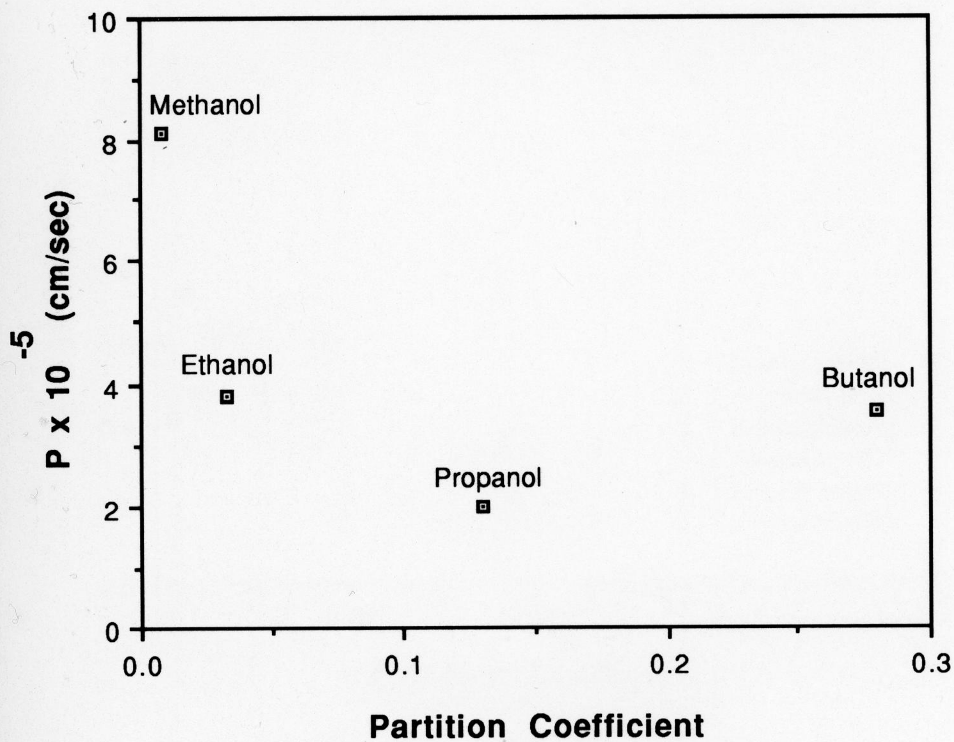


Figure I-10. Permeability coefficient versus partition coefficient of alcohol series. The oil phase is olive oil. (Data is from Siegel, 1981).

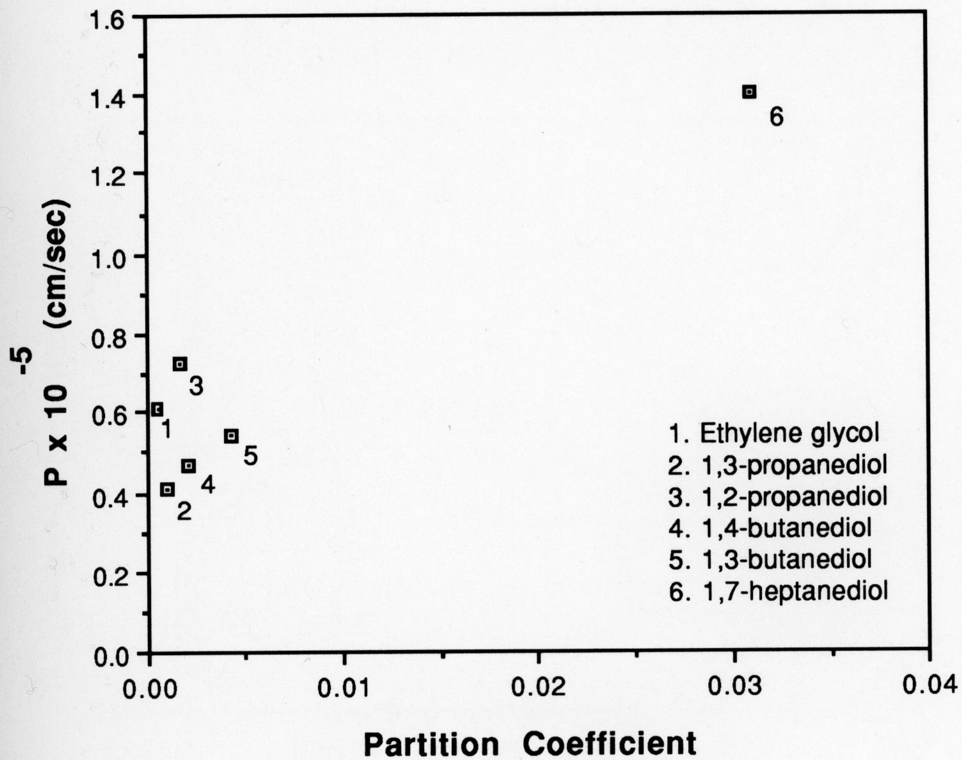


Figure I-11. Permeability coefficient versus partition coefficient of diol series. The oil phase is olive oil. (Data is from Siegel, 1981).

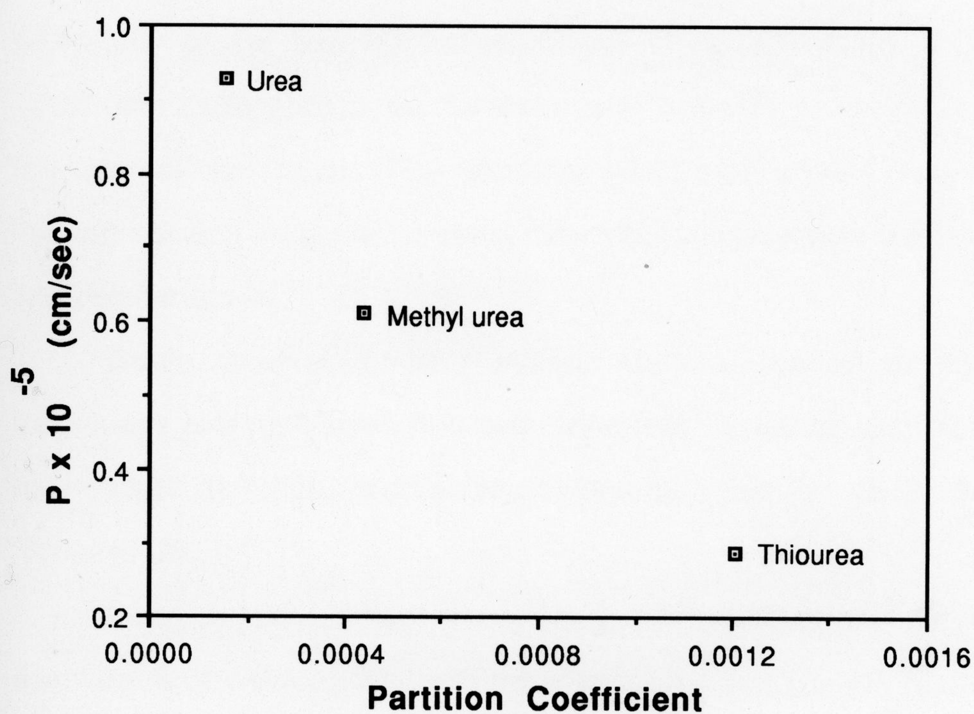


Figure I-12. Permeability coefficient versus partition coefficient of urea series. The oil phase is olive oil. (Data is from Siegel, 1981).

compounds may also diffuse directly through the cell membrane with the help of a carrier system, e.g., ions can diffuse through ion channels. However, the major penetrating route for hydrophilic compounds is the intercellular space (paracellular).

The paracellular pathway has been studied by many researchers. Dowty (1992) studied the permeability of thyrotropin releasing hormone in rabbit buccal tissue and revealed that the permeability was altered by the osmolarity of the bathing buffer solution. The permeability is higher in hypertonic bathing because the water loss from the cytoplasm opened up the intercellular space. The opposite effect was shown in a hypotonic bathing solution because the water flow into the cytoplasm closed down the intercellular space.

Squier and co-workers studied the penetration of horseradish peroxidase and lanthanum and concluded that the intercellular space is the major route for large, hydrophilic compound(s) (Squier, 1973; Squier and Rooney, 1976).

For a compound that has both hydrophilic and hydrophobic form, e.g., weak acids or weak bases, both the intracellular and paracellular route may be used. The total flux of drug is a combination of these two routes.

3. Partition Coefficient

Preferential partition of a drug into the epithelial membrane may play an important role in drug absorption, as shown in equation (19). However, using simple octanol/water or other organic solvent/water systems cannot

truly represent the partition coefficient K_p, in equation (19), because the media of the penetrating route, especially the paracellular route, is much more hydrophilic than these organic solvents. Although Beckett, et al (1971) and Moffat (1971) found a linear relation of percent Absorption versus log(Partition Coefficient) or log(Absorption) versus log(Partition Coefficient), there is no theoretical bases for this relation. Their linear relationship is probably results from the use of a logarithmic transform, which tends to make the data appear linear. As a matter of fact, Beckett himself found that "There is not always a correlation between the buccal absorption test for drugs and their partition coefficients." (Beckett, 1971). Siegel also claimed a somewhat linear relation between the partition coefficient and permeability coefficient (Fig. I-10 to Fig. I-12), which has been discussed previously.

From equation (19), it is clear that the partition coefficient K_p, is actually the tendency of the drug molecules to move into the epithelial membrane. This tendency is influenced by many factors, such as the chemical properties of the membrane (media of the penetrating route(s)) and applied solution, and the molecular nature of the drug. Some other factors may also effect this tendency. For example, Gandhi (1990) studied the electrophysiology of the rabbit buccal tissue and found that the buccal epithelium is negatively charged at biological pH (the isoelectric point is 2.7), and the tissue transports positively charged species more readily than negatively charged species. Drug molecular size, shape, binding with certain molecules, etc. may also have effects. There is no universal measure of this tendency.

Consequently, the partition coefficients used in equation (19) should

be between the media of the penetrating route and the solution.

4. Diffusion Coefficient

The diffusion coefficient is a parameter of molecular movement in a media. It is a function of temperature, molecular size and media viscosity, as shown in the Stokes-Einstein equation:

$$D = \frac{kT}{6 \pi r \eta}$$

where T is the temperature; k is Boltzmann's constant; r is the radius of the molecule and η is the viscosity of the media.

This equation is obtained by assuming that the diffusing molecule is a slowly moving solid sphere, the diffusing media is continuous, the diffusion is under steady state conditions, and the molecules of the diffusing media are isotropic and incompressible (Burnette, 1987).

The Stokes-Einstein equation is only suitable for an ideal situation. The oral epithelial drug absorption process is much more complicated. Many other factors may affect the diffusion coefficient, for example, drug molecular shape, charge type, density and distribution, molecular interaction between drug molecules and molecules in penetrating routes.

The epithelial drug absorption process is so complicated that it is difficult to use a simple model to explain the mechanism of this process. It is

feasible to use two penetrating routes, although more penetrating routes may exist. For water soluble hydrophilic compounds, a one penetrating route model may be used if this route is predominant over others. However, the partition coefficient used in the model, as shown in equation (19), must be between the media of the penetrating route and the applied solution.

H. Animal Model

The basic criterion of choosing an animal model is that it has to be able to mimic the human oral mucosa, not only in terms of morphology, but also in terms of biochemical and enzymatic properties. As mentioned earlier, human buccal and sublingual mucosa is a stratified squamous epithelium, with no coverage of the keratin layer on the superficial layer. The animal model must have similar characteristics, and must also be freely available, easy to handle, and cheaply obtainable with sufficient area for experimental study. Among the animals studied, only dogs and rabbits are generally regarded as suitable because the buccal epithelium of rats and hamster are heavily keratinized. Pig oral epithelium is similar to humans, but it is not widely available (Chen, 1970; Ebert, 1987). The rabbit is used in this study because of availability and cost.

II. STATEMENT OF OBJECTIVE

Many studies have shown that oral mucosal absorption of an ionizable compound is pH-dependent (Beckett, 1971; Hicks, 1973; Randhawa, 1986; Al-Sayed-Omar, 1987; Barsuhn, 1988; Yamahara, 1990; Zhang, 1991). This phenomenon agrees with the theory that the oral mucosa behaves as a hydrophobic membrane. The tissue prefers to transport hydrophobic compounds because, from equation (4), the permeability coefficient is a function of partition coefficient. Changing the pH of the solution will directly change the degree of ionization of the compound, as well as the hydrophobicity and partition coefficient. Beckett and Hossie (1971) also found a linear relationship between percent of drug absorbed versus log of the partition coefficient.

However, Zhang and et al (1989), using an *in vivo* dog model, did not find the same pattern for isoproterenol. Furthermore, Davis (1979) and Henry (1980) demonstrated that drug absorbed by the oral epithelium could be recovered by rinsing the mouth with an appropriate buffer solution. Therefore, drug lost from the delivery solution is not completely absorbed across the epithelium.

Explanation of these contradictory conclusions requires additional studies. The objective of this study is to evaluate the permeability of morphine in rabbit buccal and sublingual mucosa, and the dependence of permeability *in vitro* on the pH of the bathing medium. This work will also serve as a prelude to an animal model study on transport mechanisms (penetration route) of morphine, and mechanisms of penetration enhancement.

III. EXPERIMENTAL

A. Material

Unless otherwise stated, all chemicals were used as received and all solutions were prepared with distilled, deionized water (DDW) (passed through a Barnstead PCS water purification system).

Bis-Tris propane (1,3-bis[tris(hydroxymethyl)methylamino]-propane), morphine sulfate, n-octanol, and sodium pentobarbital were purchased from Sigma Chemical Company (St. Louis, MO). Potassium phosphate monobasic (KH_2PO_4) and sodium chloride (NaCl) were analytical reagent grade and purchased from Mallinckrodt Inc. (Paris, KY). Acetonitrile (for HPLC and GC) was purchased from EM Science (Gibbstown, NJ).

B. Solution Preparation

Bis-Tris propane buffer was chosen for this study because of its physiological compatibility and its useful pH range. Bis-Tris propane has two pKa's, 6.8 and 9.0, and a useful pH range of pH 6.3 to 9.5. Buffer solutions were prepared at the following pH's: 6.5, 7.5, 8.5 and 9.5 at a concentration of 25 mM. The pH of the solution was then adjusted by adding 5N HCl and the final pH was within 0.1 unit of the desired pH. The osmolarity of the solution was measured on a Wescor 5500 vapor pressure osmometer (Logan, UT) and adjusted to 300±10 mOsm by adding an appropriate amount of NaCl.

Morphine solution (~0.1 mg/ml) was prepared by dissolving morphine

sulfate in Bis-Tris propane buffer. The pH of the solution was checked and found unchanged due to the addition of morphine sulfate.

C. Sample Analysis

1. HPLC Apparatus consisted of the following components: Beckman 110B solvent delivery module and 164 variable wavelength UV detector with wavelength set at 210 nm; Rheodyne 7125 sample injector with a 100 µl sample loop; Altech Econosphere, C-18, 5 µm, 4.6 x 250 mm HPLC column with an inline C-18 guard column; Kipp & Zonen BD40 chart recorder.

2. HPLC Mobile Phase was 0.04 M KH_2PO_4 in 14% acetonitrile/H_2O (modified from Derendorf, 1984). The pH of the mobile phase was adjusted to 3.0 (after adding acetonitrile). The flow rate was 1.0 ml/min. The typical chromatogram of morphine in Bis-Tris propane buffer is shown in Fig. III-1.

3. Linearity of the HPLC assay of morphine was tested (Fig. III-2). It demonstrated that within the range of 30 to 1000 ng/ml, the assay is linear.

D. Tissue Collection

Male albino New Zealand rabbits (Bakkom's Rabbitry, Viroqua, WI), weighing 2.5 to 3.0 Kg, were maintained in standard caging facilities with

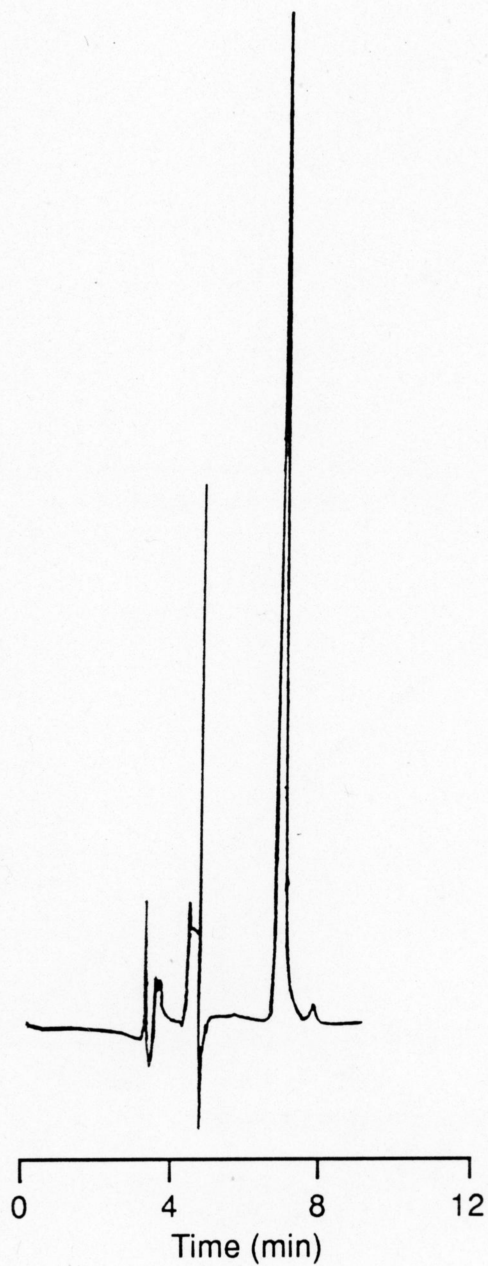


Figure III-1. The typical HPLC chromatogram of morphine in Bis-Tris Propane buffer at 0.1 AUF.

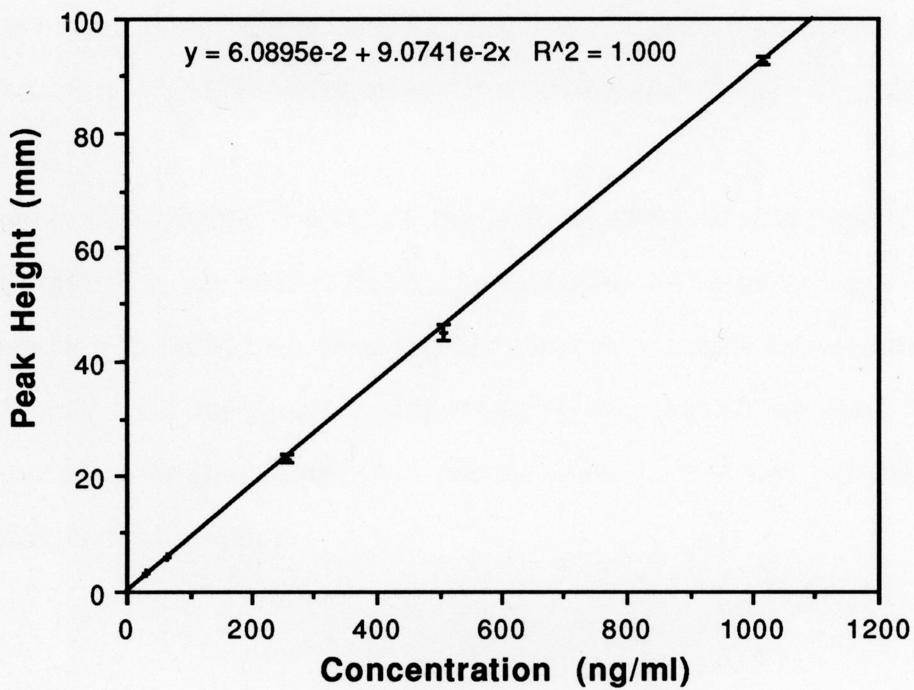


Figure III-2. Linearity study of HPLC method of morphine in a concentration range of 30-1000 ng/ml (Bis-Tris propane buffer at pH 7.5).

unrestricted diet and 12 hour light/dark cycle. The rabbits were sacrificed by an I.V. overdose injection of approximately 10 ml of 5% sodium pentobarbital into a marginal ear vein. The oral cavity was held open with rubber bands on the upper and lower front teeth. To obtain the buccal mucosa, incisions along the upper and lower teeth boundaries of the buccal region were made with a scalpel. The mucosa, with some connective tissue, was cut away from the underlying tissue. Sublingual mucosa, which was only on the floor of the mouth in this study, was cut out by a scalpel. The tissue was rinsed and stored in a buffer solution at room temperature before the clean-up procedure.

To clean the mucosa, the tissue was pinned epithelial side down onto a dissecting dish. The connective tissue was cut away, bit by bit, using a fine-pointed forceps and dissection scissors. The cleaned mucosa was stored in buffer solution at room temperature until mounting into the diffusion cell. The complete process for one rabbit (two buccal mucosa and two sublingual mucosa) took about one hour.

E. Diffusion Study

The schematic diagram of the diffusion cell (Precision Instrument Design, Los Altos, CA) is shown in Fig. III-3. The mucosa was mounted between the diffusion cells with the donor chamber facing the epithelial side. The diffusion cells were hold together firmly by a pair of steel spring rings.

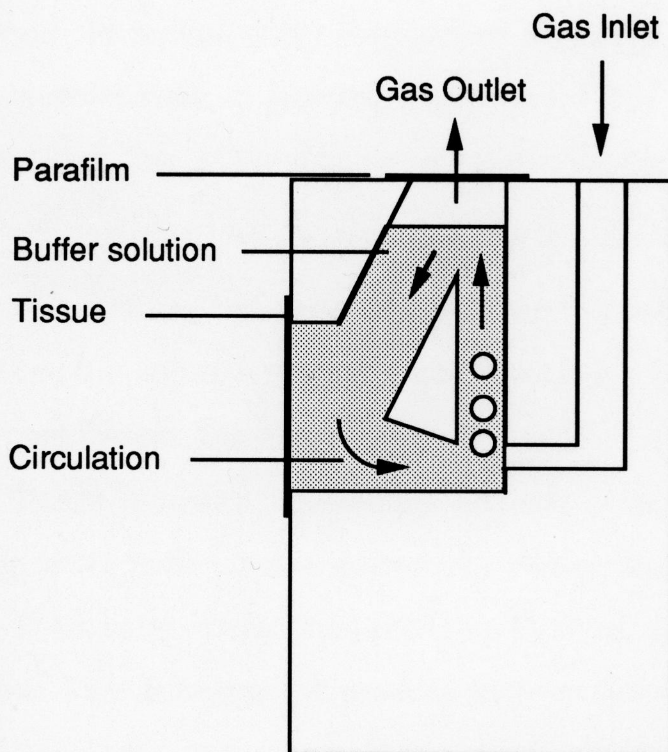


Figure III-3. Schematic diagram of diffusion cell.

The surface area of the tissue to be studied was 0.785 cm^2. The volume of the donor and receiver chambers can be varied from 1 to 1.5 ml (1.5 ml was used in this study). The solution in the chamber was stirred by purging oxygen gas through an inlet on the diffusion cell also providing aeration for the tissue. To reduce water evaporation during the study, the oxygen gas was pre-moisturized by passing it through a DDW trap before entering the diffusion cell. The opening of the diffusion cell was covered by a piece of Parafilm® to further reduce water evaporation (a needle hole was made for gas outlet). The temperature of the diffusion cells was maintained at 37±1°C by placing the diffusion cells in a water jacketed aluminum block (Precision Instrument Design, Los Altos, CA).

Tissue mounted between the diffusion cells was washed with buffer solution for 20 to 30 minutes before the diffusion study.

The rate of oxygen bubbling is very critical, especially for the receiver chamber. Fast bubbling will produce foam in the chamber and eventually spill the solution, while slow bubbling may not provide enough stirring.

Samples were taken periodically from the receiver chamber and replaced with the same amount of buffer solution. Usually the first couple of samples were 1 ml each to further clean up the tissue and reduce the foam, the latter were 0.5 ml each. The total duration of the diffusion study was four hours. A typical permeability study is shown in Fig. III-4. The fitted line represents the permeation at steady state. The permeability coefficient is calculated from equation (6):

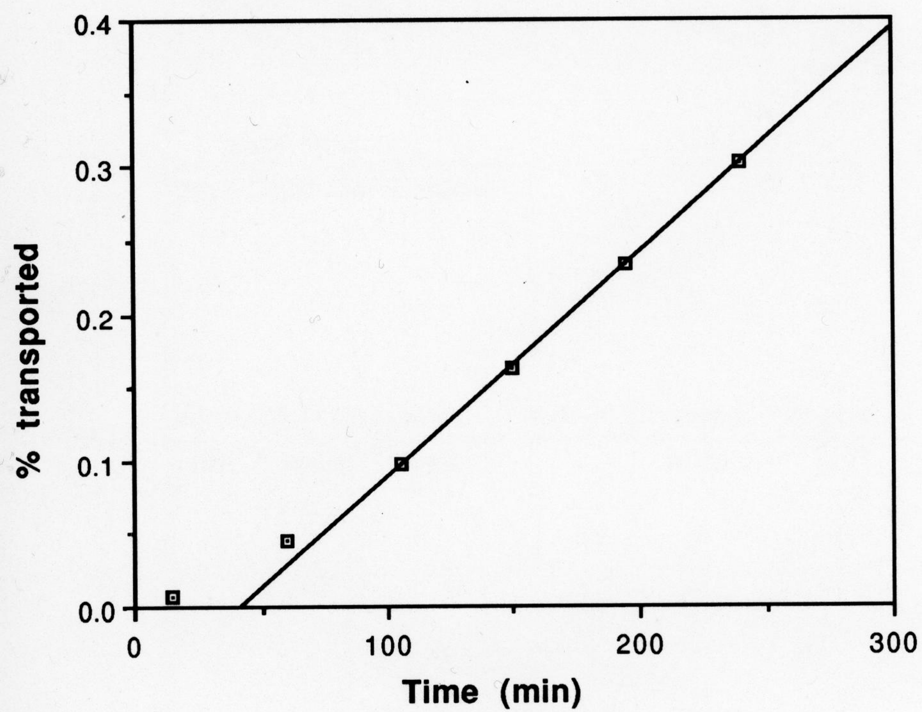


Figure III-4. Typical permeability study of the oral mucosa.

$$P = \frac{J}{C}$$

where J is the flux and C is the concentration of the donor chamber,

$$J = \frac{\text{Amount Transported}}{\text{Area} \cdot \text{Time}}$$

$$C = \frac{\text{Total Amount}}{\text{Donor Chamber Volume}}$$

thus

$$P = \frac{\text{Amount Transported}}{\text{Total Amount} \cdot \text{Time}} \; \frac{\text{Donor Chamber Volume}}{\text{Area}}$$

or

$$P = (\text{ Slope x 100 }) \; \frac{\text{Donor Chamber Volume}}{\text{Area}} \qquad (21)$$

The x-axis intercept of the fitted line represents the lag time (LT) (Burnette, 1987).

F. Partition Study

The octanol/water partition of morphine at pH 6.5, 7.5, 8.5 and 9.5 were also studied. Equal volumes of octanol and morphine buffer solution, placed in a small vial, were shaken and maintained in a 37°C water bath overnight. The aqueous phase morphine concentration was determined by HPLC and the octanol phase concentration was then calculated by subtracting the aqueous phase concentration from the original morphine solution concentration. The octanol/water partition coefficients are simply the ratio of the octanol phase concentration over the aqueous phase concentration.

G. Solubility Study

An excess amount of morphine sulfate was dissolved in small vials with 1 ml Bis-Tris propane buffer solution at pH 6.5, 7.5, 8.5 and 9.5. The vials were shaken and placed in 37°C water bath overnight. The supernatant was filtered by a 0.2 μm Acrodisc® filter (Gelman Sciences, Ann Arbor, MI), diluted properly and the concentration of morphine determined by HPLC.

IV. RESULTS

A. Diffusion Study

The percent fluxes of morphine versus time for buccal and sublingual tissue at pH 6.5, 7.5, 8.5 and 9.5 are shown in Fig. IV-1 A-H. The error bar represents standard deviation of five or more replicates. The standard deviation is somewhat high presumably due to the individual variation, although other factors, such as tissue preparation, tissue mounting, solution stirring, etc., may effect the permeation as well.

The permeability coefficient of morphine is calculated from the slope, the donor chamber volume and the surface area of the tissue, using equation (21). Fig. IV-2 shows the buccal permeability coefficient of morphine at all pH's, and that there is no significant difference between them (95% confidence level). A similar result is shown for the sublingual permeability coefficient (Fig. IV-3). The permeability coefficient at pH 9.5 is higher than those at other pH's probably due to tissue "damage" caused by the higher pH. However there is no significant difference statistically (95% confidence level) between pH 9.5 and pH 8.5 data because of the large standard deviation.

A similar situation occurs for the lag time (Fig. IV-4, Fig. IV-5). The differences between adjacent pH's are not statistically significant (95% confidence level). From equation (10), the lag time is proportional to the square of the thickness of the tissue. The buccal tissue is three to four times thicker than sublingual tissue (Squier, 1975), therefore the lag time of the buccal tissue would be nine to sixteen times longer than that of sublingual tissue if the whole tissue were the permeability barrier. This result agrees

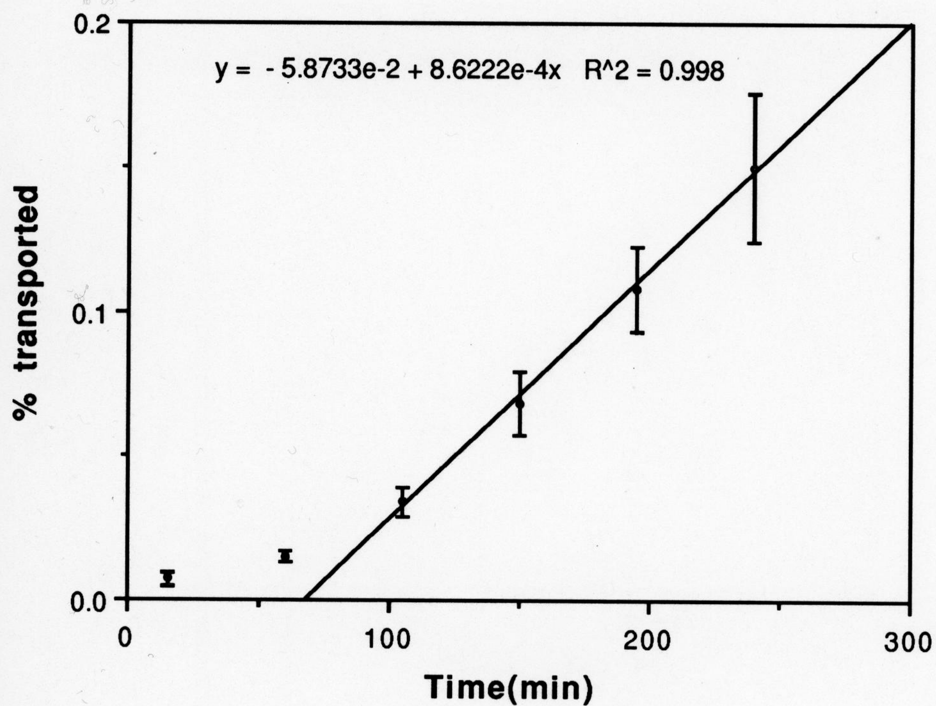


Figure IV-1A. Permeability study of the buccal tissue at pH 6.5. The error bar represents the standard deviation of five replicates.

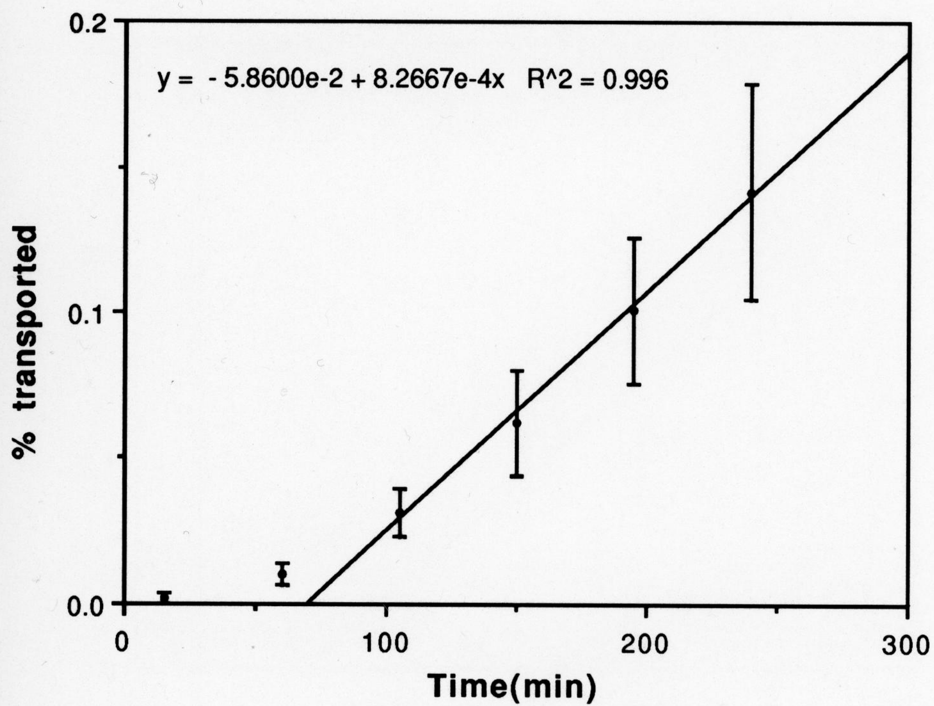


Figure IV-1B. Permeability study of the buccal tissue at pH 7.5. The error bar represents the standard deviation of five replicates.

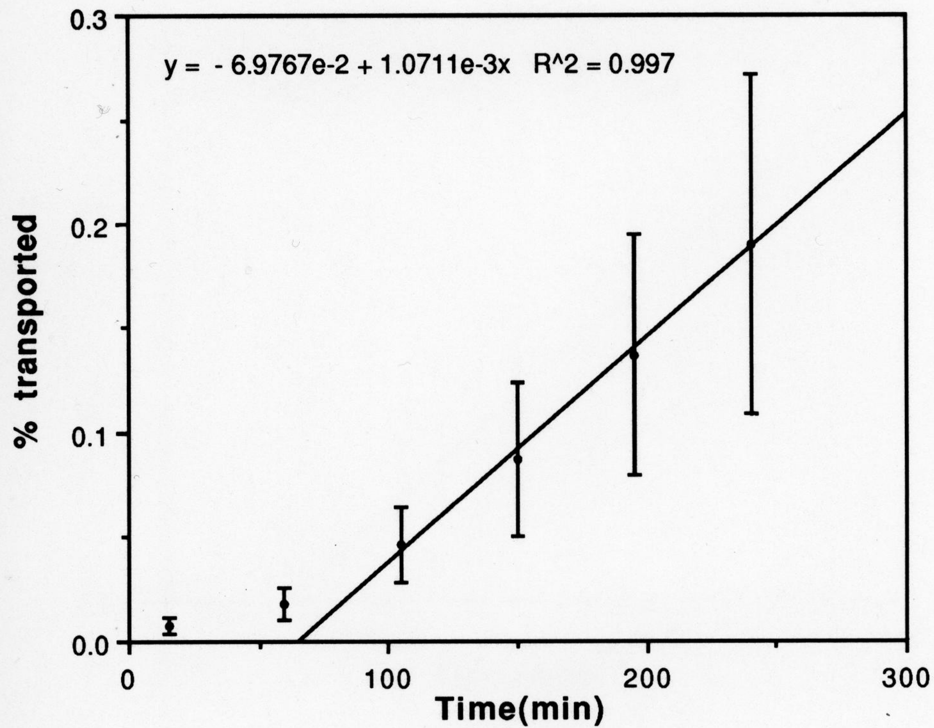


Figure IV-1C. Permeability study of the buccal tissue at pH 8.5. The error bar represents the standard deviation of six replicates.

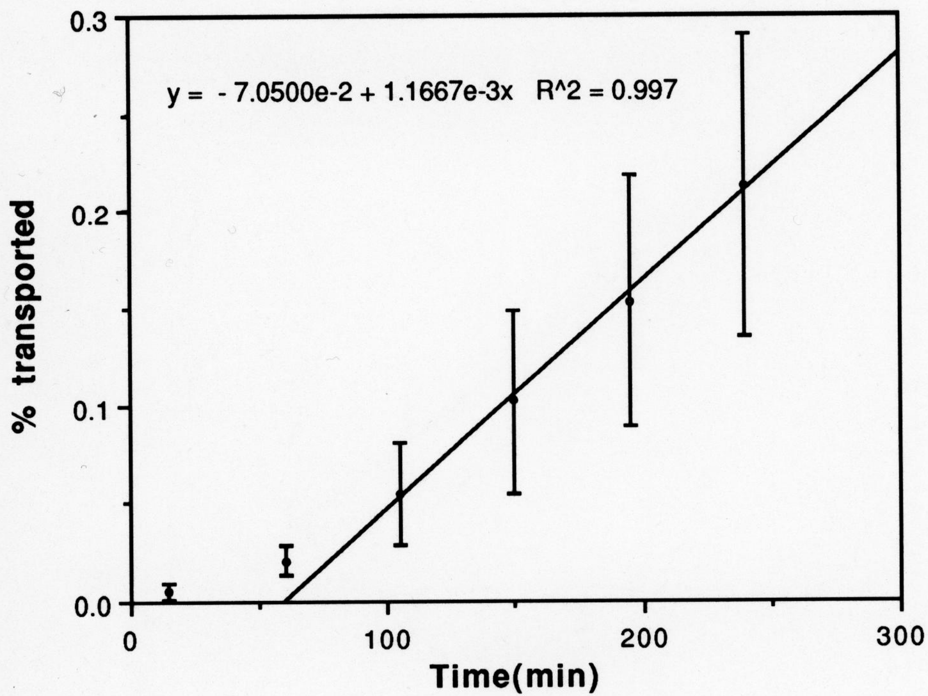


Figure IV-1D. Permeability study of the buccal tissue at pH 9.5. The error bar represents the standard deviation of five replicates.

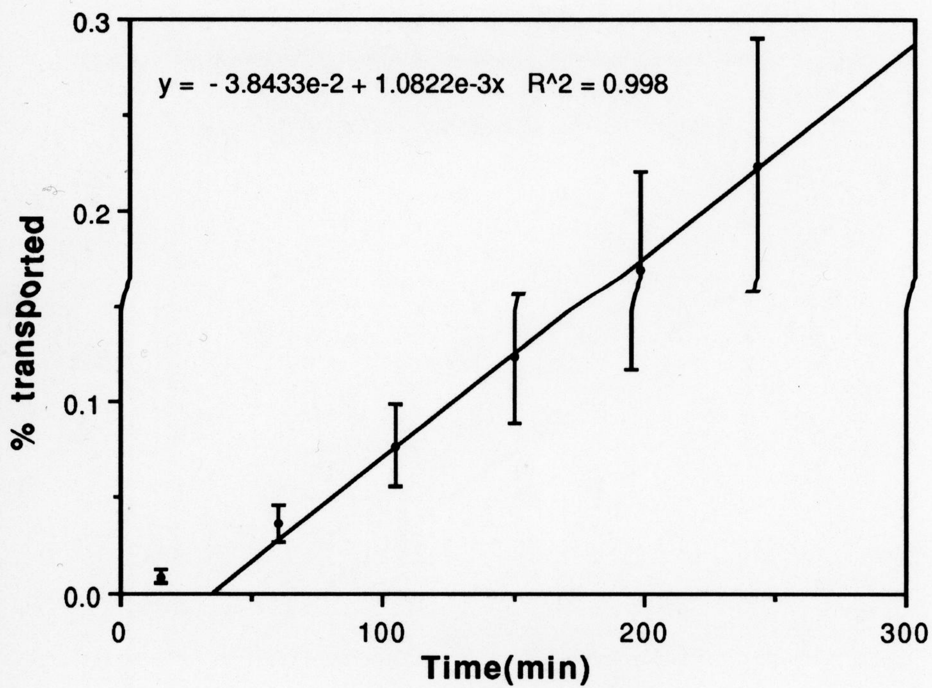


Figure IV-1E. Permeability study of the sublingual tissue at pH 6.5. The error bar represents the standard deviation of five replicates.

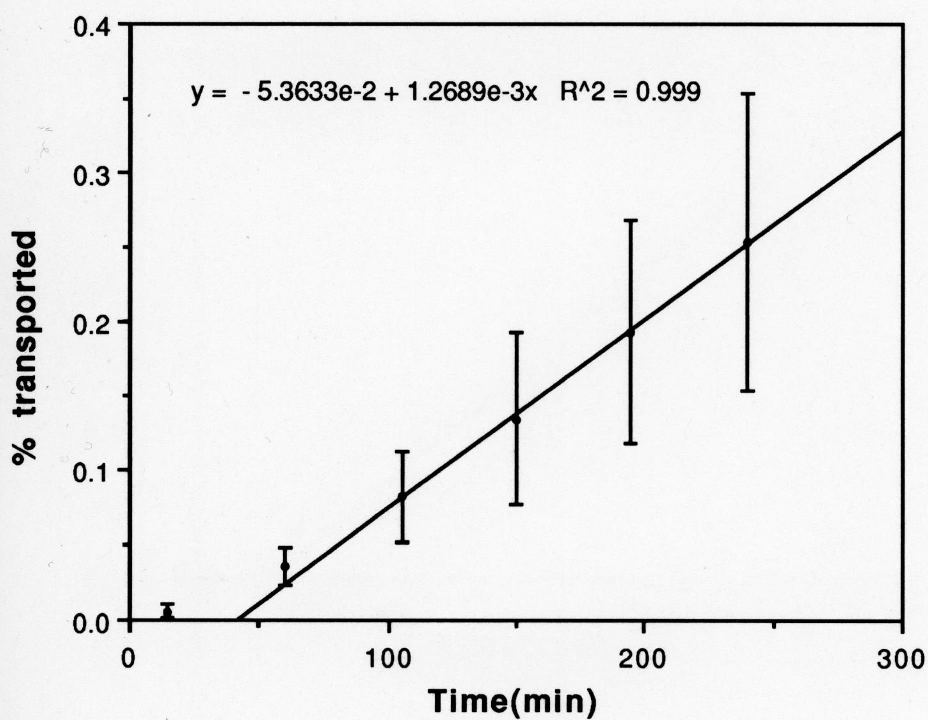


Figure IV-1F. Permeability study of the sublingual tissue at pH 7.5. The error bar represents the standard deviation of four replicates.

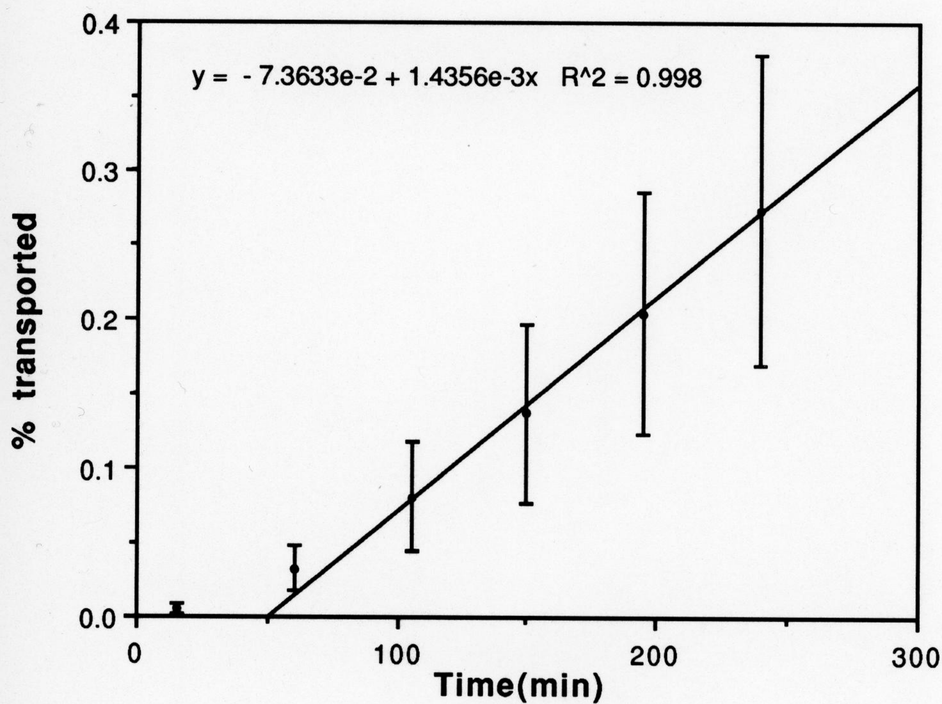


Figure IV-1G. Permeability study of the sublingual tissue at pH 8.5. The error bar represents the standard deviation of five replicates.

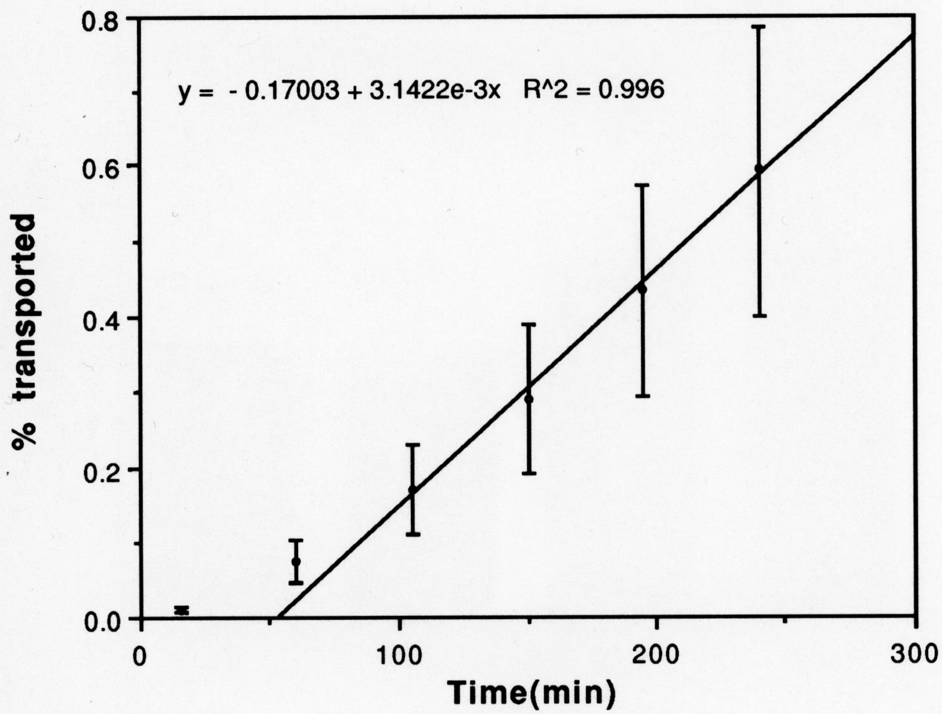


Figure IV-1H. Permeability study of the sublingual tissue at pH 9.5. The error bar represents the standard deviation of five replicates.

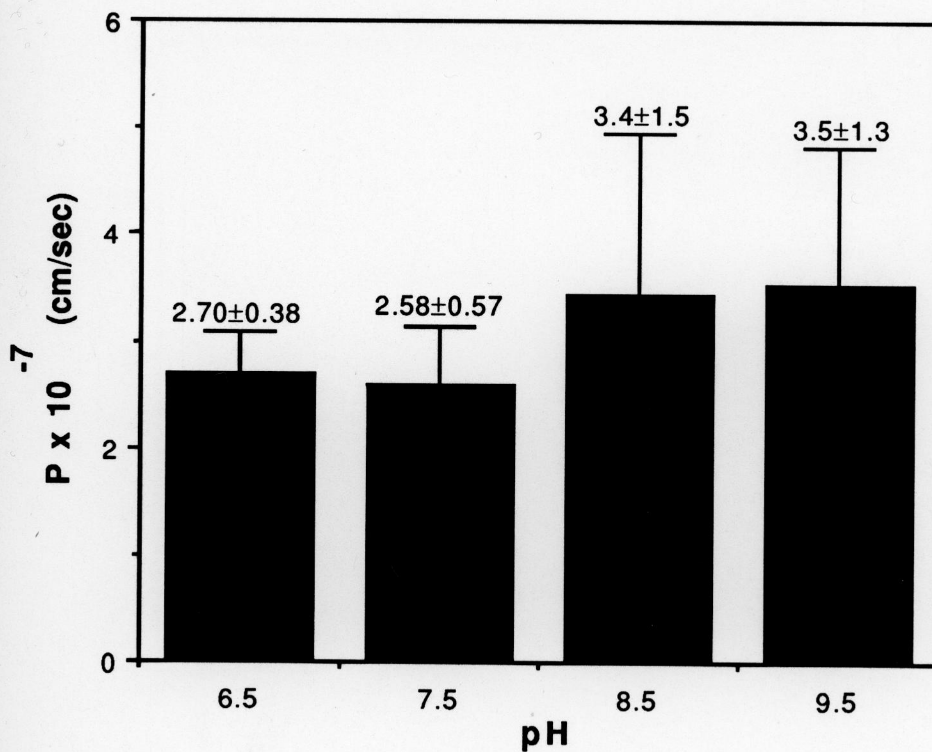


Figure IV-2. Permeability coefficients of the buccal tissue at various pH's. The permeability coefficient was calculated from the slope of % transported versus time.

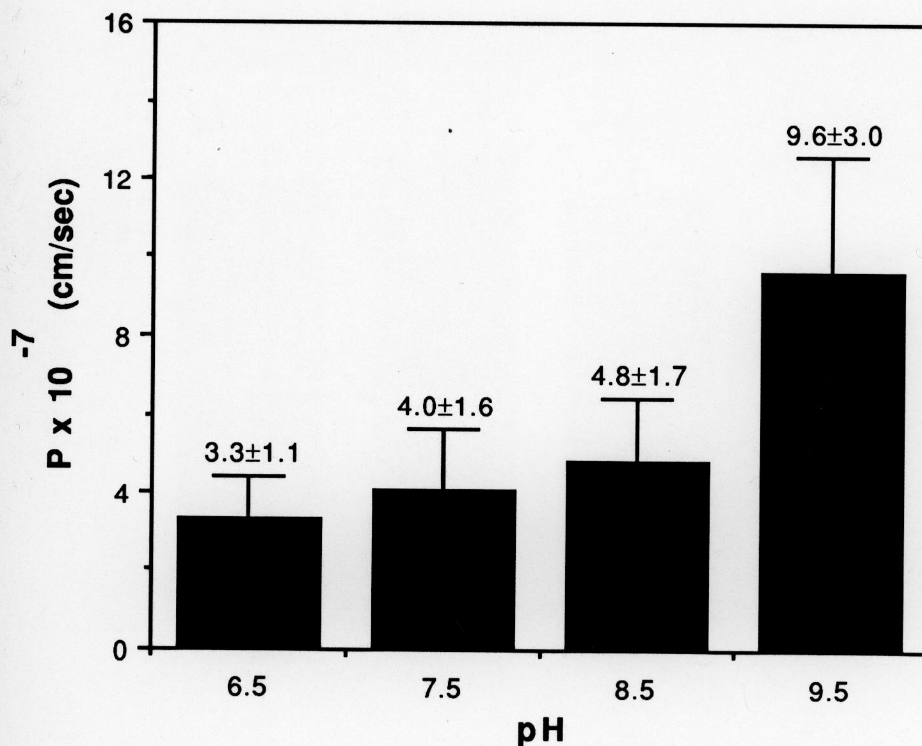


Figure IV-3. Permeability coefficients of the sublingual tissue at various pH's. The permeability coefficient was calculated from the slope of % transported versus time.

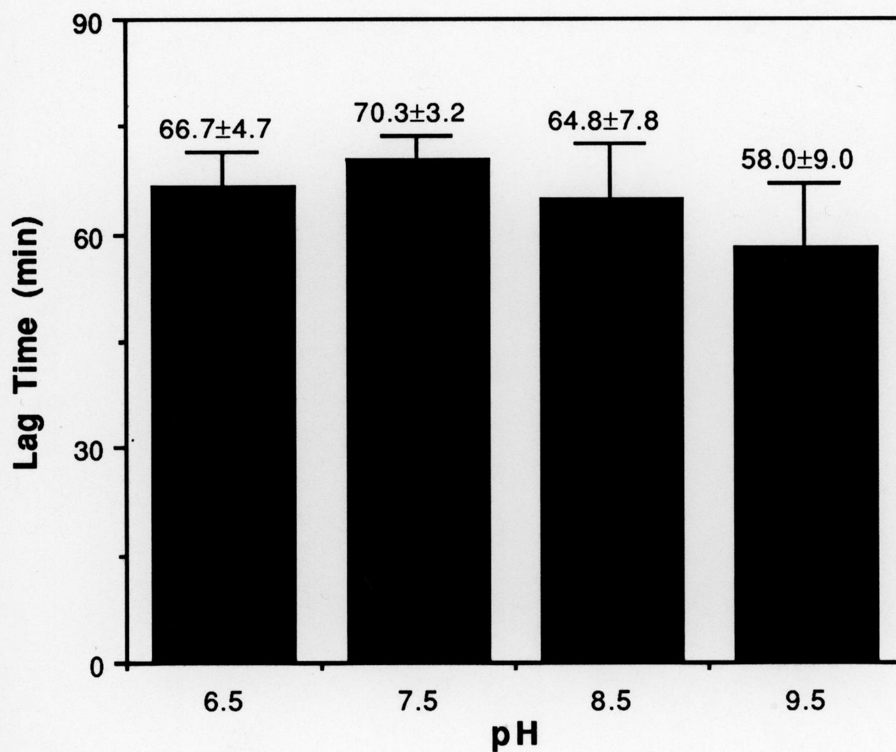


Figure IV-4. Lag times of the buccal permeability study at various pH's. The lag time is the x-axis intercepts of steady state curve of %transported versus time.

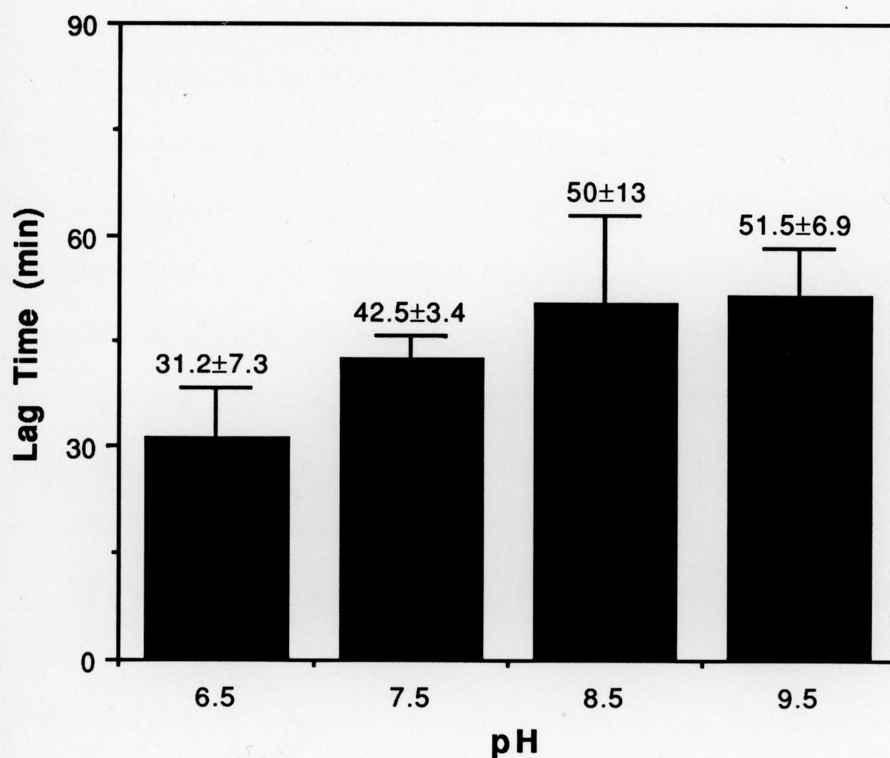


Figure IV-5. Lag times of the sublingual permeability study at various pH's. The lag time is the x-axis intercepts of steady state curve of % transported versus time.

with the theory that only the superficial layer of the epithelium is the permeability barrier (Squier, 1973; Squier and Rooney, 1976; Squier and Hall, 1985).

B. Partition Study

The partition coefficient is a measure showing the hydrophilicity and/or lipophilicity of a compound. If the compound has several molecular forms in solution, the apparent partition coefficient is the total of all the molecular forms. The apparent partition coefficient is also called the distribution coefficient. Octanol is used in this work as the oil phase. Fig. IV-6 shows that the hydrophilicity of morphine is greatest at pH 6.5 and gets smaller as the degree of ionization decreases, up to pH 8.5, where the hydrophilicity of morphine reaches its lowest point.

There are many criteria to measure hydrophilicity of a compound. The octanol/water partition coefficient gives only a general idea about the hydrophilicity of morphine in this system. Changing the oil phase will change the partition coefficient of the compound. Therefore the octanol/water partition coefficient is considered a relative measure of hydrophilicity.

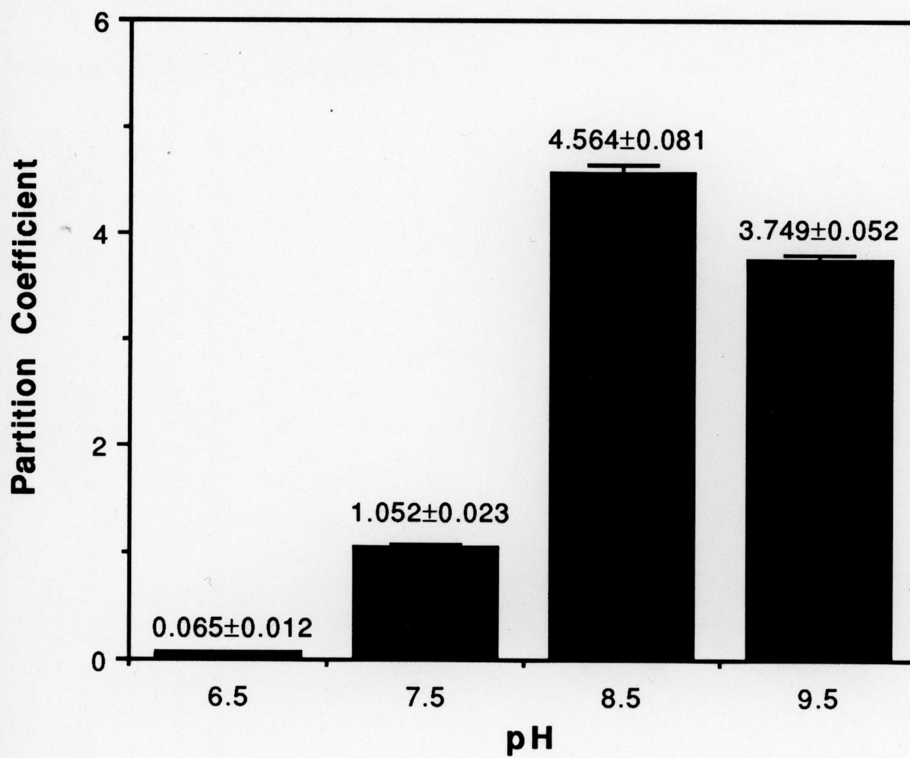


Figure IV-6. Octanol/water partition coefficient of morphine at various pH's.

C. Solubility Study

Solubility of a compound in water is another measure of hydrophilicity. Morphine solubility at various pH's are shown in Fig. IV-7. The result is similar to the partition study except that the solubility of morphine at pH 9.5 is lower than that at pH 8.5. This is not contradictory with the partition study because both partition and solubility study indirectly measure the hydrophilicity of a compound.

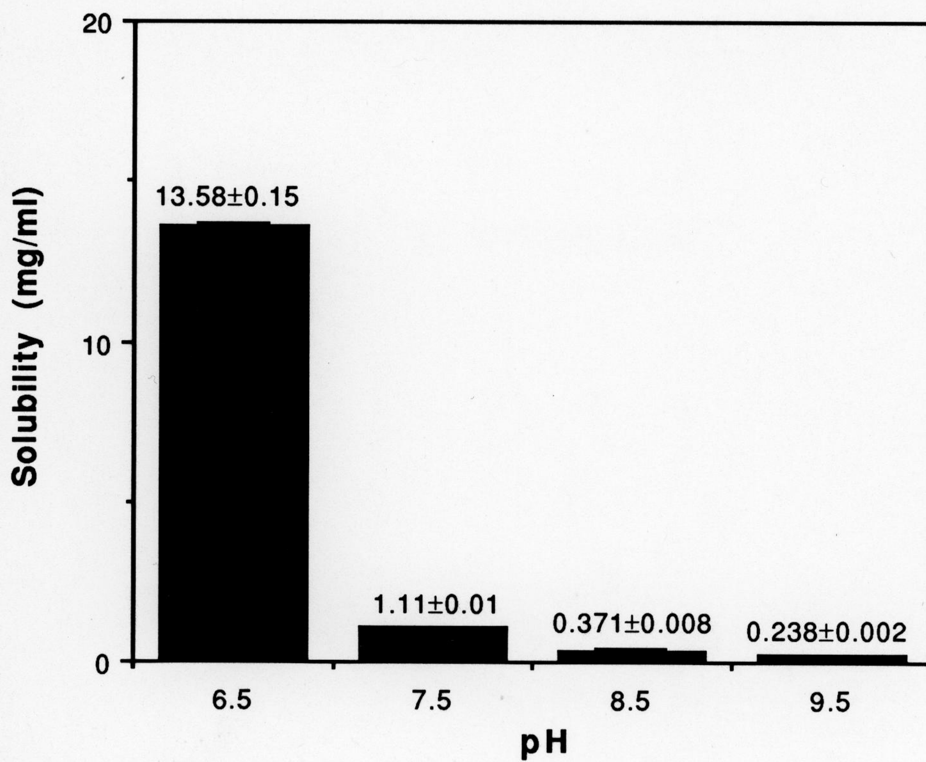
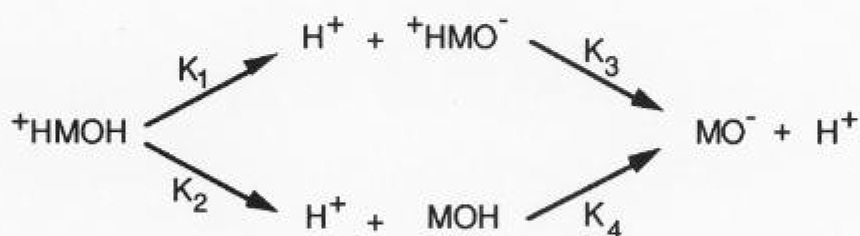


Figure IV-7. Morphine solubility in water at various pH's.

V. DISCUSSION

A. Dissociation Constants of Morphine

The structure of the morphine molecule is shown in Fig. V-1. There are two potential charged groups, the alkylamino group and the phenol group. The acidic dissociation constants of these two groups are so close that they actually overlap. Therefore there are four forms of morphine molecules: the morphinium ion $^{+}HMOH$, the morphinate ion MO^{-}, the uncharged form MOH and the zwitterion $^{+}HMO^{-}$. The complete protolysis of morphine can be depicted as below:



where K_1, K_2 , K_3 , K_4 are microscopic dissociation constants. The apparent macroscopic constants of morphine are defined by

$$Ka_1 = \frac{[H^+]([^+HMO^-] + [MOH])}{[^+HMOH]}$$

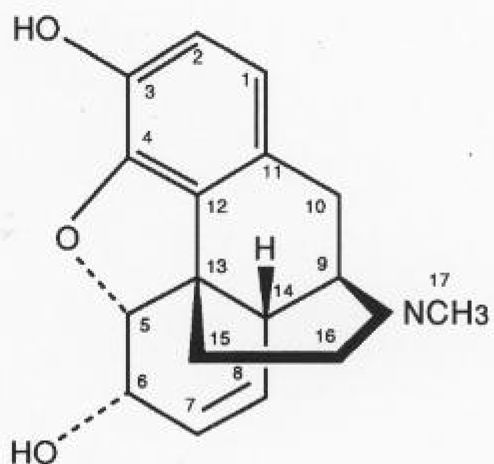


Figure V-1. Morphine molecular structure. There are two possible charged groups: the positive charged alkylamino group and the negative charged phenol group.

$$Ka_2 = \frac{[H][MO]}{[^{+}HMO^{-}] + [MOH]}$$

The relationship between micro- and macroscopic dissociation constants are

$$Ka_1 = K_1 + K_2$$

$$\frac{1}{Ka_2} = \frac{1}{K_3} + \frac{1}{K_4}$$

$$K_1 \cdot K_2 = K_3 \cdot K_4$$

The macroscopic dissociation constants, K_{a1}, K_{a2} are used most often in practice to calculate the degree of ionization of compounds.

There are many ways to measure morphine dissociation constants (Schill, 1964; Kaufman, 1975). However, due to the low solubility of morphine in water, the pK_a values from different references vary. In addition, temperature, ionic strength and other factors may effect the pK_a values. Kaufman et al, in 1975, measured morphine dissociation constants at 37°C by a microelectrometric titration method and found the dissociation constant of the alkylamino group, pK_{a1} is 7.93 and the dissociation constant of the phenol group, pK_{a2} to be 9.63. Since it is difficult to measure the microscopic dissociation constants, K_1 , K_2 , K_3 , K_4 accurately, it is difficult

to determine the percent of unionized and zwitterion morphine at a given pH. However, the percent of morphinium ion $^{+}HMOH$, morphinate ion MO^{-} and the combination of unionized MOH and zwitterion morphine $^{+}HMO^{-}$ can be determined from the pH profile (Fig. V-2). It shows that the combination of $^{+}HMO^{-}$ and MOH reaches a peak at pH 8.8. At the pH's selected in this study, the percentage of each form is shown in Table V-1.

B. pH Dependence of Hydrophilicity

The hydrophilicity of a weak acid or a weak base is highly pH dependent. This phenomenon is shown by the pH dependence of the apparent partition coefficient (distribution coefficient) and/or aqueous solubility. Although the skeleton of a compound plays a major role in hydrophilicity, the charged side group(s) may also effect the hydrophilicity within a certain range. Presumably, the unionized form has a lower hydrophilicity than the ionized form. The hydrophilicity of morphine is a good example of this in that the octanol/water partition coefficient (Fig. IV-6) and the aqueous solubility (Fig. IV-7) data show good agreement with the molecular forms (Fig. V-2).

Many publications have reported the apparent partition coefficient and solubility of a weak acid or a weak base without knowledge of aqueous phase pH. These are actually misleading statements because pH plays an

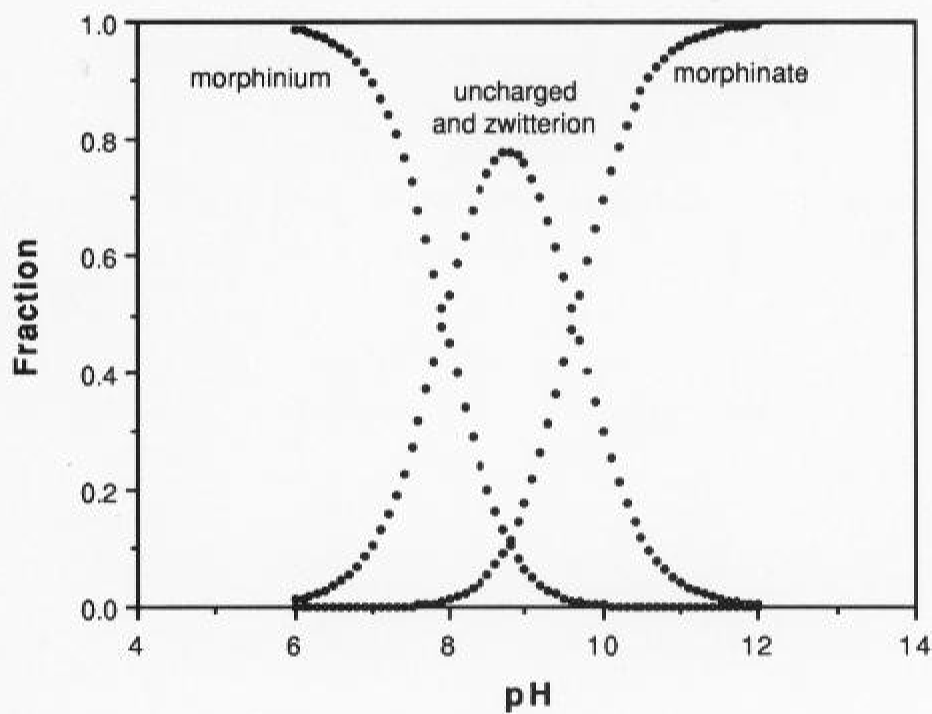


Figure V-2. Morphine pH profile at 37°C. pK_{a1} is 7.93, pK_{a2} is 9.63 (Kaufman, 1975).

Table V-1. Percent of morphine molecular forms at pH 6.5, 7.5, 8.5 and 9.5 at 37°C. pKa1 is 7.93. pKa2 is 9.63 (Kaufman, 1975).

pH	$^{+}HMOH$	$MOH/^{+}HMO^{-}$	MO^{-}
6.5	96.4	3.6	0.0
7.5	72.8	27.0	0.2
8.5	20.0	74.5	5.5
9.5	1.5	56.6	41.9

important role in the apparent partition coefficient and solubility, especially when the pH is close to the pK_a. Kaufman et al, conducted the partition coefficient study of morphine in the pH range from 7.10 to 7.70 (Table V-2) and showed that within the range of only 0.6 unit, the apparent partition coefficient changes from 0.80 to 2.30.

C. pH Dependence of Oral Epithelial Drug Absorption

Many studies have shown that oral epithelial drug absorption is pH dependent (Beckett, 1971; Hicks, 1973; Randhawa, 1986, Al-Sayed-Omar, 1987; Barsuhn, 1988, Yamahara, 1990; Zhang, 1991). To verify this conclusion, it is important to examine how the experiments were conducted.

Among the studies showing the pH dependence of oral epithelial drug absorption, four of them used the Beckett and Trigg's *in vivo* drug absorption test and the drug absorption was calculated from drug loss of the applied solution (Beckett, 1971; Hicks,1973; Randhawa, 1986; Al-Sayed-Omar,1987). The other three studies used *in situ* perfusion study (Barsuhn, 1988; Yamahara, 1990; Zhang, 1991). However, drug adsorption was also calculated from drug loss of the applied solution. Apparently, all of them measured drug absorption indirectly and none of them have evidence that all drug lost from the applied solution was transported through the oral epithelium. On the other hand, as mentioned in the introductory section, Davis (1979) and Henry (1980) demonstrated that the oral epithelium has high storage capacity, and the amount of drug stored to epithelial tissue can

Table V-2. Distribution coefficient of morphine at 37°C (Kaufman, 1975).

pH 7.10	pH 7.35	pH 7.40	pH 7.45	pH 7.50	pH 7.60	pH 7.70
0.80	1.30	1.42	1.54	1.68	1.98	2.30

be as much as 60% of the total drug lost from the applied solution. Obviously, the conclusion drawn from the previously mentioned studies is questionable.

Alternatively, other studies show that drug transport through the oral epithelium is pH independent (Zhang, 1989; Dowty, 1992). Zhang and et al (1989), using an *in situ* perfusion dog model, studied absorption of isoproterenol at pH 4.0 and 7.0 and found that the permeability coefficients at these two pH's are similar The permeability coefficient was estimated by comparing the biological effect of *in situ* transbuccal perfusion to I.V. infusion. Dowty and et al (1992), studied the permeability of Thyrotropin Releasing Hormone (TRH) using an *in vitro* rabbit model and found that the permeability coefficients are the same at pH 4.0 and 8.0. The similarity of these two studies is that the permeability coefficient is estimated from drug on the receiver side, which is transported completely through the epithelial tissue. All the other factors that can decrease the amount of drug on the donor side, such as adsorption, binding, metabolism, etc., will not effect the permeability result if it is calculated from samples in the receiver chamber.

Similar results are shown in this work. More importantly, a similar experimental concept was used in this work, where the morphine permeability coefficient was calculated from the concentration in the receiver chamber. The morphine permeability study clearly demonstrated that epithelial drug absorption is pH independent, or, at worst, small enough to be buried in the standard deviation.

D. Mechanism of Morphine Oral Mucosal Absorption

The study of morphine oral mucosal absorption was conducted by using an *in vitro* diffusion apparatus, as described in the introductory section. The apparent morphine permeability coefficient was determined by measuring the concentration of morphine on the receiver side and making the necessary calculation. However, there is no direct evidence to prove that the paracellular route was the preferred pathway for the drug, and whether the pH inside the cell and in the intercellular space are the same as the pH in the bathing solution. In addition, there is no evidence that the different forms of morphine molecules use the same penetrating route. It thus seems that there is no way of knowing the mechanism of the transport process from the data in the present study. However, if all the possibilities are listed, it is not difficult to, at least, speculate on the most possible mechanism.

Fig. V-3 A to E list all the possibilities of morphine transport through epithelium if different forms of morphine use the same pathway. Fick's first law can be used to calculate the flux of morphine transport, as described in the introductory section. It is concluded that only model A has no pH dependence whereas other models will introduce a partitioning step into the system and, thus, are pH dependent.

If morphine uses more than one penetrating route at the same time, and each route has its own partition coefficient, diffusion coefficient and length of molecular movement, the apparent flux is the sum of all the pathways. Then

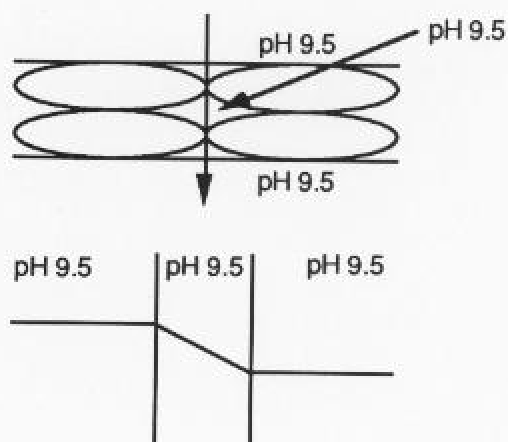


Figure V-3 A. Model of paracellular pathway permeation. The pH in the intercellular space is the same as the pH in the solution. The media of the intercellular space is hydrophilic and similar to the solution.

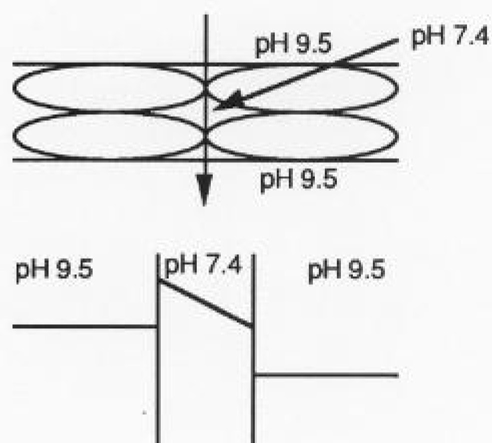


Figure V-3 B. Model of paracellular pathway permeation. The pH in the intercellular space is regulated and differs from the pH in the solution. A preferential partition occurs at the solution-membrane interface.

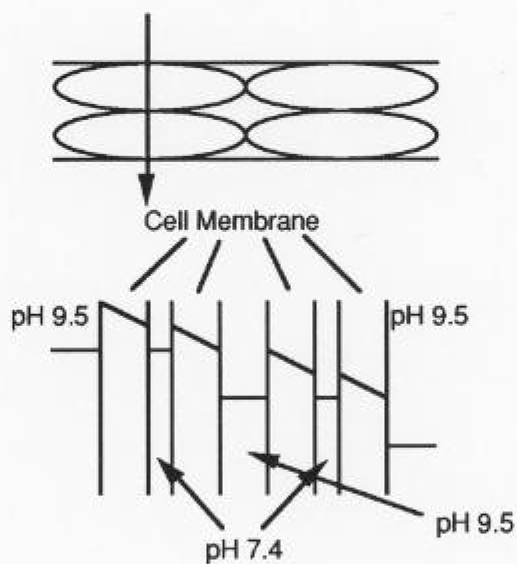


Figure V-3 C. Model of intracellular pathway permeation. pH of the cytoplasm is regulated at 7.4, but pH in the intercellular space is the same as pH in the solution.

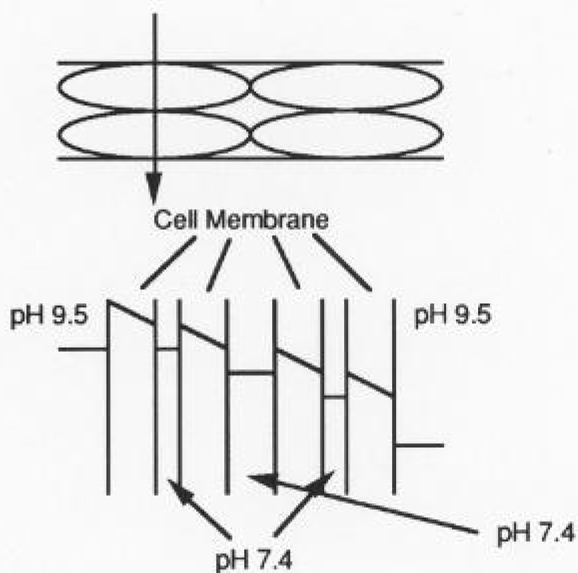


Figure V-3 D. Model of intracellular pathway permeation. The pH inside the membrane, both the cytoplasm and intercellular space, is regulated at 7.4.

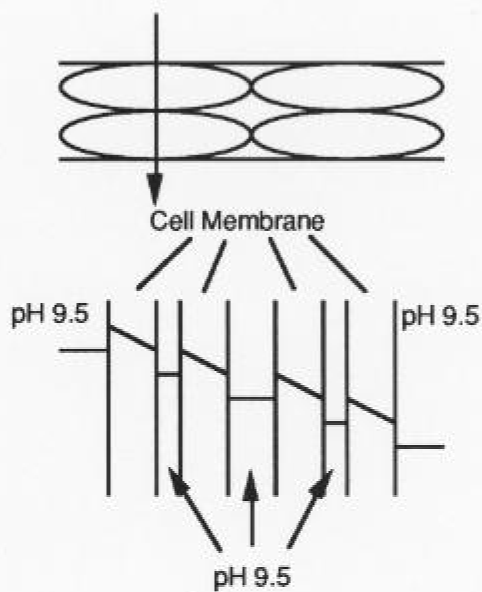


Figure V-3 E. Model of intracellular pathway permeation. The pH inside the membrane is the same as the pH in solution.

$$J = J_1 + J_2 + \cdots\cdots$$

or

$$J = \left(\frac{D_1 K_{p1}}{h_1} x_1 + \frac{D_2 K_{p2}}{h_2} x_2 + \cdots\cdots \right) (C_D - C_R)$$

where x1, x2 ··· are the fractions of molecular forms. In order to keep the apparent flux unchanged, while the pH is changed as well as the fractions of the molecular forms, the increase and decrease of each form must be fully compensated,

$$\frac{D_1 K_{p1}}{h_1} dx_1 + \frac{D_2 K_{p2}}{h_2} dx_2 + \cdots\cdots = 0$$

This is very coincidental and unlikely.

Apparently, results of the morphine oral mucosal permeability study suggest that morphine is transported by a paracellular route and, further, the intercellular media has the similar hydrophilicity and pH as the bathing solution. The pH inside the cell is unknown and is difficult to predict using the data of this study. However, another study has shown that the pH of cytoplasm is well regulated (Dowty and Braquet, 1991).

E. Molecular Basis of Barrier Function in Oral Epithelium

Squier and co-workers studied the penetration of horseradish peroxidase and lanthanum and found that the penetration barrier appears at the top quarter to one third of the epithelium, where the contents of the membrane coating granules were expelled into the intercellular membrane (Squier, 1973; Squier and Rooney, 1976). This study suggests that the barrier function of the epithelium is the result of intercellular material(s) secreted by membrane coating granules (MCG). The chemical nature of the oral epithelial intercellular space has not been well defined yet. Many researchers studied the lipid content of the whole oral epithelium (Squier,1991; Wertz, 1986). However, these studies cannot identify whether the lipids are inside or outside the cells. Therefore the distribution of the lipids is unknown, as well as the lipids in the intercellular space. Histochemical staining for carbohydrate and the behavior of the MCG suggests that the glycosylceramides may be extruded from the cored MCG into the intercellular space (Innes, 1973). Proteins may also exist in the intercellular space (Hay, 1981).

It should be emphasized that not only the contents of the intercellular media is important, but also the distribution and arrangement of these contents. Unfortunately, very little is known about this particular area.

F. Penetration Enhancement

Many theories about penetration enhancement have been proposed and many substances studied. However, with limited knowledge of the mechanism of drug penetration and the molecular basis of inter and intra cellular pathways, it is difficult to find an efficient penetration enhancer, which must not damage the normal function of the epithelium. Generally, there are two approaches, changing chemical properties of the applied solution or device to increase the partition coefficient of the drug, e.g., adding co-solvent to the applied solution, etc., and treating the oral tissue with certain agent(s) to alter the chemical and/or physical properties of the paracellular medium, and consequently change the partition coefficient and diffusion coefficient of the membrane. Dowty (1991) proposed that the polysaccharide matrix could be the major intercellular permeability barrier of rabbit buccal epithelium. A list of enzymes, which can break up polysaccharide chains, have been employed to examine the make-up of the intercellular media and its contribution to the permeability barrier. The electrophysiology studies show a significant decrease of resistance for the tissue treated with chondroitinase, trypsin and heparitinase. The permeability of horseradish peroxidase was increased with the treatment of chondroitinase and trypsin. Although it is difficult to verify which part of the intercellular space is "opened up" and to demonstrate that the same result would happen in an *in vivo* system, it is the correct direction for the study of penetration enhancement.

In order to find an efficient penetration enhancer, more details are

needed about: 1) The mechanism of drug transport; 2) The chemical, biological nature of intercellular media, and 3) The physical arrangement of molecules in the intercellular space.

VI. CONCLUSION

It appears that oral mucosa permeability of morphine is pH independent even though morphine hydrophilicity is highly pH dependent. This suggests that the traditional view of considering the oral mucosa as a hydrophobic membrane is inadequate. It also suggests that morphine molecules may use the paracellular pathway regardless of their hydrophilicity. Since the intercellular media is hydrophilic as compared to the cell membrane, the permeability coefficient should not be proportional to the octanol/water partition coefficient. The partition coefficient should be the preferential partition between the intercellular media and the bathing buffer solution. Therefore, the chemical and biochemical nature of the intercellular media and its physical arrangement is essential for studying drug permeability and to ultimately enhance drug permeability.

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