PHARMACOKINETICS OF ALBUTEROL AND BUTORPHANOL ADMINISTERED INTRAVENOUSLY AND VIA A BUCCAL PATCH

A Thesis

by

DEIRDRE FAYE VAUGHAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2003

Major Subject: Veterinary Physiology

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Approved as to style and content by:

Dawn M. Boothe (Chair of Committee)	Gordon Brumbaugh (Member)
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ABSTRACT

Pharmacokinetics of Albuterol and Butorphanol Administered Intravenously and via a Buccal Patch. (May 2003)

Deirdre Faye Vaughan, B.S., Auburn University

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Conventional routes of drug administration have several disadvantages. The rate and extent of absorption can vary greatly depending on the drug, its formulation, the presence of food, drug interactions, first-pass metabolism, and gastrointestinal pH. Better dosage forms or drug delivery mechanisms could minimize these problems.

The pharmaceutical industry has recognized the need for, and has developed many new, novel drug delivery systems. Drugs that previously had decreased effective concentrations can be given by novel routes, reducing the dosing frequency of many drugs. Transmucosal drug delivery can result in rapid drug absorption and systemic delivery. This study utilized a buccal patch to deliver albuterol and butorphanol.

The purpose of this study was to establish pharmacokinetic parameters and the bioavailability of albuterol and butorphanol when administered intravenously and buccally. Three dogs weighing 20 kg were studied. Each received albuterol and butorphanol by buccal and intravenous administration. Blood samples were collected and analyzed by ELISA. Values for pharmacokinetic parameters were determined using non-compartmental modeling.

For albuterol, extrapolated C_{max} and C_o after buccal and IV administration were 10.28 ± 2.77 and 57.74 ± 9.04 ng/ml, respectively. Volume of distribution was 2.13 ± 1.30 L/kg and clearance was 4.73 ± 3.91 ml/min/kg. A significant difference existed between the disappearance rate constant of buccal and intravenous albuterol administration. The half-lives of buccal and IV albuterol were 160.96 ± 24.19 and 364.20 ± 115.20 min, respectively. The bioavailability of buccally administered albuterol was 35%.

Maximal concentration (C_{max}) and C_o after buccal and IV butorphanol administration were 6.66 ± 1.65 and 8.24 ± 5.55 ng/ml, respectively. Volume of distribution was 27.58 ± 10.14 L/kg and Cl was 137.87 ± 19.55 ml/min/kg. The half-life of buccally administered butorphanol was 259.15 ± 33.12 min and 172.12 ± 94.95 min for intravenous butorphanol. The bioavailability of buccally administered butorphanol was 606%.

The buccal patch used in this study achieved systemic concentrations for both albuterol and butorphanol. Further studies are needed to determine if therapeutic drug concentrations can be achieved with the buccal patch and if the patch can result in clinical efficacy.

DEDICATION

This manuscript is dedicated to all animals that have donated their time, their freedom, and sometimes their lives in order to improve the welfare of creatures everywhere. Their sacrifice will never be forgotten—by science or by their Creator.

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There are too many individuals I need to thank, to recognize, and be indebted to. As such, I suppose I shall start where it all began—my parents and my family.

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Jon, you are easily the most sane person in our family. Gary, Mom, and I owe you a great deal. Thank you for your infinite patience and generous heart. Mam Maw, you are truly a wonderful grandmother. I owe my interest in cooking and gardening all to you. Thank you for the countless fried apple pies over the years, and for always having an open kitchen and heart.

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This thesis is also dedicated to Higgins, one of the sweetest dogs I have ever known. Thank you for coming into our lives and putting up with both Chaucer and my hectic schedule. Which of course, brings us to Chaucer. Chaucer, you are, without a doubt, woman's best friend. So many times I have come home upset, depressed, or angry, and one look at you completely erases the day's stresses—until you start that

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I would also like to thank Dr. Boothe for her willingness to let me finish my degree at such an overdue date, as well as all other members of my graduate committee, and the Department of Veterinary Physiology and Pharmacology

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CHAPTER I

INTRODUCTION

NOVEL DRUG DELIVERY

Many advances have been made in recent years in the area of biopharmaceutical technology. The systemic delivery of drugs through novel methods of administration is one area in which significant changes and improvements have been made. Conventional routes of drug administration such as oral, intramuscular (IM), and intravenous (IV) have, in many cases, been supplanted by the advent of new, novel drug delivery systems. Consequently, precise control of drug input into the body by a variety of routes is now possible. Controlled and sustained release formulations have been developed and are gaining in popularity and medical acceptance.[1] Drugs that normally exhibit low bioavailability after oral administration can be given by a novel route in order to improve duration of action and efficacy.[2] Examples include transdermal systems, such as patches, which been developed for a number of drugs (e.g. nicotine and fentanyl), and microencapsulation and liposomal drug preparations.[2-4]

Advantages of novel drug delivery vary with the system, but major goals include sustained drug delivery leading to less frequent dosing as well as avoidance of marked fluctuations in peak and trough plasma drug concentrations during the dosing interval which often is associated with systemic drug administration.[5, 6] Other advantages of pharmacotherapy utilizing novel delivery include: bypass of the gastrointestinal tract

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This thesis follows the model of the *American Journal of Veterinary Research.*

and hepatic portal system, thus increasing the bioavailability of orally administered drugs that otherwise undergo hepatic first-pass metabolism; improved patient compliance due to the elimination of pain associated with injections; administration of drugs in unconscious or incapacitated patients; convenience of administration as compared to injections or oral medications; and ready termination of delivery by detaching the patch.[2, 7-11] As a result, novel drug delivery systems have the potential to greatly improve the efficacy and therapeutic benefit of many existing drugs.

PROBLEMS OF CONVENTIONAL DRUG DELIVERY

Oral drug delivery is the most widely utilized route of administration for the systemic delivery of drugs.[12] The popularity of oral drug administration may be attributed to ease of administration, as well as the traditional belief that drugs delivered orally—like food—are well absorbed.[12] However, oral drug administration is limited by many disadvantages. The rate and extent of absorption can vary greatly depending on the drug, its formulation, the presence or absence of food in the stomach, drug interactions, and the pH of gastrointestinal fluids.[2] These and other factors contribute to variability in the amount of drug absorbed among patients.[2]

Extensive first-pass hepatic metabolism can greatly reduce the bioavailability of orally administered drugs.[2] Drug metabolites formed following first-pass through the liver may not be as active or as potent as the parent drug (e.g. butorphanol), thus necessitating the oral dose to be much greater than the parenteral dose required to cause the same clinical effect.[13] For some drugs, such as isoproterenol and albuterol, first pass

metabolism is so great that therapeutic concentrations cannot be achieved with oral administration.[13]

Some patients (e.g. sedated, comatose, or neonatal patients) cannot take medications orally, and some drugs are not available as oral preparations. Children or veterinary medical patients may be fractious, or otherwise difficult to medicate orally. Better dosage forms, or drugs delivered via a novel route could minimize many of these problems.

Regardless of the route of administration, an appropriate amount of drug must be absorbed and transported to the site of action in order to elicit a given therapeutic response. Drug distribution can also be non-selective, resulting in drug residue appearing in tissues (e.g. liver and kidney) other than the targeted site of action. Not only can drug non-selectivity be wasteful, but it can also contribute to toxicity.[2] As a result, the full therapeutic potential of many drugs cannot be realized by conventional methods of drug delivery. In many cases, the use of novel drug delivery systems could circumvent many of these problems, while still achieving therapeutic drug concentrations.

TRANSDERMAL DRUG DELIVERY

Drug administration across the dermis, or transdermal drug delivery, is a method gaining increasing use in both human and veterinary medicine. Transdermal systems have been utilized in human medicine for the delivery of a variety of compounds. In veterinary medicine, a wide variety of drugs have also been formulated into products that are applied directly onto the skin. Both insecticides and anthelmintics are formulated

into topically applied treatments. Furthermore, the growing interest in post-operative/traumatic pain control in small animals has led to investigations studying the pharmacokinetics and clinical application of transdermal administration of fentanyl and oxymorphone in dogs.[14]

The skin is an anatomically dynamic structure that varies among subjects and is affected by a variety of conditions. Such factors include individual, species, and breed variation, blood flow and vascular perfusion, degree of environmental exposure, body temperature, hydration state, and skin integrity; each is able to influence drug movement across the skin.[15] As a result of this variability, it is often not possible to predict an individual animal's clinical response to transdermal drug delivery.

Formulations of Transdermal Drugs In Veterinary Medicine

Pesticides are among the most common—and perhaps well-known—transdermally administered compounds in veterinary medicine. Dosing forms include backrubbers, dips, body sprays, and medicated ear tags.[16] High volume, diluted pour-on treatments, and low volume, high concentration "spot-on" formulations are also available as topical insecticide treatments. The first topical application of a pour-on insecticide was reported in 1957 to successfully treat pediculosis in chickens and sheep.[16] Pour-on formulations containing organophosphates have had tremendous impact in the cattle industry by controlling lice infestations and the cattle grub, *Hypoderma* species.[16] Examples of spot-on formulations include flea control products such as imidacloprid, selamectin, and fipronil which have revolutionized pesticide control in companion animals.

Iontophoresis is an “active” form of transdermal drug delivery whereby movement through the skin occurs as a result of an electric current. Iontophoresis increases the permeability of the stratum corneum to large and/or charged drugs that are not able to passively diffuse. The permeability is increased due to mechanical disruption of the stratum corneum caused by the low voltage current that is generated. Other means of epidermal disruption include ultrasonic (phonophoresis) energy, and high voltage electrical pulses (electroporation). Due to the electrically induced breakdown of the stratum corneum, it may be possible to deliver large molecular weight compounds, peptides, and oligonucleotides via a transdermal route.[17] Iontophoretic technology may be more appropriate to achieve rapid, immediately effective plasma drug concentrations that more passive technologies (e.g. transdermal patches) are less suited for.[18] Iontophoresis has been examined in veterinary medicine to administer dexamethasone, ketoconzole, lidocaine, 2% methylene blue, and a novel inotropic catecholamine.[19-24]

Many of the antibiotics used in veterinary medicine to treat bacterial skin infections are prepared as topical formulations. These include sulfonamides, chloramphenicol, polymyxins, and neomycin. In fact, antiseptics such as nitrofurazone, povidone iodine, and chlorhexidine are available only as topical preparations. Antifungal agents are also formulated into topical medications to treat cutaneous mycoses. In addition, glucocorticoids are often found in topical antibiotic or antifungal preparations, or they may be used alone.

Drugs suspended in gel formulations can also be applied cutaneously and absorbed through the integument. Investigations utilizing lecithin based organogels have

demonstrated their effectiveness in increasing the transport rate of scopolamine and ketoprofen in the skin.[25] These gels—as with other transdermal delivery systems—may be effective in administering drugs to patients that are unable to take oral medications, or for drugs that undergo significant first pass metabolism and are not available as oral preparations. Other advances in the transdermal delivery of drugs include the use of supersonic helium to deliver drug particles in powder form at a velocity high enough to penetrate the stratum corneum.[26]

The use of transdermal patches in veterinary medicine is rapidly gaining interest and popularity in clinical use. Fentanyl is the only drug that is currently available in patch formation that is widely used in small animal patients at this time. The primary challenge in development of these systems is based upon the species variation seen in skin structure and function.

The Skin: Physiology and Histology

In addition to being the largest organ in the body, the skin is an actual physical barrier that protects the body from environmental and chemical insults. On a physiological level, the skin is vital to thermal, hormonal, immunologic, metabolic, and electrolyte regulation.[27] The skin is composed of two primary layers separated by a basement membrane: an outer epidermis and the underlying dermis. The junction between the two layers is formed by raised, undulating ridges, called rete ridges. Capillaries found in the rete ridges provide the blood supply to the avascular epidermis.[27] Hair follicles, sebaceous, and sweat glands all originate in the dermis before traversing the epidermal layers. Beneath the dermis is the hypodermis—or

subcutaneous layer, which attaches the dermis to underlying muscle or bone.[16] The skin is also a dynamic organ, differing in texture and thickness in various regions throughout the body.[28] For example, although basic skin architecture is similar between all mammalian species, differences do exist and can impact the rate and extent of TDD.[16] For instance, rats, mice, and rabbits have more hair follicles than humans, but lack sweat glands.[16] Also, the presence of hair, fur, or wool must be accounted for when using a veterinary species to investigate transdermal drug delivery, since these structures can interfere with drug movement through the skin.[16]

Histologically, the epidermis is classified as stratified squamous keratinized epithelium and is comprised of five layers. The stratum basale is the deepest layer and consists of a single layer of mitotically active cells, thus is partially responsible for epithelial cell renewal.[27] It is supported by a basal lamina and rests on the dermis. The stratum spinosum is the thickest layer of the epidermis, and like the stratum basale, assists in epithelial cell turnover. The stratum granulosum contains cells that possess membrane-coating granules.[27] These granules are released by exocytosis, forming a waterproof, lipid barrier that represents one of the protective mechanisms provided by the skin. The stratum lucidum is a clear, thin layer of cells that is superficial to the stratum granulosum. The outer-most layer of the epidermis is the stratum corneum, containing many flattened layers of keratinized cells surrounded by lipid bilayers with hydrophilic regions in between. The stratum corneum is the major barrier to systemic delivery of drugs applied to the skin.

A network of arterial and venous blood vessels is interspersed throughout the dermis. This blood flow nourishes both the dermis and epidermis, and is the site of percutaneous uptake of compounds delivered transdermally. In humans, blood supply to the epidermis is provided via two artery types: a musculocutaneous branch that runs perpendicular to the skin and supplies the skin and underlying muscle; and a cutaneous branch that travels parallel to the skin and directly supplies blood to the skin. Blood flow rates are believed to be one of the factors affecting passive drug perfusion through the skin. Increased flow that occurs with vasodilation, increases systemic delivery of topically applied drugs, while decreasing local accumulation. Vasoconstriction has the opposite effect, decreasing systemic delivery and increasing localized drug. In addition, flow rates vary between anatomic sites and the species in question. For example, the ventral abdomen of the dog exhibits a blood flow rate of 8.78 ± 1.40 ml/min/100g tissue.[17] In contrast, the humero-scapular joint has a flow rate of 5.51 ± 2.32 ml/min/100g tissue.[17] Thus, the anatomic site of drug application can play a critical role in achieving systemic and therapeutic drug concentrations.

Comparative Anatomy of the Integument

Though minor differences do exist, in general, skin structure and function are analogous among species. Avian integument, unlike mammalian skin, contains no skin glands.[17] Aquatic mammals, such as dolphins, have an epidermis that lacks the stratum granulosum, but possess a thickened, parakeratotic appearing, stratum corneum.[17] The integument of pigs is the most similar to human skin, and is thought to be most valuable for extrapolation of results into human medicine.[17, 29]

Blood flow to the skin also differs among species. Dogs and cats lack musculocutaneous arteries; all vessels involved in cutaneous supply therefore travel parallel to the skin. In contrast, the musculocutaneous arteries are the primary vascular supply to human, ape, and swine integument.[17]

The barrier function of the skin in food-producing animals is not understood as well as in humans. Few investigations have addressed the mechanisms that determine percutaneous absorption of compounds in these animals. Studies conducted by Pitman and Rostas[30] have found considerable variability in the barrier function of large animals. For instance, temperature differences exist between black and white-haired regions, and climatic changes can induce alterations in sebum output and skin thickness.[31] The variability in skin morphology that exists within breeds further complicates the interpretation of drug movement across different species. Other factors complicating transdermal drug delivery include the presence or absence of hair follicles, wool, body weight, age, and sex. Since the role these factors have in drug transport across the integument is not well characterized, further investigations are needed to determine their relative import.

Principles of Transdermal Drug Movement

For drug to be delivered transdermally, it must pass through the integument and into the underlying systemic circulation. Absorption begins in the epidermis, with the major barrier being the stratum corneum. Once the stratum corneum has been penetrated, drugs can diffuse into the deeper layers of the epidermis and the dermis, respectively. At the level of the dermis, the drug is absorbed by blood vessels and

travels into the systemic circulation. However, drugs that either do not penetrate the stratum corneum or that fail to partition out of the vehicle, are removed by physical exfoliation.[17] The vehicle is the medium in which an active drug or chemical is topically administered. Drugs must be able to partition out of the vehicle in order to penetrate the stratum corneum. Thus, the vehicle must have more affinity for the stratum corneum that it has affinity for the drug.[17] Therefore the nature of the vehicle controls, to a great extent, the degree of success a particular drug will have in penetrating the integument and reaching the systemic circulation.

Systemic drug administration is not the intention of all topically applied drugs. Indeed, most topically applied preparations are meant to accumulate in the epidermis and exert their effects locally. Penetration enhancers that can augment drug movement through the epidermal layers, generally are absent in these formulations.[16] A simplified, schematic view of the fate of topically applied drugs is exemplified in (**Fig 1**).[17]

Transdermal absorption of drugs occurs primarily through an intercellular route through the lipid matrix of the stratum corneum.[17, 32] Drugs move by passive diffusion according to Fick's Law of Diffusion which states that the steady state of drug flux across a membrane can be defined as follows:

$$\text{Flux } (J) = \frac{DP}{h} \text{ (Concentration Gradient) (Surface Area)}$$

where *D* is the diffusivity of the drug in the intercellular lipids of the stratum corneum, *P* is the partition coefficient for the drug between the skin surface and the stratum corneum, and *h* is the skin thickness.[18] The catalyst for this dynamic process is the concentration gradient that exists between the applied dose of drug and the degree to

which the dermis is perfused.[18] Transdermal flux is defined in terms of surface area. Accordingly, the two critical points of transdermal dosage are the concentration of drug applied, and the surface area at the site of application.[18]

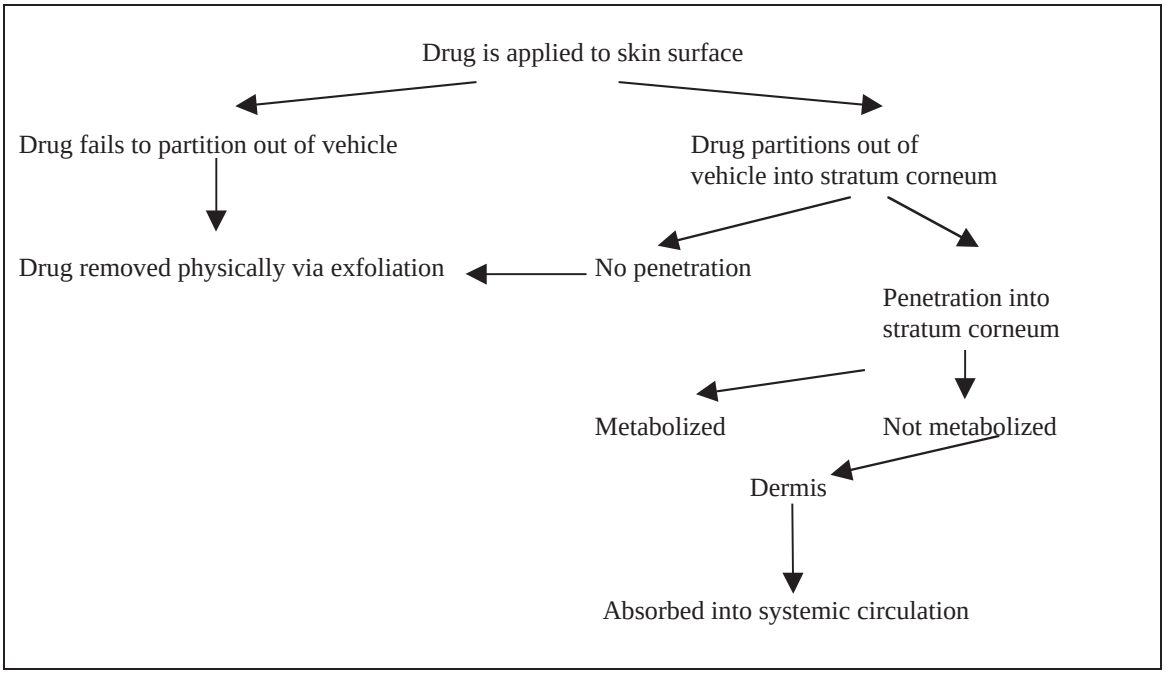

Figure 1—The fate of topically applied drugs.[17]

The ability of a drug to diffuse through the skin is a function of its molecular weight, molecular interactions with skin components (hydrophobic or hydrophilic regions), the drug's solubility, and the degree of drug ionization. Large molecular weight drugs exhibit a low degree of diffusivity.[16, 18, 32] Only non-ionized fractions of weak acids or bases are available for passive diffusion across the stratum corneum.[18]

Absorption through the skin is also dependent on the condition of the skin itself. The rate-limiting structure of transdermal drug absorption is the stratum corneum,

disruption, injury, or removal of this layer can result in a dramatic increase in permeability.[33]

Barriers to Drug Penetration

Although the barrier function of the stratum corneum is essential to maintaining internal homeostasis, it can be a major impediment to drug penetration. The stratum corneum exhibits low permeability, with a relatively uniform thickness of 30 microns across domestic animal species (**Table 1**).[16]

Table 1—Stratum corneum thickness for several species.[16]

Species	Stratum corneum thickness (μm)
Hairless mouse	8.8
Hairless rat	15.4
Guinea pig	18.6
Dog	19.9
Pig	17.5
Human	18.2
Sheep	31.4
Cattle	30.9

Due to the lipid composition of the stratum corneum, lipophilic compounds are best able to penetrate.[32] However, hydrophilic regions in the layer will deter strongly lipid-soluble molecules.[32] As a result, ideal transdermal drugs should have both lipophilic and aqueous characteristics. There is also species variation in the amount of lipid contained within the stratum corneum, a fact that must be considered when formulating transdermal drugs.[34]

The structure of the skin guards against penetration of large molecular weight compounds. Based on the fact that most of the common contact allergens in human medicine are below 500 Da, it has been proposed that molecules larger than 500 Da

cannot effectively penetrate the skin.[32] With the exception of few, most drugs administered topically are also smaller than 500 Da (**Table 2**).[32]

Table 2—Commonly applied transdermal drugs and their molecular weight. [32]

Compound	Molecular Weight (Dalton)
Topical antifungals	
Ketoconazole	531
Clotrimazole	345
Miconazole	416
Topical Corticosteroids	
Hydrocortisone	404.5
Bethamethasone	477
Difflucortolone	394
Topical anti-infectives	
Gentamicin	478
Acyclovir	225
Transdermal drug-delivery systems *	
Nitroglycerine	227
Nicotine	162
Fentanyl	336
Topical parasiticides *	
Fipronil	437.15
Imidacloprid	255.7
Ivermectin	875.1

* denotes drugs intended for systemic delivery

The presence, quantity, and type of hair follicles are important considerations that impact transdermal drug delivery. Hair density in pigs and humans is considerably less than that of the rodent. Other species differences are also important. Sheep wool is coated with an emulsion of sweat and sebum that has been reported to act as a solvent for topically applied chemicals. This emulsion has been reported to directly compete and interfere with drug diffusion through the skin.[35] Hair follicles, sebaceous, and sweat glands are often thought to be channels through which compounds can be

shunted—therefore bypassing the rate-limiting stratum corneum.[16] Thus, areas covered with hair will have a greater skin surface area for transdermal drug absorption to occur.[16] Species with high hair density also have reduced interfollicular epidermis, which may lessen the barrier to drug penetration.[18] These are important considerations when dealing with species that have sparse versus dense hair coats. In humans, for instance, sweat gland and hair follicle openings only represent 0.1% of the total skin surface, probably attenuating their significance in drug delivery.[32]

The skin also has the ability to metabolize compounds before they are absorbed systemically.[18] Investigations have shown that the epidermis is capable of both phase I and II biotransformation pathways. [18] Although cutaneous first-pass metabolism utilizes cellular enzymes and soluble esterases, compared to hepatic metabolism, it has only a minor role in the metabolism and degradation of drugs. [18]

The wide range of body surface areas among and within species impacts drug movement. The body mass of humans often only varies by a factor of 2-3 fold. [18] Veterinarians, however, deal with great differences in body size, from laboratory mice to elephants. This variability in size can complicate drug administration. In particular, a single “one size fits all” transdermal patch is both impractical and virtually impossible to develop for veterinary medicine. The most important factor in transdermal patch-based drug delivery is the ratio of the patch area to total body mass of the animal.[18] Transdermal patches are designed to deliver precise amounts of drug directly proportional to the surface area of the patch. For example, fentanyl is delivered at a rate of 25 μg per hour per 10cm^2 patch.[14] Patch sizes sufficient to deliver therapeutic

concentrations of a drug via the greater surface area of large animals would be unrealistic to develop, except for very potent drugs, for which low effective plasma concentrations are therapeutic—as is the case with pesticides. [18]

Enhancing Drug Penetration

Transdermal drug movement can be facilitated with the use of penetration enhancers or adjuvants included in the drug formulation.[16, 34] These substances appear to increase fluidity in the intercellular lipid of the stratum corneum, and cause the stratum corneum to swell and/or exude structural components that might otherwise hinder drug passage. This causes a change in the permeability coefficient of the lipid relative to the drug, thereby increasing drug penetration.[16, 18] Enhancers include lipophilic compounds such as ethanol, oleic acid, and terpenes. Solvents such as dimethyl sulfoxide (DMSO) with a molecular weight of 78.14 Da, also facilitate the passage of molecules through the skin.[27] Alternative methods to disrupt or alter the stratum corneum are through the use of ultrasound or iontophoresis.

The dermis is a vascular structure with its blood supply under complex neural and local control. Dermal perfusion varies in regard to body temperature and for certain compounds, modification of perfusion may alter drug delivery through the skin. [18, 36] For instance, vasoconstriction will result in decreased dermal perfusion, thereby reducing systemic absorption, but enhancing local drug activity. [18] Vasodilation, on the other hand, will increase blood supply to the dermis, maximizing systemic delivery while minimizing local accumulation.[18, 36] This principle is often used in local anesthesia with the inclusion of a vasoconstrictor, such as epinephrine, in the anesthetic solution.

Epinephrine decreases local perfusion and thus delays vascular absorption of the anesthetic, thus prolonging anesthetic action.

Transdermal Patches In Veterinary Medicine

In veterinary medicine, the delivery of drugs through the skin has been widely employed. Topical medications have been used locally to treat bacterial infections, seborrhea, keratinization disorders, and allergic dermatoses.[17, 36] Most of these formulations, however, are used to treat specific, local diseases. Therapy to achieve systemic drug concentrations is also commonly utilized—most often in the form of pesticides. Several flea, tick, and heartworm preventatives containing fipronil, imidacloprid, and selamectin are applied to a local area of skin and are widely used and promoted by veterinarians. In fact, the veterinary "dermatopharmacologic" industry is rapidly expanding to include other drugs available for systemic transdermal delivery. In anesthesia, transdermal fentanyl patches have been investigated as an alternative route to deliver opioid analgesic agents. Because fentanyl provides only short-term analgesia when administered subcutaneously (SC) or as a bolus IV dose, transdermal patches were developed to outweigh these limitations. It has been suggested that one of the advantages of transdermal systems is that they offer continuous drug release that is slower than absorption, thereby maintaining a relative constant plasma drug concentration and prolonging the analgesic interval.[15, 37] Because the degree of analgesia can be maintained for extended periods of time, the disadvantages of frequent dosing, including resultant fluctuations in peak and trough plasma concentrations can be avoided. Also, many analgesics may require large loading doses to attain immediate,

effective plasma drug concentrations, thus potentially increasing the risk of toxicosis.[15] Additional benefits of transdermally delivered fentanyl include the elimination of repeated oral doses, avoidance of injection pain, reduction in first pass hepatic clearance, and a decrease in the equipment and labor costs that accompany a continuous intravenous infusion.[38, 39] However, the animal must be clipped and prepped at the site of patch application. Also, some animals may require an Elizabethan collar to prevent chewing, biting, or scratching the patch, causing terminated or interrupted drug delivery. In addition, there is some liability in sending a fentanyl patch home with owners due to the abuse potential of the drug. Timing is also critical in patch application. It is recommended to apply the patch at least 24 hours prior to surgery or expected trauma in the dog.[15]

Fentanyl is the only drug available as a transdermal patch and used clinically in small animals at this time. Transdermal fentanyl systems have four components: a protective polyester backing, a fentanyl reservoir, a semipermeable membrane that controls/limits the rate of drug release, and an adhesive layer.[15] The fentanyl patch has been shown *in vivo* to demonstrate large variations in delivery rate, plasma drug concentration, and epidermal drug absorption among dogs.[15, 37] Similar pharmacodynamic variations have also been shown in humans.[15] Variation in drug behavior is also evident among different species. For example, it often takes 24 to 36 hours to achieve steady state plasma and therapeutic concentrations in dogs following patch application.[6, 15, 40] In horses, however, fentanyl is rapidly absorbed within 4 hours after application of the patch.[40] Studies involving cats have resulted in conflicting

findings. In one study, steady state concentrations were reached within 2 to 6 hours following application of the patch.[41] In another study, however, steady state concentrations were not achieved until after a 12 to 18 hour delay.[40]

The clinical efficacy of transdermal fentanyl patches has been investigated in several studies for application in veterinary medicine. Many evaluate fentanyl patches in the context of achieving a balanced anesthesia protocol, either alone or in comparison to other drugs. In one study, transdermal fentanyl was compared to injectable butorphanol in cats following ochyectomy.[40] The two analgesic protocols were compared using a pressure-sensitive mat to evaluate post-surgical lameness. Since the pressure mat was unable to detect a difference between the two protocols, these results either suggest analgesic equivalence of transdermal fentanyl and butorphanol or could indicate the pressure mat was not sensitive enough to identify a difference.[40] This study also refuted earlier claims regarding the economic benefit of using transdermal fentanyl patches as opposed to other formulations. In fact, the cost of using transdermal patches in this investigation was 2.5 times the cost of using butorphanol.[40] However, the investigator did note that the increased cost might be justified by the benefits in using non-invasive patches, which include ease of application and maintenance, and improved patient tolerance for patches as opposed to periodic injections and administration of pills.

Another study compared transdermal fentanyl to epidural morphine for analgesic effectiveness following orthopedic surgery in dogs.[14] Heart rate, respiratory rate, body temperature, and pain score were recorded both pre and post-surgery. Fentanyl patches were applied 24 hours prior to surgery. When variables were analyzed post-surgery, the

dogs in the transdermal fentanyl group experienced significantly less pain after surgery than dogs given epidural morphine.[14]

TRANSMUCOSAL DRUG DELIVERY

The disadvantages of traditional routes of drug administration have led clinicians and researchers to search for new, novel alternatives in pharmacologic dosing. As is the case with the integument, the oral cavity is another example of a novel site for drug delivery. The oral mucosa has been investigated in several studies as a means to give both local and systemic amounts of drug.[12] Drug delivery across mucosal membranes, such as the oral mucosa, is termed transmucosal drug delivery (TMDD). TMDD can be divided into three different target areas based on the characteristics of the oral cavity: (1) sublingual delivery, consisting of administration through the membrane of the ventral surface of the tongue and the floor of the mouth, (2) buccal delivery, consisting of administration through the buccal mucosa, mainly composed of the lining of the cheeks, and (3) gingival delivery, consisting of administration through the gingival mucosa.[7] These sites differ anatomically in their permeability to drugs, rate of drug delivery, and ability to maintain a TMDD system for the time required for drug release out of the delivery apparatus and into the mucosa.[42] This study focuses on the suitability of the buccal mucosa to deliver systemic drug concentrations.

Transmucosal drug delivery via the buccal lining has proven particularly useful and offers several advantages over other drug delivery systems including: bypass of the gastrointestinal tract and hepatic portal system, increasing the bioavailability of orally administered drugs that otherwise undergo hepatic first-pass metabolism; improved

patient compliance due to the elimination of associated pain with injections; administration of drugs in unconscious or incapacitated patients; convenience of administration as compared to injections or oral medications; sustained drug delivery; increased ease of drug administration; and ready termination of delivery by detaching the patch.[2, 8-11] Though less permeable than the sublingual area, the buccal mucosa is well vascularized, and drugs can be rapidly absorbed into the venous system underneath the oral mucosa.[7, 8, 42, 43] The large contact surface of the oral cavity contributes to rapid and extensive drug absorption.[7, 9, 43] Additionally, mucosal surfaces do not have a stratum corneum. Thus, the major barrier layer to transdermal drug delivery is not a factor in transmucosal routes of administration.

In comparison with transdermal drug delivery systems, TMDD systems exhibit a faster initiation and decline of delivery than do transdermal patches.[8] Also, TMDD delivery occurs in a tissue that is more permeable than skin and is less variable between patients, resulting in lower intersubject variability.[8] Because of greater mucosal permeability, TMDD can also be used to deliver larger molecules such as low molecular weight heparin.[8] In addition, TMDD systems could potentially be used to deliver drugs that exhibit poor or variable bioavailability, and bioavailability will be enhanced for drugs that undergo significant first-pass metabolism.[8, 9, 44] Because drug absorbed from the oral cavity avoids both first pass metabolism and enzymatic/acid degradation in the gastrointestinal tract, transmucosal administration could be of value in delivering a growing number of peptide drugs.[42]

Buccal Mucosa: Physiology and Histology

The various regions (sublingual, buccal, gingival) of the oral mucosa vary anatomically and physiologically. Due to these differences in structure as well as function, considerable variation exists in permeability among these regions.[42] This difference could make one region more or less suitable for delivery of a particular drug. In addition, just as the microstructure and function of the integumentary system differs between and within species, the buccal mucosa also exhibits some dissimilarity.

The oral mucosa is comprised of an outer layer of stratified squamous non-keratinized epithelium. Below the epithelium lies a basement membrane, a lamina propria, and submucosa, respectively (**Fig 2**). Oral epithelium is very similar to epithelium found elsewhere in the body. It consists of a basal cell layer, several intermediate layers, and a superficial layer from which cells shed. There are approximately 40-50 cell layers that make up the buccal epithelium, with a cellular turnover time of 5-6 days.[42] In humans, dogs, and rabbits, the buccal mucosa measures 500-800 μm in thickness.[42] Other areas of the oral epithelium (gingiva, hard and soft palates, floor of mouth) vary in size. Likewise, the composition of the epithelium varies in accordance with location. Areas that endure mechanical stress such as the gingiva and hard palate, like the epidermis, are keratinized. In contrast, the buccal mucosa, sublingual region, and the soft palate are not keratinized. Large quantities of protein are present in the cells of both keratinized and non-keratinized epithelium. Keratinized regions of the mucosa contain large amounts of acylcermides and ceramide, while the more permeable non-keratinized mucosal regions (buccal, floor of mouth) contain smaller quantities of lipid.

The basement membrane forms the boundary between the lamina propria and the basal layer of the epithelium. Composed of collagen, the basement membrane is thought to provide support and adherence between the epithelium and the lamina propria, and to form a mechanical barrier to cells and some large molecules across the mucosa. The lamina propria lies underneath the basement membrane and consists of a continuous sheet of collagenous connective tissue and elastic fibers. The capillaries and nerve fibers that supply the mucosa are present in this region.

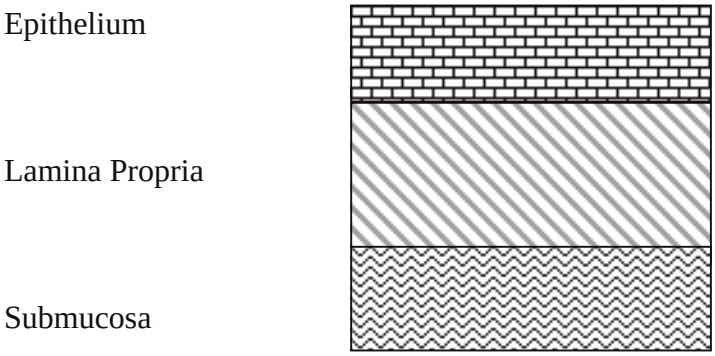

Figure 2—Generalized structural components of the oral mucosa.

Comparative Anatomy of the Buccal Mucosa

The oral lining of most laboratory animals is a thick, keratinized epithelium.[45] This is in contrast to the non-keratinized mucosa of humans, dogs, pigs, monkeys, and rabbits.[45] As a result, the data obtained from the use of laboratory animals in drug permeability studies is limited in its value, especially in studies that wish to extrapolate data to either human or other animal species.[45] Dogs are frequently used models in buccal drug delivery investigations due to their non-keratinized buccal mucosa and its similarity to human mucosa.[45]

CONSIDERATIONS FOR TRANSMUCOSAL DRUG DELIVERY

Nature of Permeant

Drugs administered via the oral mucosa gain access to systemic circulation by passive diffusion in accordance to Fick's law.[42] Less common is carrier-mediated transport or facilitated diffusion.[42] Most drugs move extracellularly through the neutral lipids and glycolipids that separate the mucosal cells. Therefore, the lipid solubility of drugs is an important determinant in TMDD suitability.

Along with lipid solubility, drugs selected for TMDD must have physiochemical properties, including size and pKa, that facilitate drug movement through the mucosa at a rate capable of producing therapeutic blood concentrations.[42] The drug must resist, or be protected by salivary and tissue enzymes that could cause inactivation.[42] Additionally, the drug and adhesive materials must not damage the teeth, oral cavity, or surrounding tissues (e.g. by keratinolysis, discoloration, and irritation).[42]

Molecular Size

The rate of absorption of hydrophilic compounds is a function of the molecular size.[42] Smaller molecules (<75-100 Da) generally exhibit rapid transport across the mucosa, with permeability decreasing as molecular size increases.[42] For hydrophilic macromolecules such as peptides, absorption enhancers (see later section) have been used to successfully alter the permeability of the buccal epithelium, causing this route to be more suitable for the delivery of larger molecules.[46] Though the relationship between permeability and size has not yet been demonstrated for lipophilic substances, based on previous investigations it is likely such a correlation exists.[32, 46]

Lipid Solubility and Partition Coefficient

Only the nonionized forms of molecules have the ability to cross lipoidal membranes in significant amounts.[45] The more lipid soluble a compound is, the higher its permeability.[42] The permeabilities for these compounds are direct functions of their oil-water partition coefficients.[42] The partition coefficient is a useful tool to determine the absorption potential of a drug.[47] In general, increasing a drug's polarity by ionization or the addition of hydroxyl, carboxyl, or amino groups, will increase the water solubility of any particular drug and cause a decrease in the lipid-water partition coefficient.[47] Conversely, decreasing the polarity of a drug (e.g. adding methyl or methylene groups) results in an increased partition coefficient and decreased water solubility.[47] The partition coefficient is also affected by pH at the site of drug absorption. With increasing pH, the partition coefficient of acidic drugs decreases, while that of basic drugs increases.[47] The partition coefficient is also an important indicator of drug storage in fat deposits. Obese individuals can store large amounts of lipid-soluble drug in fat stores.[47] These drugs are dissolved in the lipid and are a reservoir of slow release from these fat deposits.

Ionization

The ionization of a drug is directly related to both its pKa and pH at the mucosal surface.[42] Only the nonionized form of many weak acids and weak bases exhibit appreciable lipid solubility, and thus the ability to cross lipoidal membranes.[42, 45] As a result, maximal absorption of these compounds has been shown to occur at the pH at which they are unionized, with absorbability diminishing as ionization increases.[42]

PRINCIPLES OF DRUG MOVEMENT THROUGH THE BUCCAL MUCOSA

Like transdermal drug movement, drugs contacting the oral mucosa must penetrate the epithelial barrier in order to gain access to systemic circulation. The epithelium represents the primary barrier to compounds, though unlike the epidermis, there is no stratum corneum present in the oral cavity.

Drug transport across the oral mucosa is achieved by two pathways: 1) the paracellular (between cells) route, consisting of hydrophilic intercellular spaces, and 2) the transcellular route, through pores in the cell membranes or penetration through the lipid bilayers of cell membranes. [42, 45] Hydrophilic compounds, and large or highly polar molecules, follow paracellular transport, whereas transcellular transport through the lipid bilayer is followed by lipophilic drugs and by small molecules through epithelial membrane pores. [42, 45]

Buccal patches can potentially deliver a wide range of drug classes (e.g. opioids, antifungals, hormones) with differing physiochemical properties (lipophilic, hydrophilic, 200-10,000 Da), and at various concentrations.[42, 48] However, small lipophilic molecules active at low plasma concentration (e.g. are potent) are the easiest to deliver.[43] As with transdermal drug delivery studies, methods to increase overall drug permeability and to make a wider selection of compounds available and practical for buccal delivery are being investigated.

CHAPTER II

BUCCAL PATCH SYSTEMS

STRUCTURE AND DESIGN

Drug delivery systems designed for the buccal mucosa contain a polymeric adhesive component. When in contact with the saliva, the adhesive attaches to the mucosa causing immediate and rapid drug delivery. Transmucosal drug delivery systems can be unidirectional or bi-directional. [42, 45] Unidirectional patches release the drug only into the mucosa, while bi-directional patches release drug in both the mucosa and the mouth. The buccal patch is designed in either a matrix configuration with drug, adhesive, and additives mixed together (Fig 3), or a reservoir system that contains a cavity for the drug and additives separate from the adhesive.[42] An impermeable backing is applied to control the direction of drug delivery; to reduce patch deformation and disintegration while in the mouth; and to prevent drug loss.[42, 49] Additionally, the patch can be constructed to undergo minimal degradation in the mouth, or can be designed to dissolve almost immediately.[42]

Fig 3—Schematic representation of the buccal patch design.

Much less is known about the type and characterization of drug transport that occurs in the buccal epithelium as opposed to other sites of mucosal drug delivery, such as the gastrointestinal tract.[50] How these drug processes may be altered in disease or manipulated pharmaceutically in order to optimize drug absorption, is less defined.[50] Currently, buccal patches have been used to deliver a variety of drugs to dogs including buprenorphine, heparin, melatonin, theophylline, nitroglycerine, digoxin, propranolol, miconazole, insulin, morphine, fentanyl, and estradiol.[10, 45, 46, 51-53]

HISTORICAL BACKGROUND AND LITERATURE REVIEW

The absorption of drug via the oral mucosa was recognized as early as 1847 in the investigations of Sobrero, the discoverer of nitroglycerin.[42] Later studies ensued with Overton in 1902 and the first systemic studies of oral cavity absorption were conducted by Walton in 1935 and 1944.[42, 54] The investigations of Walton provided information on the importance a drug's lipid solubility and pH have in its transport through the oral mucosa.[53,54] More recently, factors such as drug ionization, improved patch design, and the use of prodrugs, have all been shown to be important in drug absorption and delivery.

Numerous *in vivo* and *in vitro* experiments have been conducted in an effort to further define the feasibility of buccal patch drug delivery systems. These studies have been important in determining the overall feasibility of developing buccal patch systems for *in vivo* drug delivery. Numerous *in vitro* investigations have centered on the buccal patch design itself, in an effort to improve or enhance mucosal drug delivery. Others have studied drug flux across mucosal membranes, compared mucosal properties across

species, or have centered on the effects of pH and penetration enhancers on drug passage.

Increasing Permeability: Penetration Enhancers, Prodrugs, Patch design and pH

Penetration Enhancers

In addition to the adhesive component, buccal patches can also incorporate additives such as solubilizers or penetration enhancers. Absorption enhancers have demonstrated their effectiveness in delivering high molecular weight compounds, such as peptides, that generally exhibit low buccal absorption rates. Although only a few buccal enhancement studies have been performed, reports show promising results using permeation enhancement agents.[42] Among these agents are Azone, ionic and nonionic surfactants, chelators, chitosan, and bile salts.[51] Azone is a type of accelerant that interacts with lipids in the stratum corneum in order to increase fluidity in the hydrophobic regions of intercellular areas, thus decreasing the diffusional resistance of skin.[16] Enzyme activity present in the mouth may also contribute to the metabolism of some drugs.[55] As such, enzymatic inhibitors have been studied to prevent drug degradation in the mouth.

Most penetration enhancers exert their effects by disrupting the membrane integrity of the mucosa, thereby increasing membrane permeability and drug penetration into mucosal tissues.[51] However, tissue irritation at the site of application is a concern. Because the oral mucosa is commonly exposed to mechanical and chemical irritants, it is an ideal region to examine the efficacy and overall safety of penetration enhancers.[46] Researchers are now investigating penetration enhancers that are reversible in action and

are inert to the cells it comes in contact with.[51] Recent investigations have looked at several types and classes of penetration enhancers.

Chitosan, a marine origin mucopolysaccharide, has not only demonstrated itself to be an effective penetration enhancer, but is also nontoxic, biocompatible, and biodegradable.[51] Chitosan was investigated for its ability to deliver transforming growth factor-β (TGF-β), a large, hydrophilic peptide molecule to which the oral mucosa was reported to be relatively resistant to penetration.[51] Results of this study showed a six to seven-fold enhancement of mucosal permeability to TGF-β with the concurrent use of chitosan.[51] A possible mechanism for enhanced penetration of TGF-β can be attributed to the bioadhesive nature of chitosan, which increases drug retention at the site of application.[51] Another scenario is based on chitosan's ability to disrupt lipid micelles in the intestine, thus attributing increased drug permeability to lipid disruption or interference within the buccal epithelium.[51, 55]

Bile salt enhancers are the class of compounds most commonly used for drug permeation enhancement.[55] Bile salts have been utilized extensively to enhance drug absorption through various types of epithelia including nasal, rectal, ocular, pulmonary, and vaginal.[55] Bile salts create aqueous channels via extraction of membrane protein or lipids, increasing membrane fluidity, and reverse micellization in membrane.[55] Many bile salts also exhibit an inhibitory effect on membrane peptidases that are found within the mucosa.[55]

In one study using dogs, the buccal administration of insulin coupled with the bile salt enhancer, sodium glycocholate, resulted in a significant decrease in blood

glucose, comparable to that seen after intravenous insulin injection.[56] In a similar study, non-diabetic beagle dogs received either insulin or insulin with sodium glycocholate. Blood glucose decreased only with insulin and sodium glycocholate combination.[57]

In a subsequent study, the co-administration of the nonapeptide buserelin (a luteinizing hormone-releasing hormone agonist), and sodium glycocholate was examined in pigs.[46] The mean bioavailability (F = 5.3%) was increased five-fold when compared to buccal administration without the enhancer.[46] Higher steady state plasma levels were also noted in the pigs treated with the combination.

The effect of sodium glycodeooxycholate on the transbuccal permeation of morphine sulfate was studied using excised non-keratinized bovine buccal mucosa as a model for human mucosa.[55] It was demonstrated *in vitro* that the permeability of bovine buccal mucosa was enhanced by a factor of 5, when 100 mM concentrations of the bile salt were used. No enhancement occurred when lower 10 mM concentrations of sodium glycodeoxycholate were used. Permeability studies were followed by histological and infrared studies to further explain how the bile salt interacted with and modified the drug.[55] Results of the studies indicate that sodium glycodeoxycholate interacts with the lipids within the epithelia, decreasing diffusional resistance to the permeants.[55]

Prodrugs

A practical consideration, but one that has been shown to affect the bioavailability of buccally administered drugs, is taste.[52] For example, many opioid agonists and antagonists taste bitter—a feature that could negatively affect buccal administration and subsequent absorption.[52] Hussain et al[52] examined the possibility of

delivering opioid agonists and antagonists in bitterless prodrug forms and the subsequent effect these dosage forms had on bioavailability. When nalbuphine and naloxone were administered to dogs via the buccal mucosa, the bitter taste of the drugs caused excess salivation and swallowing. As a result, the drug exhibited low bioavailability. Administration of nalbuphine and naloxone in prodrug form caused no adverse effects, with bioavailability ranging from 35 to 50%. This is a marked improvement over the oral bioavailability of these compounds, which is generally 5% or less. [52] It should be noted, however, that the absorption of prodrugs must be more rapid than their dissolution, in order to prevent the development of a bitter taste. [52]

pH

In a recent study, Shojaei et al[45] utilized porcine mucosa in order to determine the major routes of buccal transport of acyclovir and to examine the effects of pH and permeation enhancer on drug absorption. Buccal mucosa was excised from porcine tissue (approximate area of 0.75 cm^2) and mounted on side-by-side flow-through diffusion cells bathed in isotonic buffer solution. The permeability of acyclovir was evaluated at pH ranges of 3.3 to 8.8, and in the presence of the absorption enhancer, sodium glycocholate. The *in vitro* permeability of acyclovir was found to be pH dependent with an increase in flux and permeability coefficient at both pH extremes (pH 3.3 and 8.8), as compared to the mid-range values (pH 4.1, 5.8, and 7.0). In contrast, the permeation enhancement was pH independent: acyclovir absorption increased 2 to 9 times in the presence of sodium glycocholate regardless of the pH.

Buccal administration of fentanyl has been studied using a specially constructed Teflon cell attached to the buccal mucosa of six dogs. Streisand et al[53] hypothesized that the transmucosal bioavailability and absorption of fentanyl could be improved if more of the drug was converted to its unionized form. Because fentanyl is a basic drug, the pH of the delivery vehicle could be increased, thus potentially converting more fentanyl to the unionized form. This was achieved using pH buffered fentanyl solutions with pHs of 6.6, 7.2, and 7.7, respectively. Arterial blood samples were collected at frequent intervals over a period of eight hours. Peak plasma concentration, bioavailability, and permeability coefficient demonstrated a three-to five-fold increase as the pH of the fentanyl solution increased. In each case, regardless of pH, time to peak plasma concentration occurred within ten minutes of removal of the fentanyl cells from the buccal mucosa. The mean C_{max} for the pH 7.7 drug solution was nearly three times that of the mean C_{max} at pH 6.6. Based on these results, higher fentanyl concentrations could occur simply by altering the pH of the environment or by buffering the fentanyl solution.[56]

Although this study was geared toward eventual clinical use and application in human medicine, it does have relevance in veterinary clinical medicine. Already, the transdermal fentanyl patch is gaining broader popularity and acceptance in veterinary medicine. This method of fentanyl delivery has demonstrated its safety and efficacy in both dogs and cats. However, the patch must have adequate contact with the skin for a variable, but sustained period of time. The skin must be clipped and dried first, and bandaging material should be applied to assist in patch placement and adhesion. As is

the case, and often the frustration of many veterinarians, animals sometimes are able to remove bandaging material. Should this occur, drug delivery and subsequent pain alleviation are also terminated. Also, if the animal ingests the removed patch, there is a risk (though minimal) for toxicity.[37] Thus, a dissolvable buccal patch that provides sustained, therapeutic drug concentrations without the need for bandaging, skin prep, and prolonged adhesion times would be advantageous.

Patch design

Several *in vitro* studies have been conducted regarding buccal patch bioadhesive properties, the influence of application site, and drug release characteristics. From these studies, information was gathered on variables that affect drug absorption and delivery via the buccal mucosa. In one report, it was found that the type and amount of backing materials altered the adhesion characteristics of buccal patches, and these changes could alter the drug release profile.[49] Also, the drug release pattern was different between single-layered and multi-layered patches.[49]

Specific Drugs Delivered to Animals via a Buccal Patch

Several drugs and drug classes have been studied in an effort to determine the feasibility of using buccal patches as a novel route of drug delivery. These studies have explored the consequences of altering patch design, pH, and including permeation enhancers in the patch formulation The sheer variation in class of compounds illustrates the interest the medical, veterinary, and pharmaceutic industries have on alternative, more feasible routes of administration for existing drugs.

Steroids

The oral and parenteral bioavailability of testosterone is rapidly absorbed and metabolized by the liver.[54] Due to this high first-pass effect, the half-life of testosterone is very short. [54] As a result, the more lipophilic testosterone esters are used instead of testosterone. However, no current testosterone therapy results in sustained, therapeutic drug levels. Recent medical need for a sustained testosterone plasma level in human males has generated interest in alternative forms of administration that will achieve this goal. Bioavailability of testosterone in the form of a bioadhesive tablet was determined in a study conducted by Voorspels et al.[54] Tablets containing 60 mg of testosterone were affixed to the buccal mucosa of six dogs. Testosterone was also administered orally and intravenously, with bioavailability and additional pharmacokinetic parameters analyzed for three formulations. Oral administration of testosterone had a significantly lower absolute bioavailability (1.03 % ± 0.75) when compared to buccal administration (14.14% ± 0.75).[54] In addition, only the bioadhesive tablet was able to sustain target drug concentrations for 20 hours.[54]

A systemic amount of drug, however, is not always the desired effect of all formulations. The need for local activity to treat specific areas of inflammation or infection is also of interest. Local treatment is based on high concentrations of drug being maintained at the site of administration, with minimal or absent systemic effects. Hydrocortisone acetate is an anti-inflammatory agent contained in many topical products intended for local application on the skin. Previous studies have shown topical buccal therapy of steroids is useful in treating local ulcerative and inflammatory mucosal

conditions.[58] A buccal mucoadhesive formulation of hydrocortisone was developed in order to elicit a controlled amount of drug at the site of action, enhance bioavailability, and ensure optimal contact with the absorbing surface.[58]

Antimicrobials

A bioadhesive tablet containing miconazole was used to examine the influence of application site on bioadhesion and release characteristics.[59] The study was undertaken to determine if a novel method of drug therapy could reduce typical nosocomial infections in intensive care patients. The treatment of using a combination of antifungals in paste form has not been shown to reduce these infections.[11, 59] In this investigation, 10 mg miconazole nitrate tablets were attached to the buccal mucosa or gingiva of 8 comatose, intubated human patients. It was concluded that the buccal mucosa was the better application site for bioadhesive miconazole tablets.[59] When applied to the gingiva, salivary miconazole concentrations could only be observed 660 minutes post-application. In contrast, drug concentrations were detected much earlier and at a higher concentration when attached to the buccal mucosa.

Peptides

Peptide delivery via a buccal patch has been examined in a number of investigations. In a randomized crossover study, Hoogstraate et al[46] administered the luteinizing hormone-releasing hormone antagonist, buserelin, intravenously and buccally, with and without absorption enhancer, to six pigs. Buccal administration of the drug resulted in rapid steady state plasma levels. The mean bioavailability of buccal delivery without enhancer was 1.0%. With enhancer, mean absolute bioavailability

increased to 5.3%. This study not only indicates the potential for peptides to be buccally delivered, but illustrates that therapy could theoretically be improved by altering the composition of the delivery device, in this case, using an absorption enhancer.[46]

Further peptide delivery studies were undertaken by DeGrande et al[42] to examine the potential use of a TMDD system to deliver low molecular weight heparin to dogs. In this study, three dogs received two patches placed on the right and left buccal mucosa for 8 hours. Each patch contained 13.4 mg of heparin. Maximal plasma concentration of heparin reached 0.8 units/ml at 6 hours, and declined slowly until patch removal at 8 hours. Since the therapeutic drug concentration for heparin to prevent thromboembolism is 0.1 to 2 U/ml, this study indicates the potential for the buccal patch to deliver therapeutic drug doses in patients and provide adequate thromboembolic prophylaxis.[42] In addition, this data suggests the possibility of delivering other peptide macromolecules via the buccal route as an alternative to traditional parenteral administration.[42]

In an additional investigation,[10] human insulin was administered buccally to streptozocin-induced diabetic rats. Although the data did not suggest a significant therapeutic benefit from using the buccal mucosa as a site for insulin delivery, the study did demonstrate that a pharmacologic effect (decrease in blood glucose level) could be achieved following buccal administration.[10]

Anesthetics and analgesics

Further clinical applications of buccally administered drugs focus on anesthesia and analgesia. One of the challenges of anesthesia and analgesia is delivering the ideal dose of drug to control an individual patient's pain, or to maintain sedation without

fluctuations into and out of consciousness. Due to extensive inter-patient variability, over- and underdosing often occurs with injections or oral administration of drugs.[39, 60] Thus, the development of a titratable drug formulation for a patient's individual needs would be of significant clinical interest.

In one such study, [60] the sedative-hypnotic drug etomidate was administered across the oral mucosa of dogs in a solid dose form. Though etomidate is used mainly for the intravenous induction of general anesthesia, it was the investigators' purpose to study etomidate's practical use as a premedication and sedative in conscious patients. For an oral transmucosal system to deliver titratable amounts of drug, rapid onset must occur when the drug is applied to the oral mucosa, as well as rapid termination upon removal of the dose form.[60] Both rapid onset and termination of etomidate occurred with buccal mucosal absorption. Canine buccal mucosa was also highly permeable to etomidate. These results suggest the clinical use of buccally administered etomidate to achieve a specific, tailored, and titratable dosing regimen for individual patient needs.[60]

DeGrande et al[42] also investigated the use of buccal patches to deliver buprenorphine to dogs in order to provide a more stable and sustained serum drug concentration. Buprenorphine is a partial opiate agonist used clinically in the management of acute and chronic pain. Oral doses undergo significant first-pass metabolism and rapid clearance, resulting in poor bioavailability in both dogs and humans.[4, 61, 62] Buccal patches (0.5 cm^2) containing 1 to 4 mg of drug were applied to four Beagle dogs in a crossover study. Single patches of each dosage were applied for 8 hours to the lip or gingiva of the dogs. Measurable drug concentrations were present

within 30 minutes after patch application, with C_{max} occurring by 4 hours. Although there was notable inter-animal variability in both C_{max} and AUC within treatment groups, all dogs exhibited opiate related clinical signs (miosis, sedation, unsteady gait, vomiting).

Drugs affecting the cardiovascular system

The angiotensin-converting enzyme inhibitors enalapril and linisopril have both been studied as to their extent and precise mechanism of buccal absorption.[63] Results showed enalapril to be absorbed to a slightly greater extent than is linisopril. However, it was noted that the extent of buccal absorption was much less than 60%--the percentage of enalapril absorbed after oral administration. Based on these results, enalapril would probably not be absorbed to a large enough extent from the oral mucosa to produce therapeutic drug levels, such as that needed for treatment of a hypertensive crisis.[63]

In contrast, the buccal mucosa is a more than adequate site for the absorption of other drugs that impact the cardiovascular system. Its suitability to deliver clinically effective amounts of drug was demonstrated in a study examining application of transdermal nicotine patches to the buccal cavity of dogs in order to evaluate cardiovascular effects.[64] The study was conducted due to public safety concerns over inappropriate use or exposure to transdermal nicotine patches (e.g. children biting or chewing patches). In fact, application of the patches to the oral cavity for a period of only five minutes resulted in plasma nicotine levels greater than 1000 times that of previously reported levels following either oral or transdermal routes in dogs.[64]

Cardiovascular effects were significant, with systolic arterial pressures rising as high as 400 mmHg within the five minute period of exposure. Ventricular arrthymias and tachycardia were also observed. Investigators hypothesize that the cause of higher nicotine levels was multifactorial. The first-pass hepatic metabolism that would occur with oral administration (e.g. swallowing) was avoided following buccal exposure. Also, the composition of the transdermal patches seemed to be ideal for the rapid delivery of significant amounts of nicotine via the buccal mucosa.[64] The researchers did note, however, the possibility that the proximity of the jugular vein sample site to the capillary system of the buccal cavity may have attributed to the higher plasma levels of nicotine.[64] This study implicates the buccal surface as an alternate route of drug administration that not only delivers detectable, pharmacologic levels of drug, but quantitative clinical effects in arterial blood pressure and heart rate as well.

DRUGS TO BE INVESTIGATED IN THIS STUDY

This study will focus on the systemic delivery of butorphanol and albuterol via a buccal patch. The two drugs differ in their chemical composition, physical properties, and their clinical use in medicine. As such, results of this study will provide information on the feasibility of delivering these drugs that have slightly different physiochemical properties across the oral mucosa.

Albuterol

Albuterol sulfate is a synthetic, sympathomimetic β_2-agonist that causes relaxation of bronchial, uterine, and vascular smooth muscles. It is one of several adrenergic compounds developed for the treatment of asthma in humans.[65] Albuterol is

available in both oral and aerosol forms, although intravenous, intramuscular, and subcutaneous methods of administration have been reported in the literature.[65]

Chemistry

Albuterol occurs as a white, crystalline powder. It is soluble in water, slightly soluble in alcohol, and has a molecular weight of 576.7.[66] Albuterol's β_2-receptor selectivity is achieved by modifying the basic catecholamine structure that is common to the physiologic compounds epinephrine and norepinephrine (**Fig 4**). For albuterol, this modification consists of a tertiary butyl substitution on the nitrogen and the inclusion of a hydroxymethyl group instead of the 3-hydroxyl group.[65] Other β-agonists such as terbutaline and fenoterol, can be produced with similar substitutions. The result is a compound that possesses specific β_2 effects and negligible action on either α or β_1 receptors.[65]

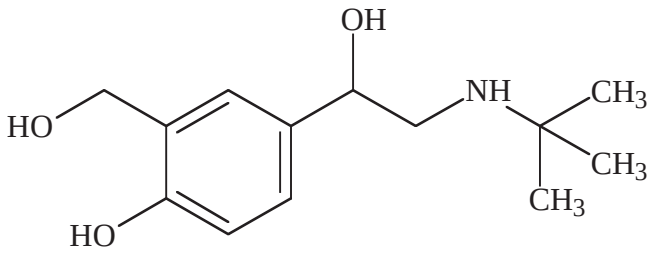

Figure 4—Chemical structure of albuterol.

Mechanism of Action

There are at least two types of β-adrenergic receptors: β_1- and β_2-receptors. β_1-receptors are primarily found in cardiac muscle and adipose tissue.[67] When activated, cardiovascular stimulation occurs. β_2-receptors are predominately located in bronchial

smooth muscle, the gastrointestinal tract, the blood vessels of skeletal muscle, and the uterus.[65, 66] β-adrenergic agonists exert their effects on these receptors by activating adenyl cyclase, an enzyme present in the cell membrane.[65] When activated, ATP is converted to cyclic AMP. Cyclic AMP then initiates a sequence of intracellular events, eventually leading to a physiologic effect—in this case, inhibiting contraction of bronchial smooth muscle, thereby causing smooth muscle relaxation and bronchodilation.[65, 67] In addition, bronchodilators decrease mucosal edema; are anti-inflammatory; and stimulate airway mucosal secretion, resulting in a less-viscous secretion and improved ciliary activity.[68]

Clinical Use

Albuterol is used in the management of asthma. Asthma is a pathological lung state characterized by bronchoconstriction and inflammation.[68] In the treatment of asthma, the most important therapeutic effect is the β_2-receptor-mediated relaxation of smooth muscle in the airways.[65] β_2-receptor agonists are the most effective bronchodilators available because they block airway constriction, despite the inciting cause.

Albuterol is widely used for the treatment of bronchial asthma in adults and children.[5, 69] However, in veterinary medicine, albuterol is infrequently used. When the drug is used, its primary indications are for the alleviation of bronchospasm or cough in dogs and cats. Albuterol reduced the cough in one-half of dogs with chronic bronchitis.[70] Routes of administration to small animals include aerosolization, oral syrup, and tablets.[68]

Recently, albuterol and other β_2-agonists have been used in human medicine to treat hyperkalemia caused by chronic or acute renal failure.[71] Albuterol is effective in lowering extracellular potassium by facilitating the intracellular uptake of potassium in muscle and hepatic cells.[71]

Pharmacokinetics

Albuterol undergoes rapid and complete first-pass metabolism following oral administration, resulting in reduced systemic bioavailability.[68] The excretion and bioavailability of albuterol is primarily affected by hepatic metabolism.[5] Thus, if the amount of drug presented to the liver was significantly decreased by using a novel dosing method, with the reduction of subsequent hepatic metabolism, bioavailability may be altered in comparison with conventional dosing forms. Such modification in pharmacokinetic parameters could offer several advantages including: maintenance of therapeutic drug concentrations, a less frequent dosing regimen, and improved patient/client compliance.

The average oral bioavailability of albuterol administered in four different preparations in dogs was determined in a study by Hernandez et al[5] to be 80%.[5] Elimination (disappearance) half-life was 1.2 hours after IV administration, 3.0 hours for an oral immediate-release formulation, and ranged between 5.4 and 7.2 hours in an orally administered sustained-release preparation.

Adverse Effects and Drug Interactions

Since β_2-adrenergic receptors are present in sites other than the lungs, systemic concentrations of albuterol can stimulate these receptors and cause a number of

undesirable “extrapulmonary”effects.[65] For instance, though normally the β_1 effects of albuterol are minimal, at increased doses, albuterol can stimulate these receptors, resulting in tachycardia.[65] Tachycardia may also be caused by the β_2-mediated arteriolar dilation, resulting in decreased peripheral vascular resistance.[65]

Albuterol also enhances skeletal muscle tremor, primarily in the extremities of human patients. The tremors are thought to be the result of β_2-receptor-mediated decreases in the recovery period following muscle fiber contraction.[65]

Other adverse effects of albuterol include dose-related metabolic alterations, resulting in increased concentrations of plasma glucose, renin, insulin, lactate, and ketone.[65] Decreases in plasma potassium, phosphate, calcium, and magnesium concentrations have also been reported. [65]

Sympathomimetic amines used in conjunction with albuterol may increase the risk of adverse cardiovascular effects. Ventricular arrthymias can occur—especially in patients with preexisting cardiac disease—with the use of inhalation anesthetics such as halothane, isoflurane, and methoxyflurane. Digitalis glycosides may also increase the risk of cardiac arrthymias. The vascular effects of albuterol can be potentiated with the use of tricyclic antidepressants or monoamine oxidase inhibitors. In contrast, drugs such as propranolol that are β-adrenergic blockers, can antagonize the effects of albuterol.

Toxicity and Overdose

Clinical signs consistent with systemic albuterol overdose include hypokalemia, weakness, tachycardia, tachypnea, hypersalivation, hyperthermia, and delirium.[71] In one

published report, [71] a dog developed albuterol intoxication after it had punctured and decompressed the metal albuterol inhaler of a family member.

In cases of known albuterol overdosage, potassium supplementation is recommended.[71] Propranolol should be administered if cardiac arrthymias are noted in order to cause a β-adrenergic blockade. ECG, serum potassium, and blood pressure monitoring are also recommended.[71]

In humans, albuterol administered in the form of an inhalation aerosol spray has shown effectiveness in the treatment of bronchospasm with reversible obstructive airway disease in adults and children, providing bronchodilation for a duration of 3 to 8 hours.[61, 62] As a result, the drug must be taken 3-4 times daily in order to provide therapeutic relief for a sufficient period of time.[61, 62]

Clinical Relevance

In the study by Hernandez et al, [5] the elimination (disappearance) half-life of intravenously administered albuterol (1.2 hours) indicates that approximately 90% of the dose is eliminated in four hours. This fact illustrates the need to administer the drug three to four times daily, and further indicates the need for the design of sustained-release formulations.[5] An alternate route of drug delivery that could provide convenience and longer duration of activity—thereby reducing the number of doses that must be taken daily—would be of interest to both clinicians and patients.

Although therapeutic albuterol concentrations in dogs and cats are not defined, current-dosing recommendations for dogs is 0.05 mg/kg PO every 8 hours.[61] A buccal formulation could potentially provide sustained plasma concentrations, perhaps allowing

decreased dosing intervals, and increasing the drug's use in veterinary medicine by eliminating the inconvenience of multiple oral dosing.

Butorphanol

Butorphanol tartrate is a synthetic opiate agonist-antagonist, commonly used in small animal medicine for its analgesic, antitussive, and sedative effects.[48, 72, 73] Butorphanol is available in both oral and injectable preparations.

Chemistry

Butorphanol tartrate is a lipophilic drug that occurs as a crystalline powder and is somewhat soluble in water and is insoluble in alcohol.[66] The molecular weight for butorphanol is 477.55.[72] Butorphanol is a morphine derivative with kappa receptor agonist and mu receptor antagonist properties.[73-75] Opioids have multiple receptors that are widely distributed throughout the body.[73] These receptors are not specific to opioids, but rather have "preferences" to different exogenous and endogenous opioids.

The chemical structure of the opioid derivatives is the determining factor on the drug's "fit" and action at the receptor site.[73] Butorphanol differs from morphine by chemical substitutions at the C3, C6, C14, and C17 positions (**Fig 5**) .[76] There is also an oxygen molecule lacking between positions C3 and C4.[76] In addition, opioids exhibit a great degree of stereo-specificity. Thus, one optical isomer may be an agonist, but its enantiomorph can be either inactive or an antagonist. As a result, not only is the chemical structure of opioids an important determinant on site of drug action, but their arrangement in three-dimensional space is also crucial.[73]

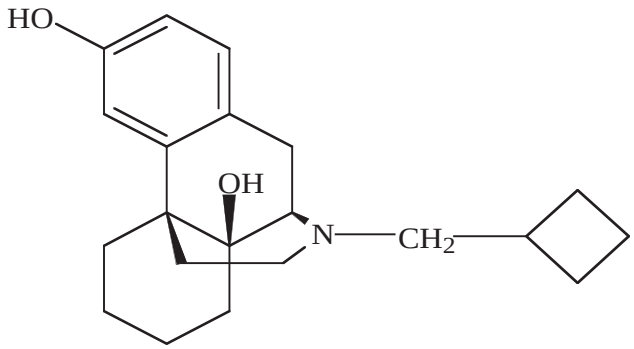

Figure 5—Chemical structure of butorphanol.

Mechanism of Action

Butorphanol, like other opioids, exerts its pharmacologic effects by the interaction with the opioid receptors found in the body.[37, 74] There are three major opioid receptors (mu, kappa, and delta) in the body. The sigma receptor may no longer be considered as a separate class or receptor, though its current status remains unclear. Sigma receptors do not appear to cause analgesic effects.[74] The delta opioid receptor appears to result in analgesia primarily at the level of the spinal cord.[74]

Most of the actions of morphine-like drugs seem to be mediated through the mu opioid receptor.[74] There are two subtypes of mu receptors that are responsible for analgesia and sedation: mu_1-receptors that act above the level of the spinal cord, and mu_2-receptors that act within the spinal cord.[37] Mu_2-receptors are also thought to be responsible for respiratory depression and suppression of gastrointestinal motility.[74]

The kappa opioid receptor is involved in spinal and supraspinal analgesia, miosis, and sedation.[74] Although both kappa and mu receptors mediate analgesia, mu agonists cause euphoria, while kappa agonists produce sedation and dysphoria.[74] Butorphanol is

an opioid agonist-antagonist that exhibits agonist effects at kappa receptor sites, and antagonist effects at mu receptors.

Clinical Use

Butorphanol is commonly used in veterinary medicine as a preanesthetic and perioperative analgesic agent in cats and dogs.[37, 74] It is also used as an antitussive, an antiemetic prior to cisplatin treatment, and for the control of post-operative pain in small animals.[37] In horses, butorphanol is commonly used as an analgesic, and after xylazine, is the next best drug at controlling visceral pain.[74] Butorphanol can also be used to partially reverse the sedative or respiratory depressant effects caused by oxymorphone, a pure opioid agonist.[37]

Pharmacokinetics

In veterinary medicine, the pharmacokinetics of butorphanol have been reported in dogs, cats, rabbits, goats, llamas, and horses.[76-80] In dogs, several different routes of administration, including subcutaneous, intramuscular, and epidural have been studied.[73, 77] No intravenous butorphanol studies have been conducted in dogs.

The subcutaneous and intramuscular routes of administration were found to be bioequivalent, based on the lack of a statistically significant difference between AUC values.[77] For SC and IM, the mean serum elimination (disappearance) half-life was 102.6 ± 24 and 91.8 ± 14.4 min, respectively.[77] Mean C_{max} was 33.3 ± 16.9 ng/ml at T_{max} 28.65 ± 13 min following SC administration. After IM administration, C_{max} was 25.1 ± 6.7 at T_{max} 42.2 ± 13 min.[77] Following epidural administration of butorphanol, mean elimination (disappearance) half-life was 186 ± 60 min.[73] Maximum

concentration of butorphanol and time to obtain this concentration was 42.28 ± 7.46 ng/ml and 13.88 ± 7.62 min, respectively.[73]

Adverse Effects and Drug Interactions

The primary adverse effects caused by opioids are CNS depression, respiratory depression, and to a lesser extent, cardiac depression. In ruminants, opioids may cause excitement.[80] Respiratory depression is generally dose-related, and more likely to occur with mu receptor activation than with kappa activation.[78] As such, butorphanol has a high safety profile. In dogs and cats, respiratory depression caused by butorphanol displays a ceiling effect so that increasing the dose does not increase the respiratory depression.[78] Effects on the gastrointestinal system are also minimal.[78]

The administration of other CNS depressants such as tricyclic antidepressants and the phenothiazines, may intensify or prolong the depressant effects of opioids.[37] Some phenothiazines, however, can potentially reduce the concentration of opioid required for analgesia, while others have the opposite effect.[37]

Toxicity and Overdose

Butorphanol is, in general, a very safe drug with a greater margin of safety than morphine.[73] As such, risk of acute overdose or toxicity with butorphanol is low.[73] Toxicity studies have indicated the LD_{50} in dogs following oral administration to be greater than 50 mg/kg.[79, 80] Clinical signs of butorphanol intoxication include CNS depression, cardiovascular changes, and respiratory depression. In case of overdose, treatment with intravenous naloxone should be initiated immediately. Fluid and oxygen

supplementation, mechanical ventilation, and the administration of vasopressor agents may also be required.

Clinical Relevance

Butorphanol is completely absorbed following oral administration, but with a high first-pass effect—only 16.67% of the administered dose reaches the systemic circulation.[61] Butorphanol is also absorbed completely following intramuscular injection.[61] The development of a buccal patch delivery system for butorphanol could be useful due to the potential of a longer duration of effect compared with injectable formulations, and without the significant first-pass effect of orally administered butorphanol.

PHYSIOCHEMICAL COMPARISON

The physiochemical properties of the drug to be studied are perhaps the greatest determinant of rate and extent of oral mucosal drug absorption.[43] In general, molecules larger than 100 Da have a more difficult time in penetrating the oral epithelium.[43] It is probable in the case of hydrophilic drugs that the rate of absorption is directly related to its molecular size.[43] Such a relationship has not yet been demonstrated across the oral mucosa for lipophilic drugs.[43] Hydrophilic drugs also appear to be more effective in partitioning into the oral epithelium and basal lamina.[43] The more hydrophilic layers of the basal lamina can be a potential barrier in the transport of extremely lipophilic drugs.[43]

Results of this study will provide information on systemic oral delivery of butorphanol, and the larger, more hydrophilic drug, albuterol. Data will be analyzed in

order to determine the feasibility of delivering these drugs with slightly different physiochemical properties across the oral mucosa.

ViroTex Buccal Patch

The patch to be utilized in this experiment is manufactured by ViroTex Corporation[a]. The patch, less than 1.5 cm diameter, is designed for application inside of the mouth on the buccal mucosa (**Fig 6**).

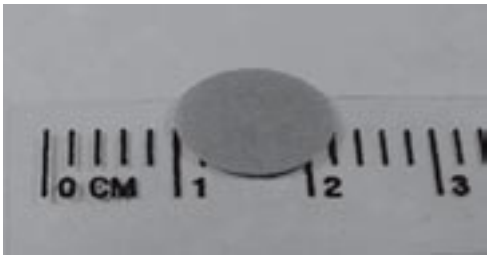

Figure 6—ViroTex buccal patch

The patch is comprised of the drug and an adhesive polymer in a matrix-type configuration such that systemic delivery of drug will achieve therapeutic effect. When in contact with the oral mucosa, the hydrophilic adhesive causes gradual dissolution of the patch. The design of the patch causes immediate adherence to the mucosa, and immediate and rapid drug delivery.

CHAPTER III

STUDY PURPOSE AND PROCEDURE

STUDY PURPOSE

The overall purpose of this study is to determine the feasibility of drug delivery in dogs using a TMDD system. Feasibility will be based on anticipated therapeutic efficacy, which in turn will be based on likelihood of achieving targeted drug concentrations; and convenience, which will be based on the operation of the delivery system and animal tolerance to drug administration via this route.

OBJECTIVES

Two drugs, albuterol and butorphanol, will be studied. The bioavailability of each drug when administered buccally will be compared to intravenous administration. Bioavailability (F) is determined by the following equation:

$$F = \frac{\text{AUC buccal} * \text{dose(IV)}}{\text{AUC}_{IV} * \text{dose(buccal)}}$$

where F is the proportion of an administered dose that enters systemic circulation.[81] By definition, after intravenous administration, bioavailability is 100% (F = 1.0). Drugs administered via other routes may exhibit incomplete bioavailability (F = < 1.0), due to hepatic, gastrointestinal, or other forms of metabolism.[81] Although bioavailability is not a criterion of clinical effectiveness, it is a parameter that must be determined for any new drug or drug product.[81] Bioavailability, itself, only demonstrates the amount and rate of drug appearing in systemic circulation. Bioavailability can be used to assess the bioequivalence of different pharmaceutical preparations, but it does not mean the

different forms are bioequivalent.[81] Variations in bioavailability are primarily caused by differences in the efficiency or rate of absorption—factors that originate either with the patient or with the dosage form.[81, 82]

Due to first-pass metabolism, the routes by which these drugs can be administered are limited. Multiple dosing is often necessary to provide sustained and clinically effective plasma concentrations. Fractious, excitable, or painful animals may not tolerate excessive or repeated handling for the purpose of drug administration or re-dosing. If these drugs can be successfully administered, absorbed, and distributed via a novel buccal patch system, transmucosal delivery of these drugs may be a potentially rewarding alternative to traditional routes of administration.

The study will also provide information on whether therapeutic concentrations of albuterol and butorphanol can be successfully delivered using a buccal patch system. The therapeutic dose of butorphanol for somatic analgesia in dogs has been extrapolated to be 9 ng/ml.[73] Therapeutic concentrations of albuterol in dogs have not been established in any of the literature. Data will be analyzed to determine if buccal administration of butorphanol is capable of achieving therapeutic drug concentrations.

MATERIALS AND METHODS

Subjects

Three hound dogs with a mean weight of 23 ± 1.73 kg were studied using a randomized crossover design, with each dog receiving albuterol and butorphanol by buccal and intravenous administration. Prior to the study, an Animal Use Protocol[b] was submitted to and approved by the University Laboratory Animal Care Committee

(ULACC). Guidelines of the ULACC regarding the humane care, treatment, and utilization of animals for scientific purposes were followed. Dogs were housed at the Texas A&M University Small Animal Clinic. Dogs were fed according to daily dietary requirements; no special diet was required for this study. All dogs were found to be clinically normal as evidenced by an initial physical exam. A complete blood count performed prior to experimentation revealed no abnormalities that were clinically significant (**Appendix A**). All dogs were heartworm negative and on heartworm preventative. On the day of each study, another physical exam was conducted, and a baseline heart rate, respiration rate, and body temperature were recorded.

Drug Administration

Dogs were fasted at least 12 hours before each study, with water available free-choice throughout the fasting and sampling periods. Prior to each 12-hour study, dogs were fitted with an indwelling jugular catheter[c], aseptically placed in either the left or right external jugular vein. A two-week washout period occurred between all drug studies.

In both the buccal and IV studies for albuterol and butorphanol, vital signs such as heart rate and respiration were monitored before drug administration and at various time points following drug delivery. In the event of an overdose or adverse reaction to the drugs, reversal agents for albuterol and butorphanol—propranolol and naloxone, respectively— were kept immediately on hand throughout the study period. Any negative effects or reactions were to be treated by one of the veterinarians involved in the investigation and present on the day of the study.[d]

Buccal patch study

For the buccal patch trial, a ViroTex patch formulated to contain 0.9 mg of albuterol was placed on the buccal mucosa of each dog and held in place for 10 seconds to ensure adequate contact. The patch was affixed to the mucosa of each animal in an area approximately between the upper canine and molar teeth on the left side (**Fig 7**). The mean buccal albuterol dose was 0.039 ± 0.003 mg/kg.

Figure 7—Application of buccal patch to oral mucosa.

Animals were closely observed for the first thirty minutes to ensure the patch was not removed via excess salivation, licking, or other means of mechanical removal. In addition, water was withheld for the first thirty minutes to guard against interference with patch dissolution or attachment. Animals were also observed for adverse reactions—either behavioral or physical—to the patches.

A ViroTex buccal patch containing 1.2 mg of butorphanol was attached to the oral mucosa of each dog in the same manner described in the albuterol study. Animals

were again observed for proper patch attachment, placement, and dissolution. The mean buccal butorphanol dose was 0.05 ± 0.003 mg/kg.

IV study

For the IV portion of the study, albuterol[e] (0.18 mg/ml) was slowly administered into the cephalic vein via a Butterfly infusion set.[f] Albuterol is a β-agonist and adverse effects may include tachycardia, hypertension, CNS stimulation, vomiting, and bronchodilation. There are no intravenous dosing recommendations for albuterol in the veterinary literature. Albuterol is generally used as a nebulizer in veterinary medicine with the recommended human dose as 200 μg.[68] Heart rate and respiration were closely monitored, and the β-blocker propranolol (0.25 mg/kg IV)[g] was immediately on hand and available for administration in the event of overdose. All dogs experienced tachycardia and restless upon intravenous infusion. As a result, the albuterol infusion was discontinued when approximately half the 0.9 mg buccal patch dose of albuterol was administered. The mean IV albuterol dose was 0.018 ± 0.004 mg/kg.

A bolus dose of butorphanol[h] (0.5 mg/ml) was administered intravenously according to the same procedure used in the albuterol infusion. The total dose administered IV was identical to the buccal patch dose of 1.2 mg, or 2.4 mls. The mean IV butorphanol dose administered was 0.05 ± 0.004 mg/kg. Adverse effects of butorphanol include sedation, anorexia, respiratory and cardiovascular depression, and in rare cases, diarrhea.[37, 61] Naloxone[i] (0.04 mg/kg IV) can be used to treat overdose, and was available during intravenous administration. The recommended IV dose of butorphanol in dogs ranges from 0.1-0.4 mg/kg. Based on a 23 kg dog such as those

used in these studies, the lower-end dose is approximately 5 mls of 0.5 mg/ml butorphanol IV. Since the total dose of 1.2 mg (or 2.4 mls of 0.5 mg/ml butorphanol) used in this study is approximately half of the low-end recommended dose for butorphanol, adverse effects were not expected.

Sample Collection and Handling

Blood samples for the buccal patch studies for either drug were collected via the jugular catheter at 0, 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 360, and 720 minutes. For each sample, 0.25 ml of blood was withdrawn and discarded. Another 7 ml of blood was collected, and the catheter flushed with 7 ml of 0.9% saline.[j] The 7 ml of blood was immediately put into plain, red top tubes[k] and placed on ice until centrifugation. Within two hours after initial collection, the blood was centrifuged and the serum harvested. Serum samples were stored in 1 ml aliquot tubes[l] at -90°C until analysis.

For the IV portion of the study, samples were collected via jugular catheter by the same procedure as the buccal patch trial at 0, 2, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, and 720 minutes. Because the intravenous dose peaked immediately, additional, early sampling times were included compared to the buccal patch sample collection times. Sample handling was identical to the buccal patch study, with all blood centrifuged and serum harvested within two hours.

Sample Analysis and Validation

Prior to drug analysis, samples were thawed at room temperature. Serum concentrations of albuterol and butorphanol were determined by ELISA in accordance with manufacturer's guidelines[m] and following validation in canine serum. An

individual standard curve was prepared for each compound in canine serum. In addition, individual stock solutions[n, e] for each drug were prepared. A serum stock solution for each compound was prepared by combining a known volume of pooled blank canine serum with its respective stock solution. Individual standard points were then prepared by spiking a known volume of blank serum with each compound's serum stock solution. All standard curves were accompanied by controls that spanned the width of the standard curve. All standard curves and controls were analyzed in duplicate with the mean value serving as the point for the curve. All serum samples collected at each time point were also run in duplicate. The lower limit of quantitation for the albuterol assay was 0.332 ng/ml and the upper limit was 8.53 ng/ml. The upper and lower limits of quantitation for butorphanol were 8.8 and 0.23 ng/ml, respectively (**Appendix B**). Any serum samples that were above the upper limit of quantitation were diluted appropriately and re-analyzed (**Appendix C**).

PHARMACOKINETIC AND STATISTICAL ANALYSIS

Pharmacokinetic Calculations

For each drug and route, log plasma drug concentration verses time data were subjected to computer-assisted linear regression[o] to determine values for pharmacokinetic parameters. Noncompartmental analysis was implemented using either an intravascular single bolus dose or extravascular dose model. Values for parameters were estimated using a linear/log trapezoidal rule, with lambda being estimated to infinity.[83, 84] Drug concentrations are reported as average values calculated from duplicate samples that exhibited no more than 15 % difference. Area under the curve

was determined to the last time point using the log-linear trapezoidal rule. Peak plasma concentration (C_o) was extrapolated from the first non-computer-generated measurement at the actual time, T_{max}. Bioavailability (F) was calculated according to the equation:

$$F = \frac{\text{AUC buccal} * \text{dose(IV)}}{\text{AUC}_{IV} * \text{dose(buccal)}}$$

Clearance was determined using the equation:

$$Cl = \text{Dose IV}/\text{AUC}_{IV}$$

Volume of distribution at steady state was calculated using the equation:

$$Vss = \text{Dose IV}/\text{AUMC}_{IV}/(\text{AUC}_{IV})^2$$

Mean absorption time (MAT) was calculated for the buccal patch formulations of both drugs using the equation:

$$MAT = MRT\ (extravascular) - MRT\ (intravascular)$$

Means, harmonic means (for disappearance half-life), and standard deviations (pseudo-standard deviation for disappearance half-life) were determined utilizing a statistical software package.[p]

Statistical Methods

Selected pharmacokinetic parameters were compared between administration routes (buccal v. IV) using a paired Student's *t*-test to compare the first-order elimination rate constant for buccal and IV dosing routes. A value of $p \leq 0.05$ was considered statistically significant.

CHAPTER IV

RESULTS

Animals tolerated application of the buccal patch well. No adverse effects related to patch application were noted on gross exam of the oral mucosa following dissolution of the patch. Also, animals did not exhibit any clinical signs of toxicity or overdose. All patches dissolved within five minutes of application. Dissolution was verified by examining the mucosa on which the patch was applied.

No adverse effects, behavioral changes, or tachycardia were noted after buccal albuterol administration. Heart rate was monitored at periodic intervals throughout the day of experimentation, and did not vary from baseline heart rates attained prior to drug administration. In contrast, all dogs experienced tachycardia following intravenous administration of albuterol (**Table 3**). The dogs also vocalized and exhibited behavior indicating anxiety (e.g. pacing, panting). Because administration of an intravenous dose equivalent to the albuterol buccal patch (0.9mg) was determined by the investigators to represent too great a risk of toxicity and potential overdose, the dogs received approximately 0.45 mg, half of the 0.9 mg buccal albuterol dose. Despite the side effects, administration of propranolol was not deemed necessary following IV administration, as determined by the investigators.

Following intravenous administration of 1.2 mg of butorphanol, no dogs exhibited adverse effects or signs of toxicity. No opiate related clinical manifestations (e.g. sedation, miosis) were exhibited in any of the dogs.

Table 3—Heart rates after albuterol IV administration.

Time (post-injection)	Mean Heart Rate (beats/min)	SD
0 (baseline)	109	12.06
30 sec	136	38.57
1 min	180	20.00
1.5 min	201	2.31
2 min	204	4.00
3 min	203	23.44
5 min	174	22.54
15 min	171	32.08
30 min	135	8.08
60 min	141	4.62
90 min	131	4.62

PHARMACOKINETIC AND STATISTICAL RESULTS

The mean time-concentration curves ± standard deviation for albuterol and butorphanol are depicted in **Fig 8-9** and **Tables 4-7**. All pharmacokinetic results are reported as mean ± standard deviation. Individual dog drug concentration verses time curves and pharmacokinetic parameters are reported in **Appendix D-K**.

Statistical comparisons between the rate constant of elimination for buccal and IV drug administration are reported in **Appendix L**.

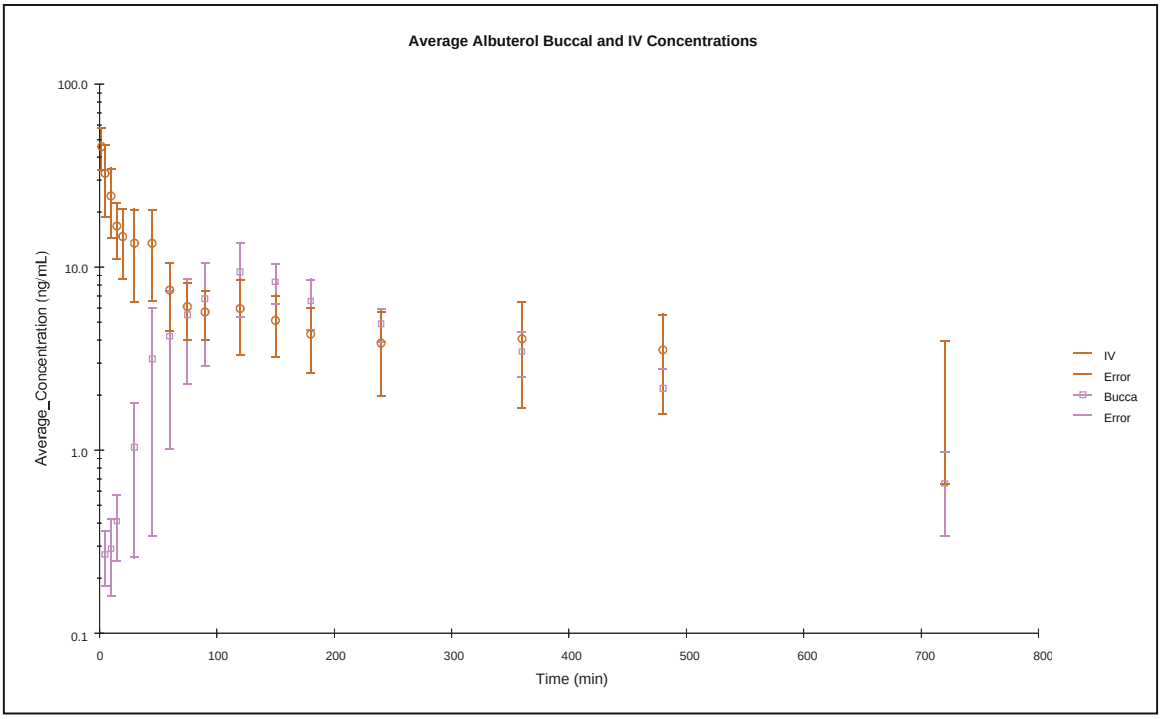

Fig 8—Average concentration ± standard deviation of albuterol.

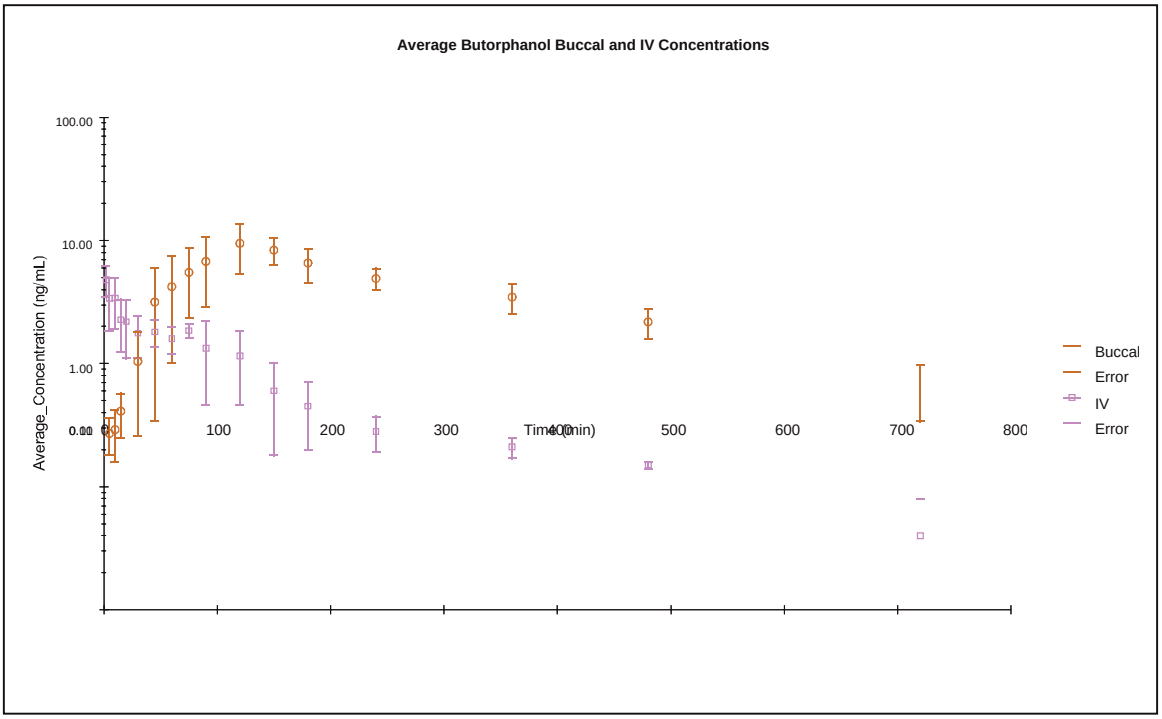

Fig 9—Average concentration ± standard deviation of butorphanol.

Albuterol

Following IV administration, the extrapolated concentration (C_o) of albuterol was 57.74 ± 9.04 ng/ml, Cl was 4.73 ± 3.91 ml/min/kg, and Vss was 2.13 ± 1.30 L/kg. Additionally, $t_{1/2}$ was 364.20 ± 115.20 min, and MRT was 240.19 ± 39.75 min (**Table 4**). Following buccal administration, a C_{max} of 10.28 ± 2.77 ng/ml occurred at T_{max} 130 ± 17.32 min (**Table 5**). Disappearance half-life was 160.96 ± 24.19 min, MRT was 260.42 ± 12.60 min, and MAT was 20.22 ± 48.80 min. Buccal bioavailability was calculated to be 34.79 ± 13.77 %

Table 4—Values for pharmacokinetic parameters of albuterol following single dose IV administration of 0.45 mg.
******indicates a significant difference ($p \leq 0.05$)

Parameter	Dog B	Dog C	Dog R	Average	SD
Co (ng/ml)	48.01	59.34	65.88	57.74	9.04
AUC (min*ng/ml)	1872.7	4539.57	4391.81	3601.36	1498.89
Lambda Z (1/min)	0.0022	0.0012	0.0023	******0.0019	0.0006
$t_{1/2}$ lambda z (min)	311.35	573.51	304.72	364.20	115.20
Cl (ml/min/kg)	9.24	2.26	2.68	4.73	3.91
AUMC (min*min*ng/ml)	401940.65	1298251.95	966023	888738.53	453125.98
MRT (min)	214.63	285.99	219.96	240.19	39.75
Vss (L/kg)	3.54	1.87	0.99	2.13	1.30

Table 5—Values for pharmacokinetic parameters of albuterol following single-dose administration of 0.9 mg in a buccal patch.
**indicates a significant difference ($p \leq 0.05$)

Parameter	Dog B	Dog C	Dog R	Average	SD
C_{max} (ng/ml)	7.24	12.66	10.95	10.28	2.77
T_{max} (min)	150	120	120	130	17.32
AUC (min*ng/ml)	1806.84	3213.44	2559.96	2526.75	703.89
Lambda Z (1/min)	0.0050	0.0037	0.0042	**0.0043	0.0007
$t_{1/2}$ lambda z (min)	138.2	184.95	166.78	160.96	24.19
AUMC (min*min*ng/ml)	496668.75	809296.6	651559.3	652508.22	156316.09
MRT (min)	274.88	251.85	254.52	260.42	12.60
MAT (min)	60.25	-34.14	34.56	20.22	48.80
Bioavailability (F) %	48.24	35.39	20.73	34.79	13.77

Butorphanol

Mean $\pm$ SD C_o for butorphanol following IV administration was 8.24 ± 5.55 ng/ml (**Table 6)**. Mean Cl was 137.87 ± 19.55 ml/min/kg, $t_{1/2}$ was 172.12 ± 94.95 min, Vss was 27.58 ± 10.14 L/kg, and MRT was 112.17 ± 6.16 min. Following buccal administration, C_{max} was 6.66 ± 1.65 ng/ml at T_{max} 170 ± 17.32 min (**Table 7**). Disappearance half-life and MRT were 259.15 ± 33.12 min and 278.96 ± 43.16 min, respectively and MAT was 166.80 ± 37.00 min. Mean bioavailability of butorphanol following buccal administration was 606.04 ± 164.09 %.

Table 6—Values for pharmacokinetic parameters of butorphanol following single dose IV administration of 1.2 mg.

Parameter	Dog B	Dog C	Dog R	Average	SD
Co (ng/ml)	4.46	5.64	14.61	8.24	5.55
AUC (min*ng/ml)	428.52	327.51	264.21	340.04	82.92
Lambda Z (1/min)	0.0066	0.0030	0.0025	0.0040	0.0022
$t_{1/2}$ lambda z (min)	105.42	230.10	277.91	172.12	94.95
Cl (ml/min/kg)	123.87	129.53	160.21	137.87	19.55
AUMC (min*min*ng/ml)	49785.73	37741.59	27762.04	38429.79	11027.96
MRT (min)	116.18	115.24	105.08	112.17	6.16
Vss (L/kg)	17.72	27.04	37.97	27.58	10.14

Table 7—Values for pharmacokinetic parameters of butorphanol following single dose administration of 1.2 mg in a buccal patch.

Parameter	Dog B	Dog C	Dog R	Average	SD
C_{max} (ng/ml)	7.15	4.82	8	6.66	1.65
T_{max} (min)	180	180	150	170	17.32
AUC (min*ng/ml)	2288.98	1605.31	1905.91	1933.40	342.66
Lambda Z (1/min)	0.0023	0.0028	0.0029	0.0027	0.0003
$t_{1/2}$ lambda z (min)	302.65	248.46	235.43	259.15	33.12
AUMC (min*min*ng/ml)	704828.3	480963.76	437137.33	540976.46	143581.88
MRT (min)	307.92	299.61	229.36	278.96	43.16
MAT (min)	191.74	184.37	124.28	166.80	37.00
Bioavailability (F) %	534.16	490.16	793.8	606.04	164.09

CHAPTER V

DISCUSSION

This study was designed as a pilot study to determine the pharmacokinetics, including bioavailability, of albuterol and butorphanol when administered via a buccal patch. The pharmacokinetic characteristics of butorphanol and albuterol have not been determined in dogs following buccal and apparently, IV, administration. The basis of future clinical studies and therapeutic effectiveness depends on the understanding and correct interpretation of pharmacokinetic parameters. The drugs selected for this study have different clinical indications that would benefit from persistent drug levels. They also contrast in physiochemical properties and molecular size, with albuterol being smaller and more hydrophilic, and butorphanol larger and lipophilic. By examining the behavior and disposition of different model drugs when administered via a buccal patch, conclusions regarding the suitability of similar drugs for buccal delivery can more likely be determined. In subsequent investigations, decisions can be made on how to enhance delivery for problematic drugs, prolong delivery of drugs with a short half-life, and alter dosing regimes of drugs that require frequent administration.

This study demonstrated that application of the buccal patch can be relatively effortless, and correct placement (and initiation of drug delivery) can be achieved in seconds. In contrast to parenteral drug administration or fentanyl patch application, no equipment or animal prepping is necessary.

Albuterol

Albuterol is a selective β_2-agonist, available as oral tablets, a syrup, or an aerosol. In humans, orally administered albuterol undergoes significant first-pass metabolism, reducing bioavailability to as low as 10%. The half-life of oral albuterol is approximately five hours, thus requiring three to four-times daily dosing to maintain bronchodilation. Buccal administration of albuterol in humans or dogs has not been examined either for clinical effectiveness or pharmacokinetic evaluation.

The C_{max} of buccal albuterol in dogs in this study was 10.28 ± 2.77 ng/ml, occurring at T_{max} 130 ± 17.32 min. The disappearance half-life was 160.96 ± 24.19 min. The estimation of half-life for the buccal dose suggests approximately 90% of the drug will be eliminated in 10-14 hours (around 4-5 half-lives). This indicates the potential of the buccal patch to allow twice daily dosing if therapeutic drug concentrations can be maintained during this time period, an ability shared by controlled release albuterol tablets currently on the market. Compared to IV administration, the disappearance half-life for buccally administered albuterol was nearly two-times greater than the elimination (disappearance) half-life reported for the IV preparation by Hernandez et al[5] (**Table 8**). Albuterol has also been studied using oral preparations intended to prolong drug concentrations, and some comparisons can be made to the buccal patch (**Table 8**). The formulations of albuterol used in the study by Hernandez et al[5] are not available in the United States. As such, differences in pharmacokinetic parameters should not be over-interpreted. It does appear, however, that buccal albuterol could provide effective and comparable plasma concentrations. After 0.9 mg of buccal albuterol was administered,

disappearance half-life values were comparable to the oral immediate release product given at a dose nearly 10-fold the buccal dose.

Intravenous administration of albuterol to dogs in this study resulted in a longer disappearance half-life than either buccal, oral, or the IV Ventolin® preparation (**Table 8**). Intravenous disappearance half-life should not be longer than extravascular administration. Possible situations that may have caused this effect include a terminal portion of the curve that is poorly defined, and thus few points used to determine the $t_{1/2}$ Lambda Z. In dog C, for instance, only three points were used in the $t_{1/2}$ Lambda Z calculation. Dog C also had the longest $t_{1/2}$ Lambda Z (573.51 min). The terminal curves of dogs B and R included twice as many points to calculate $t_{1/2}$ Lambda Z, but the calculated values (311.35 and 304. 72 min, respectively) were also longer than extravascular disappearance half-life data. The small study population used in this investigation prevented outliers from being identified. It would have been beneficial to study the pharmacokinetic behavior of IV albuterol in a greater number of dogs to determine if the results obtained in this investigation were valid . In comparison to the Hernandez et al study,[5] in which a comparable IV albuterol dose was administered to dogs similar in size to the dogs used in this study, it would appear that our results do not correlate with the elimination (disappearance) half-life values obtained in their investigation (Table 8).

When the elimination of the drug is more rapid than absorption, absorption becomes the rate-limiting step, and a flip-flop model results.[85, 86] Flip-flop models sometimes occur following topical or rectal routes of administration, and metabolite

concentration-time curves, if the metabolite is eliminated quicker than it is formed.[85] As such, the absorption half-life following extravascular dosing is mistakenly thought to represent the elimination slope of the curve. It must be recognized, however, that in flip-flop models the plasma concentration-time curve tends to parallel the rate of absorption.[87] To determine if a flip-flop model exists, comparisons should be made between the elimination phase of the intravenous plot and the extravascular concentration-time curve. The elimination rate constants between intravenous and buccal administration were compared using a paired Student's t-test with a p-value less than or equal to 0.05 considered significant. Significant differences between the mean elimination rate constants were detected for albuterol. Although the elimination rate does differ between the two routes, a flip-flop model does not exist for buccal albuterol since the disappearance half-life for IV albuterol is longer than the buccal disappearance half-life. Additionally the IV route of administration has no absorption phase.

In the study by Hernandez,[5] the elimination (disappearance) half-life of IV albuterol was 72 ± 21.6 min; much shorter than the disappearance half-life of 396.53 ± 153.31 min in this study. The previous investigation[5] detected albuterol by high performance liquid chromatography (HPLC) with a lower LOQ of 0.5 ng/ml, as compared to the lower LOQ of 0.32 ng/ml in this study. Additionally, the albuterol was only detectable for a period of approximately 6 hours, in contrast to the twelve hours in which albuterol was measurable in our study. This suggests the longer disappearance half-life obtained in our study could be because the lower LOQ of 0.32 ng/ml was able to detect albuterol at a smaller concentration and for approximately double the time

period in the previous investigation. Similarly, in the transdermal albuterol study by Gokhale et al,[88] the lower LOQ was only 2 ng/ml and detectable concentrations of drug were measured for less than eight hours. If the LOQ used in this study was able to detect a smaller quantity of drug, and thus identify a terminal component that was not evident in previous studies, the disappearance half-life could be longer if the slope of this new terminal component was not as steep as previous investigators. However, this terminal component may not reflect concentrations that are therapeutic.

Table 8—Values for pharmacokinetic parameters after buccal, IV, and oral albuterol administration in dogs [5]
NR = not reported

	Buccal	**IV**	**IV[5] (Ventolin®)**	**Oral[5] (immediate release)**	**Oral[5] (sustained release)**
Dose (mg/kg)	0.04	0.02	0.02	0.39	.39
Total mean dose (mg)	0.9	0.45	0.5	9.6	9.6
C_{max}/C_o (ng/ml)	10.28 ± 2.77	57.74 ± 9.04	NR	NR	NR
T_{max} (min)	130 ± 17.32			NR	NR
$t_{1/2}$ (min)	160.96 ± 24.19	364.20 ± 115.20	72 ± 21.6	180 ± 46.8	324 ± 79.2
Vss (L/kg)	NR	2.13 ± 1.30	NR	NR	NR
Cl (ml/min/kg)	NR	4.73 ± 3.91	NR	NR	NR
MRT (min)	260.42 ± 42	240.19 ± 39.75	66 ± 30	300 ± 102	702 ± 216
F (%)	34.79 ± 13.77			85 ± 12	82 ± 37

The bioavailability regarding albuterol administered via a buccal patch, comparisons or extrapolations among species and studies should not be over-interpreted.

Bioavailability studies have been conducted using immediate-and sustained-release oral tablets.[5] Mean bioavailability of these products was approximately 80%, more than twice the 35% value calculated in this study (**Table 8**). However, the study by Hernandez et al[5] did not report AUC data, thus AUC comparisons could not be made among formulations.

Many factors can influence the bioavailability of orally administered drugs, including: gastrointestinal motility; the presence or absence of food or other drugs; age; weight; disease state; formulation of the drug; route of administration; and the physiology of the patient.[82, 88, 89] Food, antacids, and milk all delay gastric emptying, and along with gastric acid, can directly interfere with the drug itself.[82] The buccal route of administration avoids first-pass effect, while oral drug formulations do not. Thus, buccally administered drugs should demonstrate greater bioavailability as compared to drugs administered orally. Unless the patch was swallowed, no drug should be presented to the stomach. All dogs in this study were fasted prior to drug administration, so even if drug did reach the stomach, gastric motility and food would not impact bioavailability.

Albuterol has been studied following transdermal administration in an investigation by Gokhale et al.[88] The bioavailability of transdermally administered albuterol in rhesus monkeys was 20%. The fact that the buccal formulation resulted in greater bioavailability than the transdermal patch, indicates the potential of the oral mucosa as being a suitable site for drug administration, and perhaps a more effective route of delivery than transdermal patches.

The type of drug formulation and physiochemical properties of the drug itself will impact drug movement when administered as a patch. Albuterol sulfate has a molecular weight of 576.7, slightly larger than the 500 Dalton rule of drug absorption proposed by Bos and Meinardi.[32] Albuterol also is a hydrophilic drug, potentially limiting its absorption and passage through the lipophilic regions of the oral mucosa. This can be expected to decrease bioavailability as compared to oral preparations (**Table 8**). The skin is characterized by an additional lipid barrier, the stratum corneum. The presence of this layer probably contributed to the lower bioavailability seen in the transdermal study of albuterol,[88] by lessening drug passage into the epidermal and subcutis layers.

The Vss of albuterol was 2.13 ± 1.30 L/kg (**Table 4, 8**). Depending on the intended site of action, drugs that exhibit a small Vss may require a lower dose to achieve clinical effect. The Vss of albuterol is greater than the total body water (0.6 L), suggesting it is distributed to areas other than the circulatory system. Drugs with large Vss distribute to tissues including extracellular and intracellular fluid, fat, and deep tissue, rather than being limited to the circulatory system.[75, 90] The larger the apparent volume of distribution, the smaller the concentration in plasma from a given dose, because less drug is sequestered in the plasma. Conversely, a small Vss suggests the drug remains in the plasma or serum, and will result in higher drug concentrations. The Vss of IV albuterol was not reported in the investigation by Hernandez et al[5], thus comparisons between IV routes cannot be made.

The pharmacokinetics of buccal albuterol have not previously been determined in dogs. Albuterol is used so infrequently in clinical veterinary medicine that therapeutic concentrations have not been established. Based on the lack of clinical response upon buccal albuterol administration, it is unlikely that therapeutic levels were reached in this study. In addition, the total dose of albuterol administered buccally was less than the recommended 0.05 mg/kg oral dose in dogs.[61] Future considerations may include altering the patch to contain more drug or including penetration enhancers in the patch formulation.

Butorphanol

Butorphanol is completely absorbed after oral administration, but because of extensive first-pass metabolism, oral bioavailability is low, necessitating increased dosage frequency. Butorphanol is also absorbed immediately following IM and SC administration, with peak serum concentrations occurring one hour after parenteral dosing.[76] In order to avoid first-pass metabolism, along with the negative association of injections, alternate, non-parenteral routes of drug administration have been developed. The pharmacokinetics of butorphanol administered intravenously or via a buccal patch have not previously been reported for dogs.

Following buccal administration, the disappearance half-life of butorphanol was 259.15 ± 33.12 min. This was a longer disappearance half-life than previous reports following IM, SC, or epidural dosing in dogs (**Table 9**), and after SC administration to rabbits (98.4 ± 5.4 min).[78] Thus, butorphanol given at therapeutic doses via a buccal patch may have a longer duration of effect than after parenteral or oral administration to

dogs, and parenteral administration to rabbits. The disappearance half-life of 1.2 mg of buccally administered butorphanol was longer than the elimination (disappearance) half-life of a buccal patch containing 2 mg of butorphanol (195 ± 72.6 min) and applied to the oral mucosa of human subjects.[91]

Butorphanol was absorbed slowly following buccal administration with a C_{max} of 6.66 ± 1.65 ng/ml occurring at T_{max} 170 ± 17.32 min. The 2-mg buccal butorphanol patch used by Shyu et al[91] in healthy human volunteers resulted in lower C_{max} and AUC values, 0.78 ng/ml and 315.6 min*ng/ml, respectively, as compared to the C_{max} and AUC values in this study (6.66 ± 1.65 ng/ml and 1933 ± 342.66 min*ng/ml, respectively). The 2-mg buccal patch achieved a T_{max} of 204 min, a longer time period than the T_{max} attained in this study. Normalizing dose for a 70-kg individual, the total butorphanol dose administered in the human study was approximately 0.03 mg/kg, compared to the 0.05 mg/kg dose in this investigation. Though slightly more drug was administered on a mg/kg basis in this study, it is unlikely that such small differences can account for the large disparity seen in the C_{max}, AUC, and T_{max} values between the two investigations. These comparisons would indicate that the ViroTex patch is capable of achieving higher plasma drug concentrations in a shorter duration of time.

Compared to the study conducted by Troncy et al[73] and Pfeffer et al[77], in which IM, SC, and epidural injections of 0.25 mg/kg butorphanol were administered to dogs, buccal butorphanol at 0.052 mg/kg was characterized by decreased C_{max} concentrations and later T_{max} values (**Table 9**). This indicates a slower and prolonged absorption of buccal butorphanol as compared to IM, SC, or epidural administration. However, the

plasma disappearance of buccal butorphanol remained slower than that of parenteral butorphanol, as demonstrated by the longer disappearance half-life achieved using the buccal formulation compared to previous investigators (**Table 9**). Although statistical comparisons can not be made among these studies, the magnitude of the differences among this disappearance half-life and other investigator's suggests the difference was real.

Table 9—Values for pharmacokinetic parameters after buccal, IV, SC[77], IM[77], and epidural[73] butorphanol administration in dogs.
NA = Not applicable

	Buccal	**IV**	**SC[77]**	**IM[77]**	**Epidural[73]**
Dose (mg/kg)	0.052	0.052	0.25	0.25	0.25
Total mean dose (mg)	1.2	1.2	2.75	2.75	6
C_{max}/C_o (ng/ml)	6.66 ± 1.65	8.24 ± 5.55	33.3 ± 16.9	25.1 ± 6.7	42.28 ± 7.46
T_{max} (min)	170 ± 17.32	1.67 ± 2.89	28.65 ± 13	42.2 ± 13	13.88 ± 7.62
AUC (min*ng/ml)	1933.4 ± 342.66	340.04 ± 82.92	4902 ± 2058	4062 ± 972	7866 ± 2484
$t_{1/2}$ (min)	262.18 ± 35.65	204.48 ± 89.05	102.6 ± 24	91.8 ± 14.4	186 ± 60
Vss (L/kg)	NA	27.58 ±10.14	NA	NA	NA
Cl (ml/min/kg)	NA	137.87 ± 19.55	NA	NA	NA

The AUC of buccal butorphanol was lower than the AUC of IM, SC, or epidural administration (**Table 9**). However, the total dose of buccal butorphanol was less than either the IM, SC, or epidural doses (**Table 9**). Extrapolating from this data, it would be expected that if buccal butorphanol were to be given at doses comparable to the IM and

SC dose (approximately twice the buccal dose) and epidural dose (5-times more than the buccal dose), the AUCs of all formulations should be comparable.

Absolute bioavailability for buccal butorphanol was calculated as 606.04 ± 164.09%. Bioavailabilities approximating 100% indicate complete absorption of the drug. However, bioavailabilities calculated by this study are physiologically unlikely, indicating several potential problems or scenarios. These include: analytical difficulties; trouble with patch design; physiologic interference; and drug metabolite interference.

Serum samples following buccal and intravenous drug administration were analyzed in duplicate and analysis was performed on two separate occasions with similar results and coefficients of variability were within acceptable limits. Thus, it is unlikely that analytical mistakes caused variation in drug concentrations such that would alter the AUC and cause the high bioavailabilities seen in this study. Alternatively, more drug may have been placed in the patch than cited. Because extra patches were not made available to our laboratory for analysis, the actual drug content of the patches could not be verified. Similarly, it is possible an incorrect or miscalculated intravenous dose of butorphanol was administered. Indeed, based on values of the IV pharmacokinetic parameters determined in this study as compared to those of other investigations, 1.2 mg of butorphanol IV should result in higher C_o and AUC values. For example, an IV dose of approximately 2 mg administered to rabbits[78] resulted in a C_o of 106.40 ± 19.36 ng/ml. Similarly, a 10 mg dose to llamas[92] resulted in a C_o of 94.8 ± 53.1 ng/ml.

Unfortunately, no study has reported C_o for butorphanol in dogs following IV administration.

Possible physiologic explanations include enterohepatic re-circulation, in which drugs appearing in the bile are emptied into the small intestine and are reabsorbed from the intestinal lumen into systemic circulation. Thus resulting in drug concentrations to appear higher than they actually are. However, analytical methods are not selective and this phenomenon should occur for both buccal and intravenous butorphanol. Other physiologic scenarios include liver damage, causing increased plasma drug concentrations of butorphanol, which is extensively metabolized by the liver. As such, a longer disappearance half-life and greater AUC would occur, resulting in an increased bioavailability.

Cross-reactivity between butorphanol and its metabolites could also cause altered values for pharmacokinetic parameters. Metabolite cross-reactivity has not been defined for the butorphanol ELISA assay,[93] but if interference does exist, plasma concentrations may be higher due to the presence of metabolites plus active drug. In addition to unknown metabolites, butorphanol has two recognized metabolites, hydroxybutorphanol and norbutorphanol.[93] Cross-reactivity to the unknown metabolites exists when butorphanol is analyzed using radioimmunoassay.[79] When the half-life of the drug is longer than that of the metabolite, the metabolite declines in parallel with the drug.[94] Conversely, metabolite levels build up in the body only when the half-life of the metabolite is longer than that of the drug.[93] Thus, by the time the peak metabolite level is reached, most of the drug has been eliminated. If cross-reactivity in the ELISA assay

is present, metabolite—as opposed to actual drug concentration—may be what is quantitated. Bioavailability, area under the curve, and disappearance half-life would all be increased. However, as with enterohepatic re-circulation, metabolite measurement should occur with both buccal and intravenously administered butorphanol, not just one or the other. Thus, cross-reactivity may well exist with ELISA analysis, but it would cause increased reported concentrations for both buccal and parenteral dosing. To define whether cross-reactivity exists, HPLC may be performed. High performance liquid chromatography was used to quantitate butorphanol metabolites in goats, but no evidence of metabolite formation was detected. [93]

The pharmacokinetics of intravenous butorphanol in dogs have not previously been determined. However, there is marked variability in Vss among different species. In this study, the Vss for IV butorphanol was 27.58 ± 10.14 L/kg and is larger than the Vss in llamas (0.822 L/kg),[92] rabbits (10.76 L/kg),[78] cows (4.178 L/kg),[95] and humans (8.3 L/kg).[96] Because the total body water of a 20 kg dog is approximately 12 L (60% of total body weight, or 0.6 L/kg). The Vss of IV butorphanol exceeds this value. This indicates rapid permeation of butorphanol and storage in tissues such as fat, located in peripheral compartments.[90, 97] The larger Vss indicates that increased doses are needed to achieve therapeutic plasma butorphanol concentrations in dogs, compared to that needed in other species. However, as with C_{max}, the difference also may reflect a dosing error.

The minimum therapeutic plasma analgesic concentration of butorphanol in dogs has been extrapolated to be 9 ng/ml.[73, 93] Current dosing recommendations for IV, SC,

and IM butorphanol at analgesic levels in dogs ranges from 0.1-1.2 mg/kg.[61] Dogs in this study received approximately half of the lower-end recommended dose, or 0.05 mg. A C_o of 8.24 ± 5.55 ng/ml was reached following IV dosing and a C_{max} of 6.66 ± 1.65 ng/ml after buccal administration. Both concentrations are slightly lower than the target plasma analgesic concentration of 9 ng/ml. Thus, therapeutic drug concentrations for analgesia were not reached using a butorphanol buccal patch containing 1.2 mg of drug. Although antitussive concentrations for butorphanol have not been determined, doses range from 0.05-0.11 mg/kg SC, similar to the dose administered in this study. Therefore, 0.05 mg/kg of buccal butorphanol could potentially be effective as an alternative route to provide antitussive activity in dogs.

Study Limitations

The purpose of this study was to provide a starting point for further pharmacokinetic studies utilizing buccal patches. Limitations of the information gathered here include a small sample size of three dogs, from which it is difficult to define outliers and arbitrary deviations in pharmacokinetic parameters. To increase the strength of interpretation, the sample size should be increased. However, the number of buccal patches made available by ViroTex Corporation restricted us from including more animals. Some patches were rendered unusable by mistakes made in adherence to the oral mucosa, while other patches were swallowed and data could not be analyzed.

Further compounding data interpretation, was reluctance in divulging "proprietary information" regarding patch composition. For instance, the manufacturing technique, type of vehicle used, whether the patch was unidirectional or bidirectional,

and the presence or absence of penetration enhancers was unknown us. As such, interpretation of potential problems in patch design was forced to be more speculation and conjecture than based on disclosed facts. Thus, although useful preliminary information was gained by the buccal patch study, more information is needed to appropriately characterize the pharmacokinetic parameters of buccally administered albuterol and butorphanol in dogs. In 1998, ViroTex Corporation was acquired by another company.

CHAPTER VI

CONCLUSIONS

Several useful conclusions can be made from this study. Not only was the disposition of albuterol and of butorphanol further characterized in dogs, important information was gathered regarding values for the pharmacokinetic parameters of these drugs when administered via a buccal patch. There are no reported studies that have examined the disposition of buccally administered albuterol or butorphanol, nor have the values for the pharmacokinetic parameters of IV butorphanol been described for dogs.

At present, there are no buccal patch formulations that are available for use in veterinary medicine. Indeed, novel drug delivery systems such as iontophoresis and phonophoresis are used only sparingly in the vast majority of veterinary clinics. The exception to this is the transdermal fentanyl patch for use as an analgesic. Although the fentanyl patch has demonstrated that therapeutic concentrations of drug can be achieved, it is currently the only drug available in patch formulation. Clearly, more studies need to be conducted to further explore the benefits, advantages, and convenience that novel drug delivery systems can afford the clinician both practically and economically.

Although this project was designed as a pilot study to define values for the pharmacokinetic parameters of buccally administered albuterol and butorphanol, important conclusions can be drawn regarding the drug delivery system itself. Primarily, the suitability of the oral mucosa to achieve systemic and sustained drug concentrations was clearly illustrated by this study. Higher drug concentrations of albuterol were achieved with the buccal patch than with transdermally delivered albuterol.[89]

Additional issues regarding buccal patch systems that were determined by this study include: ease of patch application, rapid dissolution of the patch, convenience of administration, and the fact that the patch was well tolerated by the dogs. The effectiveness of the buccal patch to deliver therapeutic drug concentrations is an area that must be explored in future studies. Analgesic concentrations of butorphanol were not achieved in this study. However, recommended parenteral antitussive doses of butorphanol are lower than analgesic doses, and are equivalent to the dose provided by the buccal patch used in this study (0.05 mg/kg SC).[61] As such, it is certainly feasible that this formulation could provide the necessary drug levels to attenuate and effectively control coughing. However, clinical studies are compulsory in order to ascertain this fact.

The most significant conclusion of this study is the fact that novel delivery systems are effective and convenient alternatives for traditional modes of drug administration. Although pharmaceutical companies believe oral dosage forms will likely remain the primary drug formulation, the importance and recognition of alternative routes of drug administration are likely to increase.[98] In fact, it is estimated that the annual sales for needle-free, alternative delivery systems will increase from $400 million to $1 billion by the year 2005. [98]

Despite these positive industrial projections, more studies are indicated in order to perfect, and possibly customize novel drug delivery systems for use in specific veterinary species. It is obvious from this study that although measurable drug concentrations are achieved in the blood, there is a great deal of inter-animal variation

and unpredictability that must be addressed before clinical use. Regardless, novel delivery systems such as buccal patches offer many advantages over traditional routes of drug administration. Although more work must be performed in order to accurately define pharmacokinetic and pharmacodynamic parameters in animals, buccal patches have the potential to become an important delivery system for clinical use in the future.

ENDNOTES

a ViroTex Corporation; The Woodlands, Texas
b AUP # 7-272 approved 10/17/97 (phase II approved 9/9/98); AUP # 8-199 approved August 25, 1998
c Venocath®-18; Radiopaque IV Catheter; Abbott Park, Illinois
d Dawn M. Boothe, DVM, PhD, DACVIM, DACVCP; Albert Boeckh, DVM, Veterinary Clinical Associate; Sarah Jones, DVM, Veterinary Clinical Associate
e Albuterol; Sigma-Aldrich; St. Louis, Missouri
f E-Z Set® Infusion Set; Becton Dickinson; Sandy, Utah
g Inderal®; Wyeth-Ayerst Laboratories; St. Davids, Pennsylvania
h Torbutrol®; Fort Dodge Animal Health; Overland Park, Kansas
i P/M® Naloxone HCl Injection; Schering Plough; Kenilworth, New Jersey
j Physiological Saline Solution 1000 ml; The Butler Company; Dublin, Ohio
k Monoject®; Sherood Medical; St. Louis, Missouri
l USA Scientific, Inc.®; Ocala, Florida
m Neogen Corporation; Lexington, Kentucky
n Butorphanol tartrate salt 100 mg; Sigma-Aldrich; St. Louis, Missouri
o Pharsight Corporation; Mountain View, California
p Microsoft Corporation; Redmond, Washington

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APPENDIX A

Pre-Study Complete Blood Cell Count Results

November 1997

Test	Normal Range	Dog B	Dog C	Dog R
White Cell Count	6-17 x 10^3 /μl	7.6	7.6	7.1
Red Cell Count	5.5-8.5 x 10^3 /μl	5.8	5.9	5.34^{L}
Hemoglobin	12-18 g/dl	13.6	14.1	12.0
Packed Cell Volume	37-55%	39.8	40.4	34.6^{L}
Platelet Estimate	0.2-0.5 x 10^3 /μl	**NR**	**NR**	**NR**
Segmented Neutrophil Count	3-11.5 x 10^3 /μl	4.778	4.560	5.112
Band Neutrophil Count	0-0.3 x 10^3 /μl	0.988^{H}	0	0
Lymphocyte Count	1-4.8 x 10^3 /μl	1.216	1.9	1.9
Monocyte Count	0.15-1.35 x 10^3 /μl	0.456	0.456	0.400
Eosinophil Count	0.1-1.25 x 10^3 /μl	0.76	0.532	0.284
Basophil Count	Rare	0	76^{H}	0

^{H} Indicates high value (as compared to normal)

^{L} Indicates low value (as compared to normal)

NR = not reported

APPENDIX B

STANDARD CURVE QUALITY CONTROL

Controls used for albuterol assay

Conc. (ng/ml)	8.530	5.600	3.790	2.520	1.123	0.332
Analysis #1 (ng/ml)	9.900	5.190	3.740	2.39	1.29	0.48
Analysis #2 (ng/ml)	10.450	4.670	4.540	2.440	1.290	0.240
N	2	2	2	2	2	2
Mean conc. (ng/ml)	10.175	4.930	4.140	2.415	1.290	0.360
Std Dev	0.389	0.368	0.566	0.035	0.000	0.170
Prec Err	3.8	7.5	13.7	1.5	0.0	47.1
% Acc	119.3	88.0	109.2	95.8	114.9	108.4

Controls used for butorphanol assay

Conc. (ng/ml)	8.800	5.930	3.950	*2.630	0.230
Analysis #1 (ng/ml)	7.880	5.120	4.300	1.8	0.29
Analysis #2 (ng/ ml)	8.100	5.120	3.870	2.060	0.200
n	2	2	2	2	2
Mean conc. (ng/ml)	7.990	5.120	4.085	1.930	0.245
Std Dev	0.156	0.000	0.304	0.184	0.064
Prec Err	1.9	0.0	7.4	9.5	26.0
% Acc	90.8	86.3	103.4	73.4	106.5

* Shaded area indicates a control point not used.

APPENDIX C

Intravenous albuterol assay dilutions
♦ Methodology used

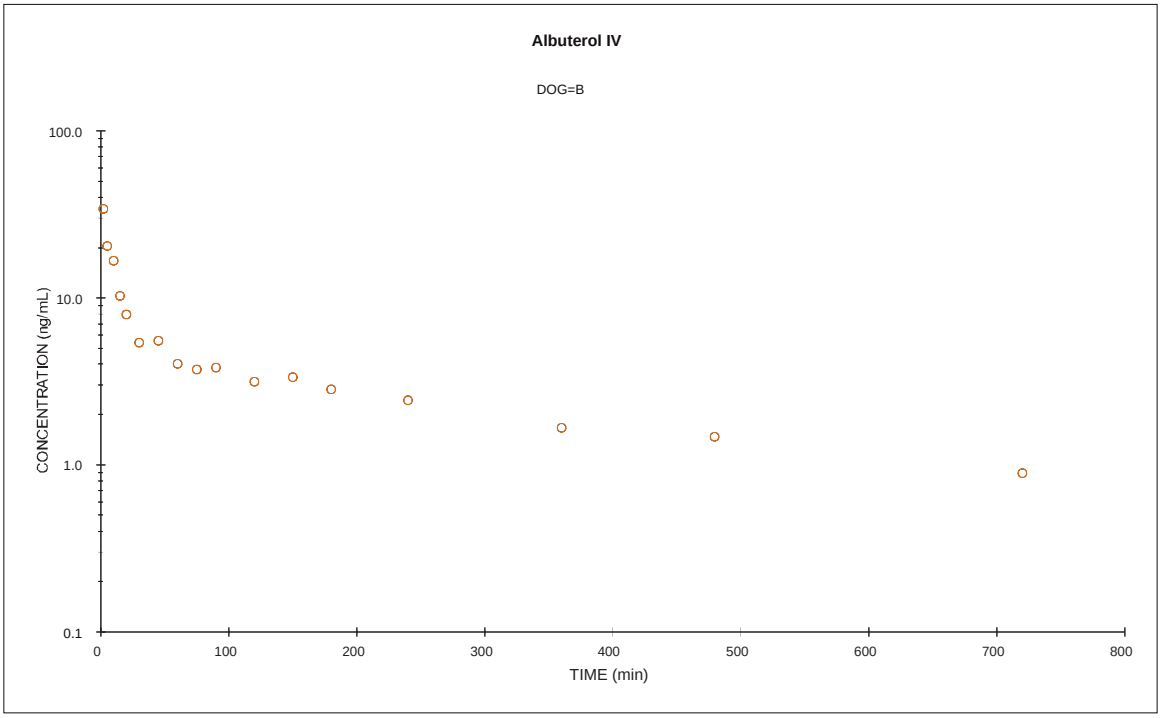
Dog	Time	Dilution Factor	Actual Conc.	Concentration
B	2	**10x**	3.412	34.12
B	5	**5x**	4.088	20.44
B	10	**5x**	3.34	16.67
B	15	**2x**	5.145	10.29
C	2	**10x**	4.5	45.06
C	5	**5x**	5.96	29.82
C	10	**5x**	4.22	21.12
C	15	**5x**	3.96	19.88
C	20	**5x**	3.31	16.53
C	30	**5x**	3.34	16.87
C	45	**5x**	3.64	18.2
C	60	**2x**	4.78	9.56
R	2	**10x**	5.79	57.9
R	5	**10x**	4.77	47.7
R	10	**10x**	3.59	35.92
R	15	**5x**	4.052	20.26
R	20	**5x**	3.94	19.72
R	30	**5x**	3.67	18.37
R	45	**5x**	3.37	16.87

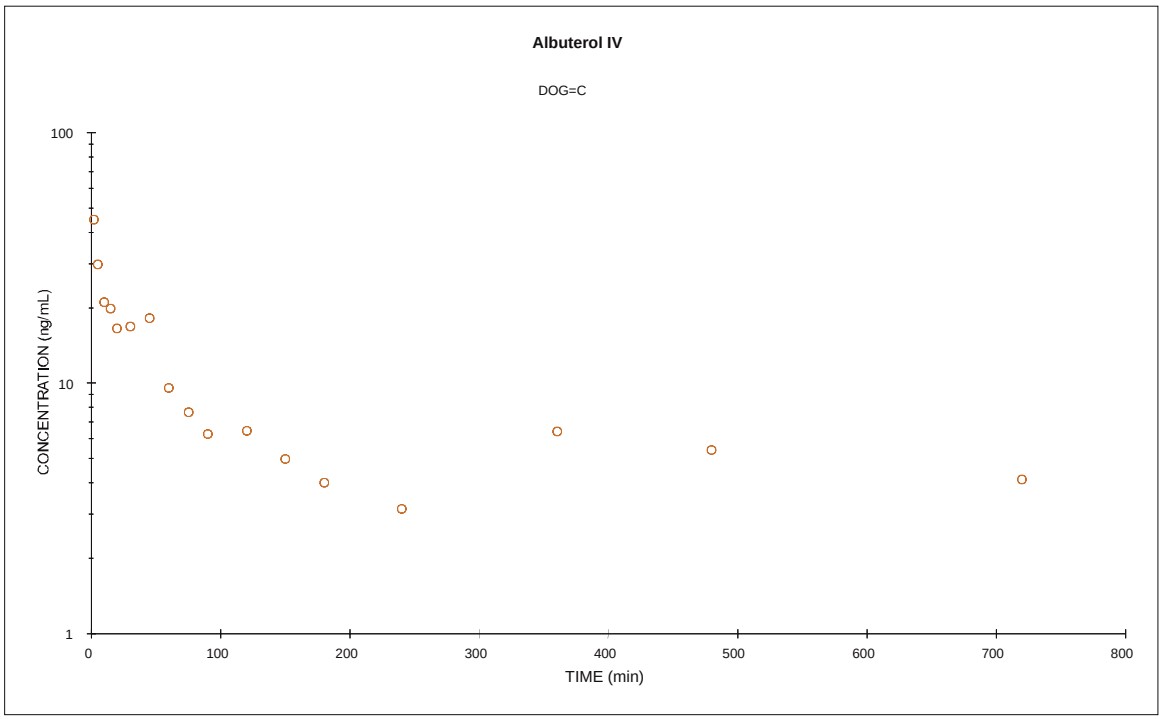
♦

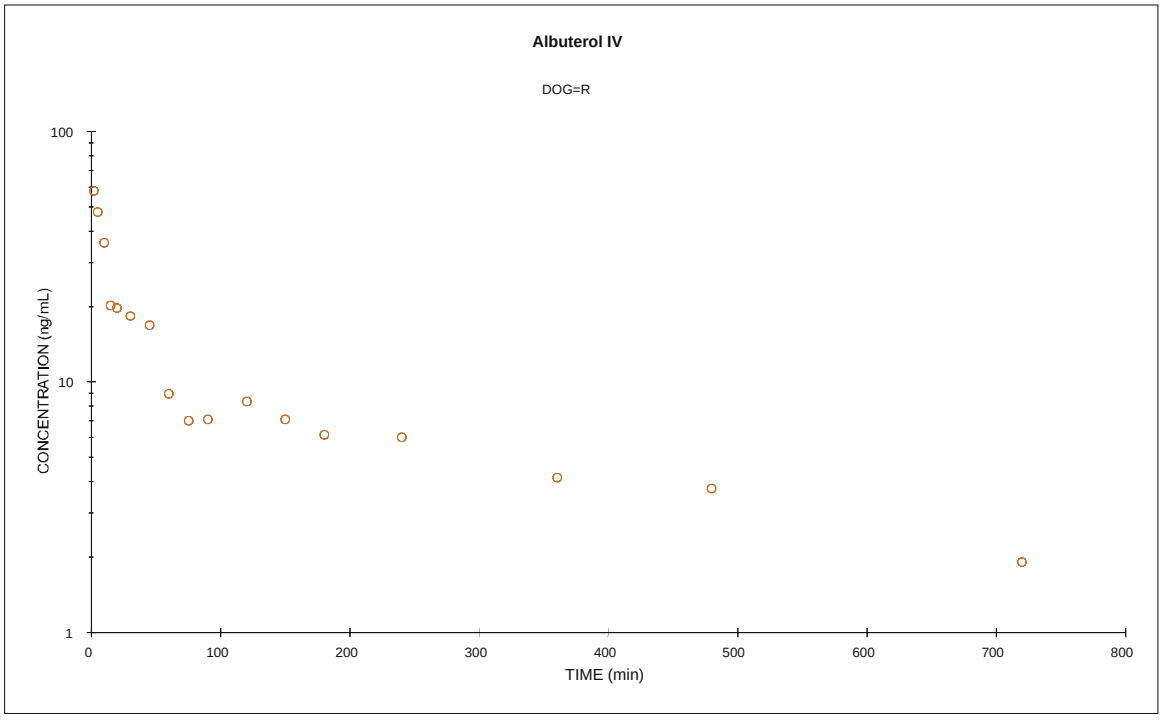
Dilution	Sample (microliters)	Blank serum (microliters)
10x	5	45
5x	20	80
3x	30	60
2x	50	50

APPENDIX D

Intravenously administered albuterol concentration versus time profiles for individual dogs

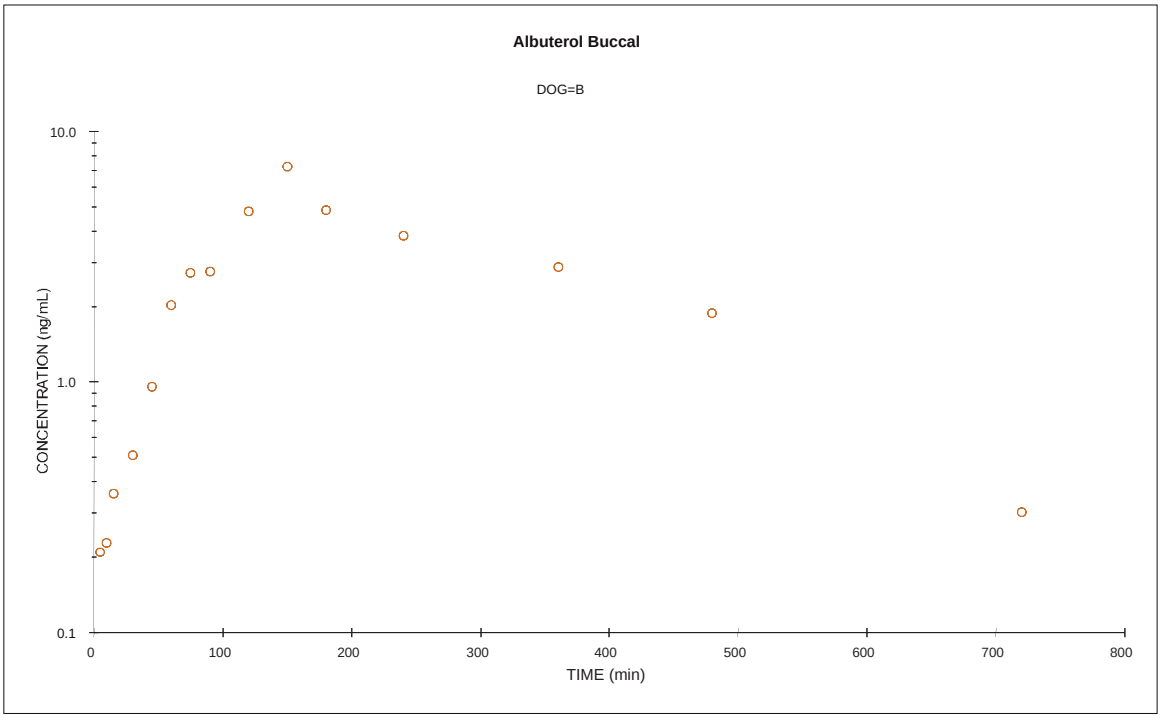


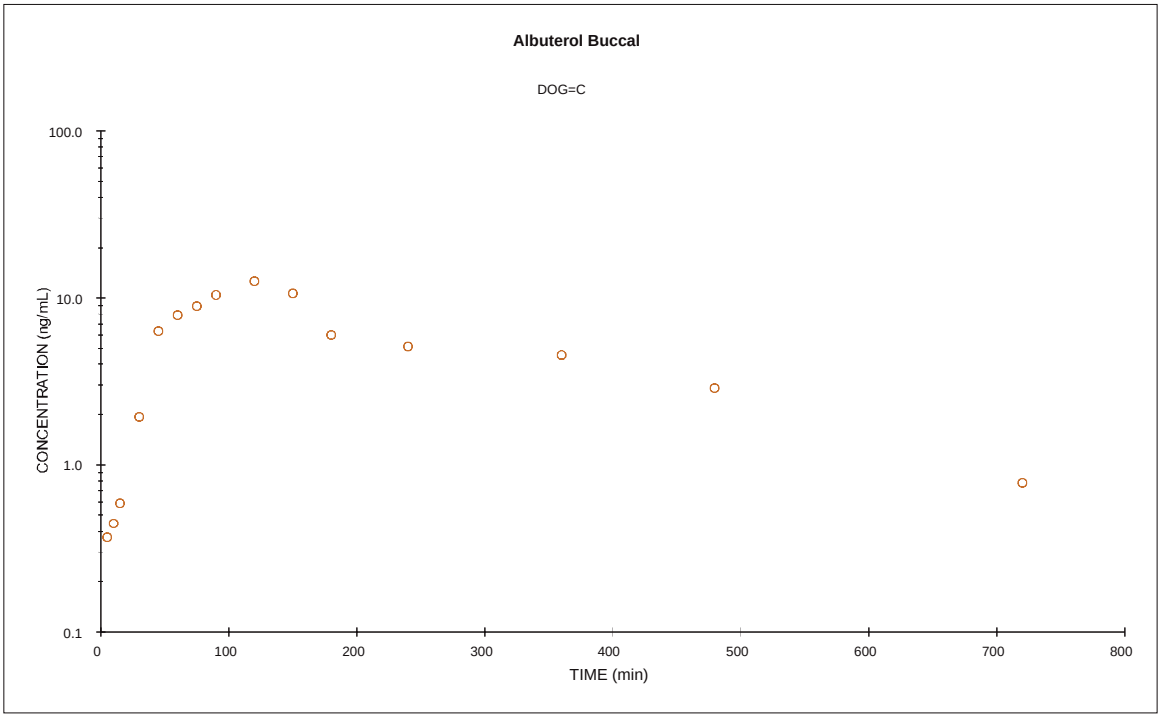

Albuterol IV
DOG=C
CONCENTRATION (ng/mL)
100
10
1
0
100
200
300
400
500
600
700
800
TIME (min)

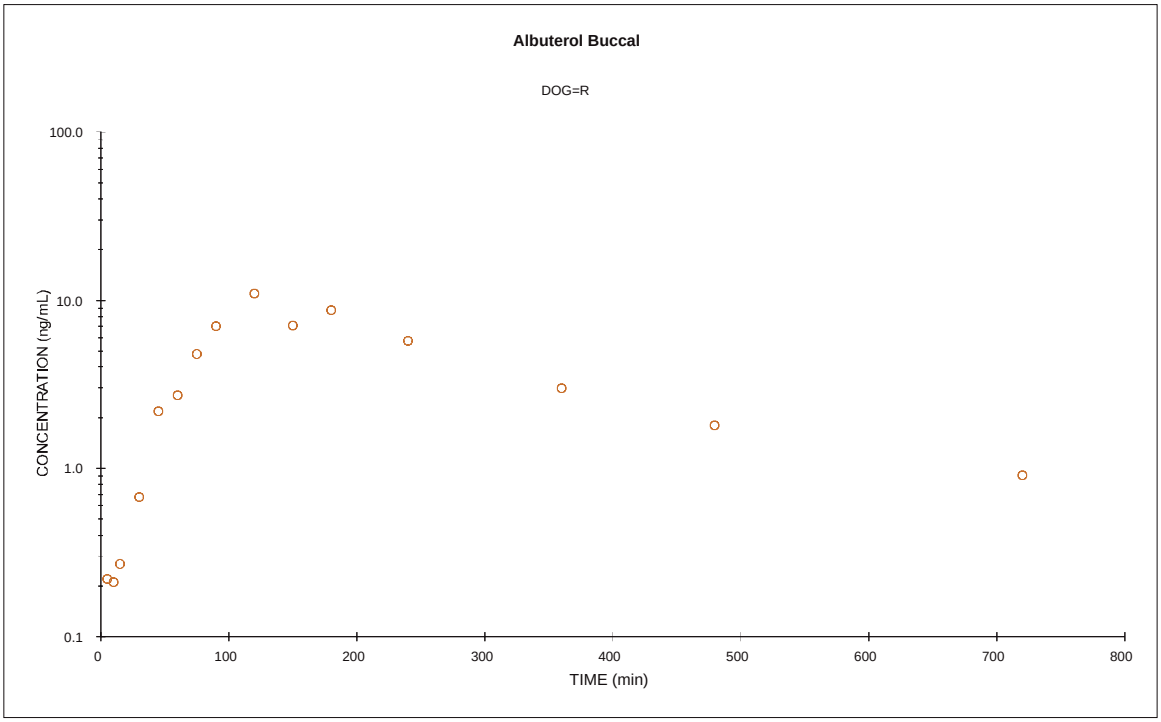

Albuterol IV
DOG=R
CONCENTRATION (ng/mL)
100
10
1
0
100
200
300
400
500
600
700
800
TIME (min)

APPENDIX E

Buccally administered albuterol concentration versus time profiles for individual dogs

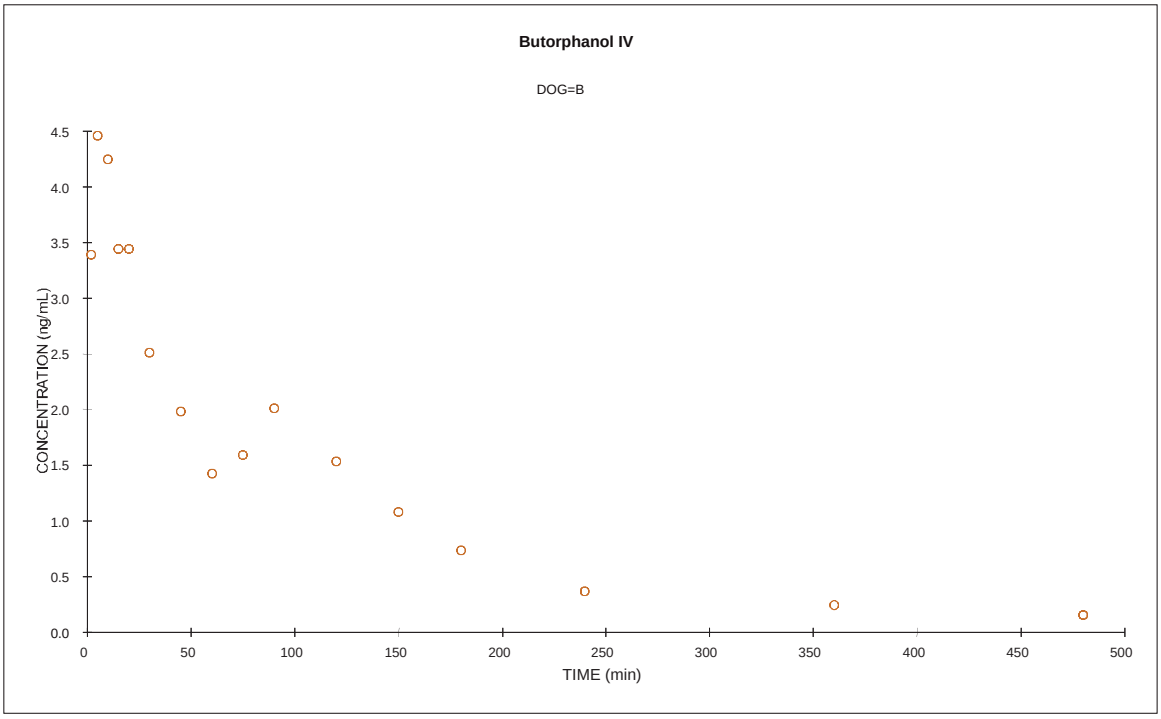


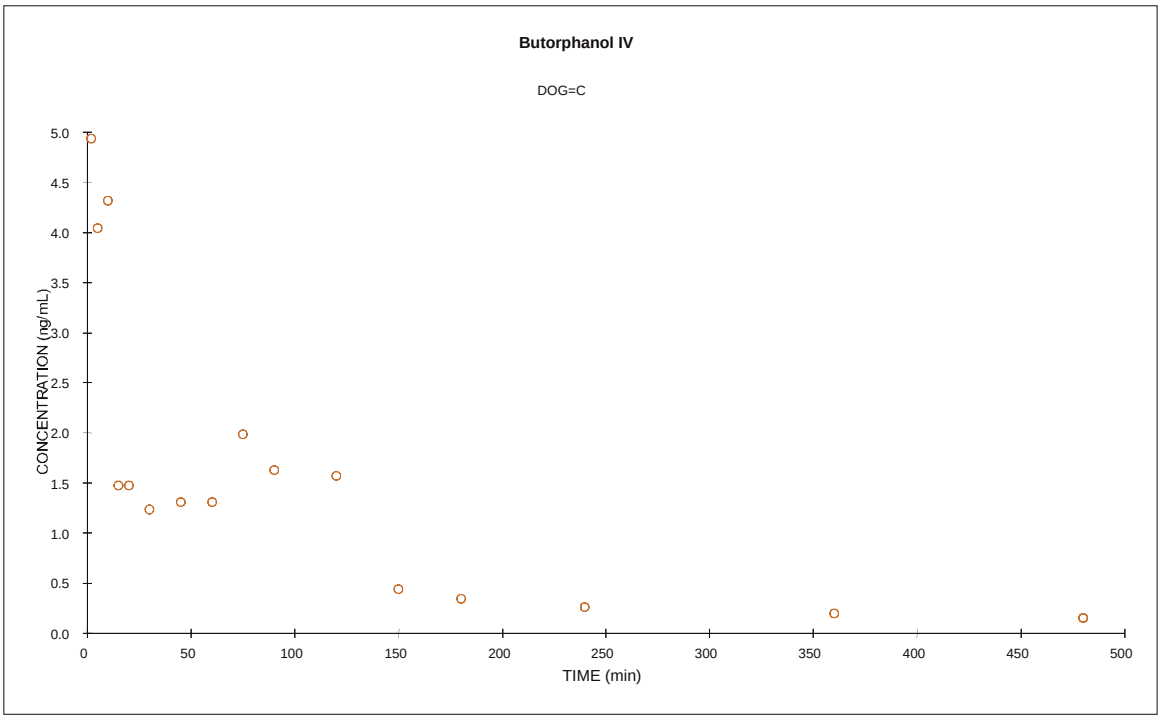

Albuterol Buccal
DOG=C
100.0
10.0
1.0
0.1
CONCENTRATION (ng/mL)
0
100
200
300
400
500
600
700
800
TIME (min)

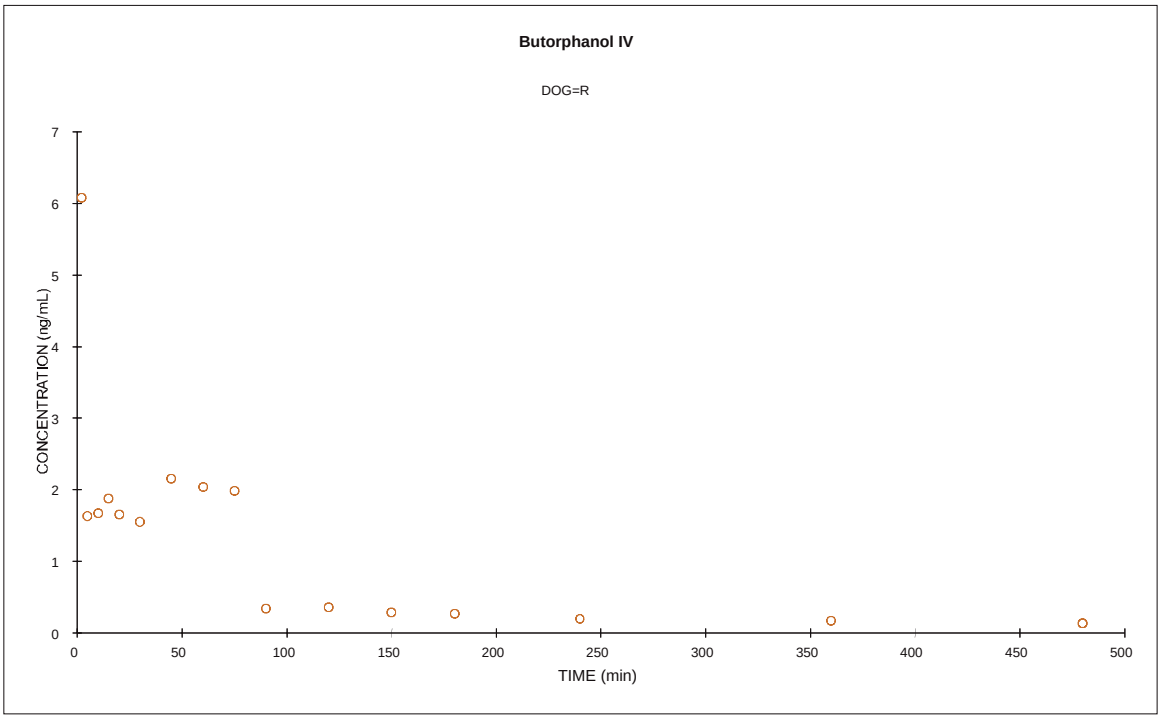

Albuterol Buccal
DOG=R
100.0
10.0
1.0
0.1
CONCENTRATION (ng/mL)
0
100
200
300
400
500
600
700
800
TIME (min)

APPENDIX F

Intravenously administered butorphanol concentration versus time profiles for individual dogs

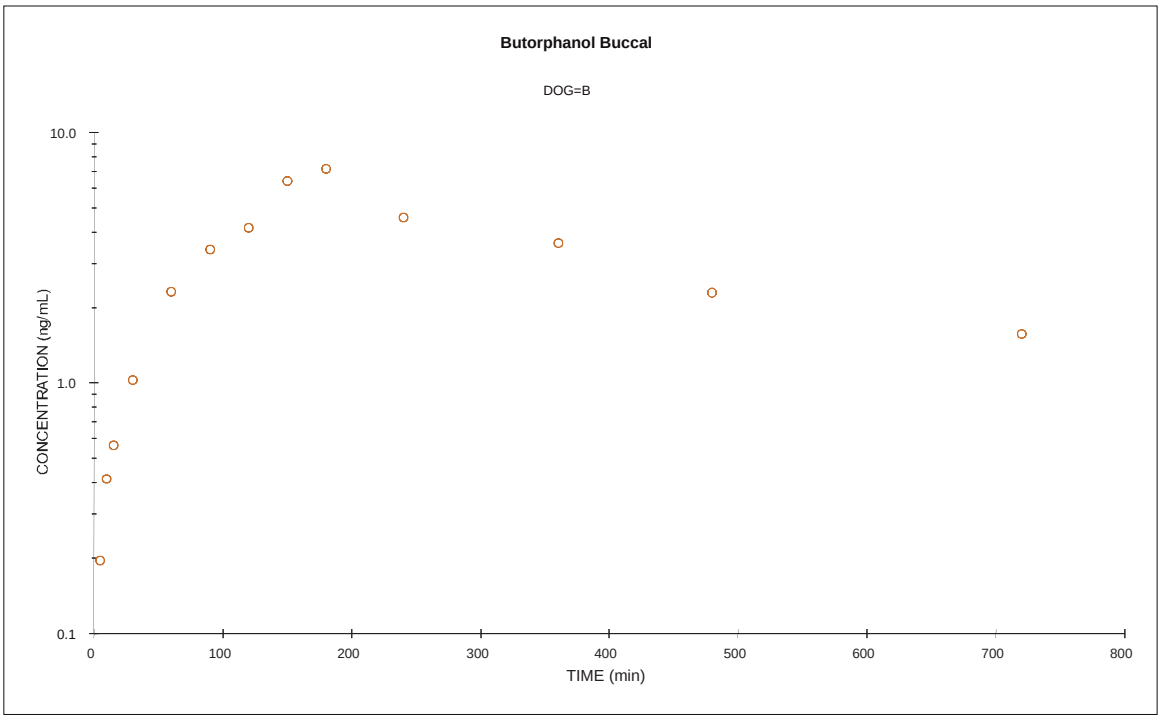


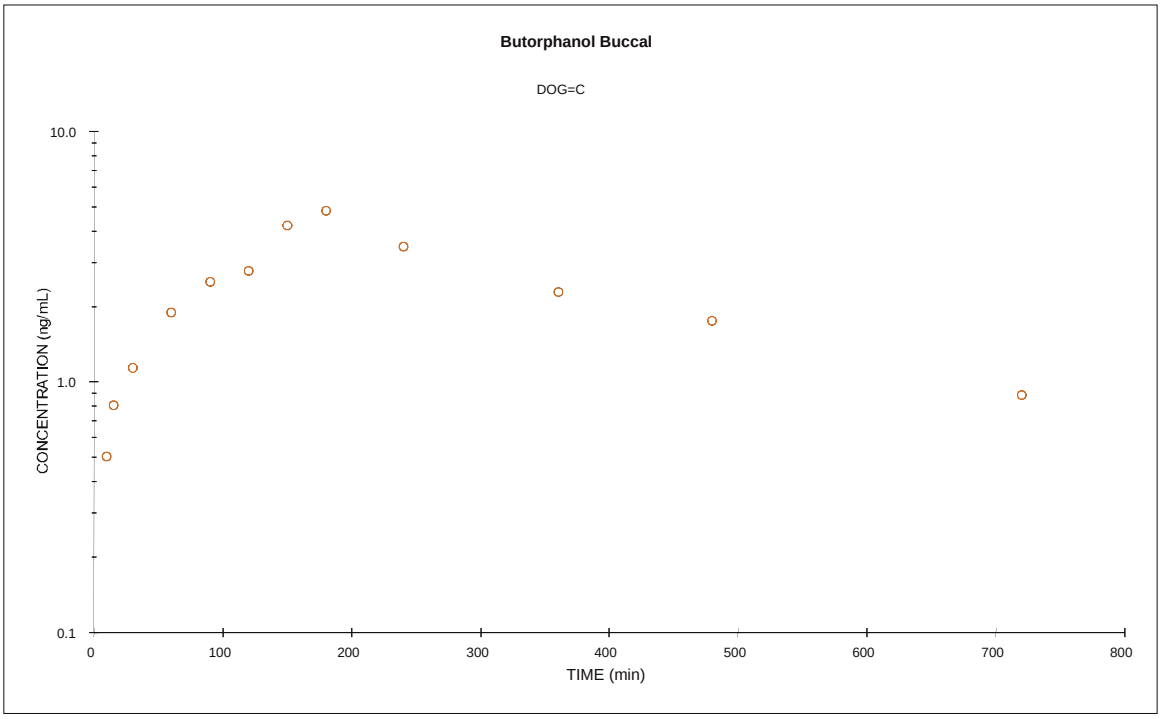

Butorphanol IV
DOG=C
CONCENTRATION (ng/mL)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
300
350
400
450
500
TIME (min)

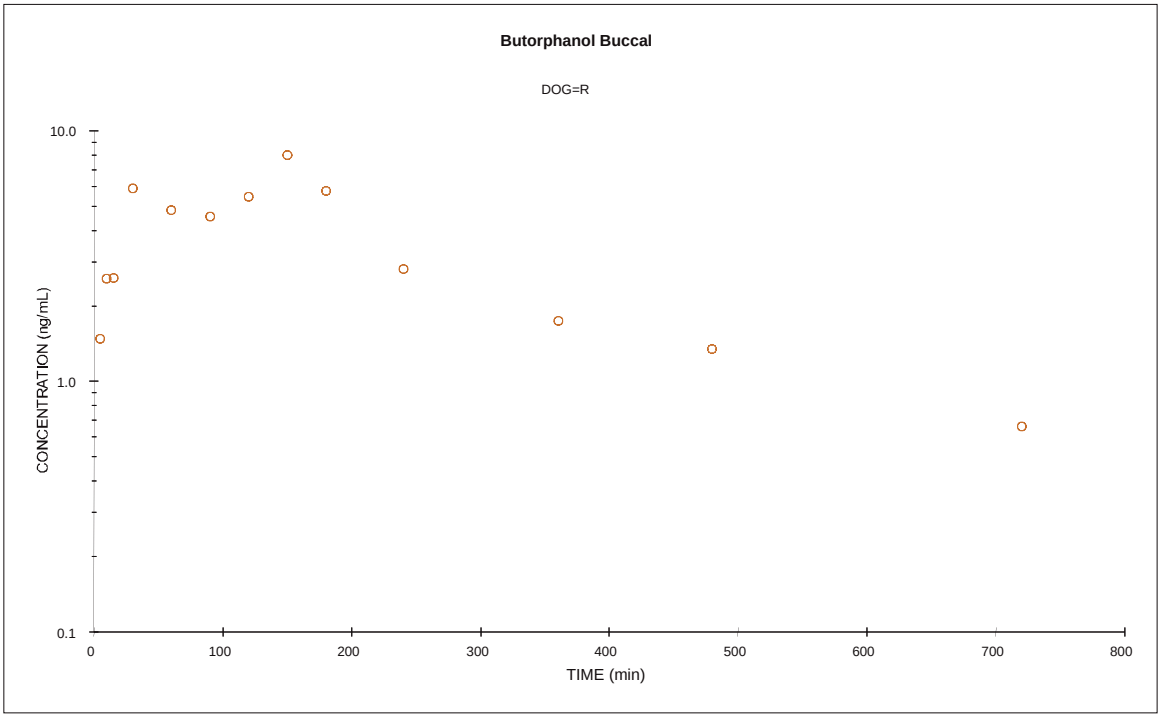

Butorphanol IV
DOG=R
CONCENTRATION (ng/mL)
7
6
5
4
3
2
1
0
0
50
100
150
200
250
300
350
400
450
500
TIME (min)

APPENDIX G

Buccally administered butorphanol concentration versus time profiles for individual dogs




Butorphanol Buccal
DOG=C
10.0
1.0
0.1
CONCENTRATION (ng/mL)
0
100
200
300
400
500
600
700
800
TIME (min)


Butorphanol Buccal
DOG=R
10.0
1.0
0.1
CONCENTRATION (ng/mL)
0
100
200
300
400
500
600
700
800
TIME (min)

APPENDIX H

Statistical comparison between the disappearance rate constant for buccal and intravenous drug administration

t-Test: Paired Two Sample for Means

P≤ 0.05

Albuterol	***IV***	***Buccal***
Mean	0.0019	0.0043
Variance	3.7E-07	4.3E-07
Observations	3	3
Pearson Correlation	0.739583	
Hypothesized Mean Difference	0	
df	2	
t Stat	-9.07115	
P(T<=t) one-tail	0.005968	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.011936	
t Critical two-tail	4.302656	

t-Test: Paired Two Sample for Means

P≤ 0.05

Butorphanol	***IV***	***Buccal***
Mean	0.004033	0.002667
Variance	5E-06	1.03E-07
Observations	3	3
Pearson Correlation	-0.99902	
Hypothesized Mean Difference	0	
df	2	
t Stat	0.925388	
P(T<=t) one-tail	0.226228	
t Critical one-tail	2.919987	
P(T<=t) two-tail	0.452457	
t Critical two-tail	4.302656	

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