A method of delivering a drug through the skin of an animal comprising applying an enzyme preparation to a localized area of the skin for a predetermined amount of time and in a sufficient quantity to alter the structure of the localized area of skin; occluding the localized area of skin with occlusion means (20) during said predetermined amount of time; removing said occlusion means (20) and applying an effective amount of drug to the localized area of the skin. Also disclosed is a percutaneous drug delivery system comprising contact means (30) for contacting an enzyme preparation to a localized area of skin; means for occluding said localized area of skin in contact with said contact means (30); and means (32) for applying a drug to said localized area of skin after said contact means and said occlusion means have been removed from said localized area of skin, wherein said contact means and said means for applying a drug are different patches (30, 32). Further disclosed is a method of delivering a drug through the skin of an animal comprising applying a percutaneous permeation enhancer comprising effective amounts of a combination of enzymatic and non-enzymatic permeation enhancers to a localized area of the skin of said animal for a predetermined amount of time and in a sufficient quantity to enhance the permeability of the localized area of skin to the drug; occluding the localized area of skin with occlusion means (20) during said predetermined amount of time; removing said occlusion means (20); and applying an effective amount of the drug to the localized area of skin so that at least some of the drug permeates through the skin.
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PERCUTANEOUS DRUG DELIVERY SYSTEM

Related Applications: This application is a continuation-in-part of U.S. Serial No. 352,926 filed on May 18, 1989, which is a continuation-in-part of U.S. Serial No. 314,819 filed on February 23, 1989, the contents of both being incorporated herein by this reference.

Background of the Invention

Field: This invention pertains to drug delivery systems generally and is more particularly directed to transdermal drug delivery systems and methods.

State of the Art: Devices for transdermal or percutaneous drug delivery are known in the art. Such devices include "patches" such as the Nitro-Dur® nitroglycerin transdermal infusion system marketed by Key Pharmaceutical of Kenilworth, New Jersey. This system consists of a "patch" containing nitroglycerin in acrylic-based polymer adhesives with a resinous cross-linking agent to provide a continuous source of nitroglycerin to the patient. The patches are available in various dosage strengths for delivering various amounts of nitroglycerin to the patient over a twenty-four hour period. These patches vary in size from five to thirty square centimeters (cm²). The rated release of the drug is dependent upon the area of the patch with 0.5 milligram (mg) being released for every square centimeter of patch per 24 hours. The patch is applied to any convenient skin area, especially the arm or chest.

Another transdermal patch is marketed by Noven Pharmaceutical of Miami, Florida. The Noven patch has been used with nitroglycerin and estrogen. It consists of a non-occlusive backing layer, a drug reservoir for containing the drug, a microporous rate controlling membrane which contacts the skin of the patient, and an adhesive formulation for keeping the patch in contact with the skin. Drug passes from the reservoir through the membrane, through the patient's skin, and into the bloodstream.
Another transdermal patch, Transderm SCOP®, is used by CIBA Consumer Pharmaceutical Co. of Summit, New Jersey. It is a film 0.2 mm thick and 2.5 cm², with four layers. Proceeding from the visible surface towards the surface attached to the patient's skin (FIG. 12), these layers are: (a) a backing layer of tan-colored, aluminized, polyester film; (b) a drug reservoir of scopolamine, mineral oil, and polyisobutylene; (c) a microporous polypropylene membrane that controls the rate of delivery of scopolamine from the system to the skin surface; and (d) an adhesive formulation of mineral oil (12.4 mg), polyisobutylene (11.4 mg) and scopolamine (1.5 mg). A protective peel strip of siliconized polyester, which covers the adhesive layer, is removed before the system is used. The inactive components, mineral oil and polyisobutylene, are not released from the system. The system is "programmed" to deliver 0.5 mg of scopolamine at an approximately constant rate of the systemic circulation over the three-day lifetime of the system.

An initial priming dose of scopolamine, released from the adhesive layer of the system is believed to saturate the skin binding sites for scopolamine and bring the plasma concentration of scopolamine to the required steady state level. A continuous controlled release of scopolamine, which flows from the drug reservoir through the rate-controlling membrane, maintains the plasma level constant.

A similar system is also used by CIBA Pharmaceutical Company in its Transderm-Nitro® nitroglycerin. In this system, the rate controlling membrane is an ethylene/vinyl acetate copolymer membrane that is permeable to nitroglycerin, and the adhesive used is a hypoallergenic silicone adhesive.

Another system to deliver drugs through the skin of a patient is disclosed in French patent 2,556,218. This patent discloses "sticks" for roll-on application of a desired drug. These sticks contain, as components, an enzymatic penetrating agent, the desired drug, and

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various excipients. The enzymatic penetrating agents disclosed include alpha-chymotrypsin and hyaluronidase. Drugs disclosed for use with these sticks include aspirin, lidocaine, lutadine, vitamin A, and tetracycline. Excipients disclosed include sodium glycerol stearate. The desired drug, enzymatic penetrating agent, and excipient can be mixed together to form a homogeneous mixture.

Alternatively, the various components of the stick can be separated from one another in a manner that brings one component (e.g., enzymatic penetrating agent) immediately after the other (e.g., drug) in contact with the local application zone of the patient's skin for treatment (e.g. a solid stick can make up two longitudinal or concentric parts of one stick). The enzymatic penetrating agents increase the penetration of the desired drug through the patient's skin or mucous membrane.

Another transdermal drug delivery system is disclosed in French Patent No. 2,448,903. This system consists of at least one antibiotic, an enzyme, an anti-inflammatory agent, and/or a local anesthetic agent, and/or a heteratolytic agent, and/or a mucolytic agent, and/or an emulsifying agent. Enzymes disclosed include hyaluronidase, streptokinase, streptodornase, trypsin, chymotrypsin, α-chymotrypsin; α-amylase, bromelain, papain, deoxyribonuclease, collagenase, and sutilain. This system is used to provide localized antibiotic therapy.

United States Patents Nos. 3,989,816, 4,316,893, and others to Rajadhyaksha, disclose the use of lactam compounds, (e.g. 1-n-Dodecylazacycloheptan-2-one Azone™ Nelson Research & Development Company, Irvine, California) for carrying physiologically active agents through the skin or other membranes of an animal or human.

The use of various penetration enhancing substances in connection with various drugs for
percutaneous delivery have been disclosed in U.S. Patent No. 4,755,535 to Minaskanian (target drug used with azacycloalkene-type substances), U.S. Patent No. 4,699,777 to Zupon, et al. (1-dodecyl-azacycloheptan-2-one and urea used to enhance penetration effect of albuterol), U.S. Patent No. 4,820,711 to Pearlman (cytotoxic compounds dissolved in Azone or Azone-related compounds to relieve actinic keratosis), and U.S. Patent No. 4,557,934 to Cooper (use of a binary mixture of Azone or Azone-related compounds and a C₃-C₄ diol to enhance penetration of various drugs).

SUMMARY OF THE INVENTION

The invention includes a method of delivering a drug to an animal having skin, comprising: 1) applying and keeping in contact a proteolytic enzyme preparation to a localized area of the animal's skin in a sufficient quantity to enhance that area of skin's permeability to a selected drug over a predetermined amount of time; 2) occluding the area of skin with occlusion means for an amount of time sufficient to allow the enzymatic preparation to enhance the skin's permeability to the selected drug; 3) removing the occlusion means and rinsing the area of the skin; and 4) applying the selected drug, in solution, to the area of skin. Optionally, the skin may be 5) occluded again after the application of the selected drug. As used herein, "animal" includes human beings.

In an alternative embodiment, the invention includes a percutaneous permeation enhancer which comprises a potentiating amount of an enzyme preparation in admixture with a non-enzymatic penetration enhancer, such as a lactam compound. Both the enzyme preparation and the non-enzymatic penetration enhancer are present in sufficient quantities and concentrations so that when they are applied to an area of the skin for a selected period of time, they enhance the penetration of chemical agents through the skin. "Skin", as used herein, refers
to the outer body integument as well as those tissues which are histologically related, such as nails and the like.

The percutaneous permeation enhancer may further include solubilizers for assisting in the admixture of the components into a liquid solvent such as water. The enhancer may also include other chemical agents, such as propylene glycol, which enhance the penetration of a chemical agent through the skin.

Non-enzymatic penetration enhancers, for purposes of this invention, are compositions which enhance the permeation of biologically active agents (e.g. drugs) through the skin of an animal. Such compositions include alcohols, such as ethanol and isopropanol; polyols, such as n-alkanols (C₆-C₈), limonene, terpenes, dioxolane, propylene glycol, ethylene glycol, other glycols, and glycerol; sulfoxides, such as dimethylsulfoxide ("DMSO") and methyl dodecyl sulfoxide; esters such as isopropyl myristate/palmiate, ethyl acetate, butyl acetate, methyl propionate, and capric/caprylic triglycerides; ketones, such as 2-alkyl cyclohexanones, t-butyl cyclohexanones, and various C₈ derivatives; amides, such as acetamides; oleates, such as triolein; various surfactants, such as Brij 96, Tweens (Atlas Chemical Company), myrj, and sodium lauryl sulfate; various alkanolic acids such as caprylic acid (C₆-C₁₀); lactam compounds, such as Azone; alkanols, such as oleyl alcohol; and admixtures thereof. These compositions are believed to enhance permeation of biologically active agents by acting at the lipid matrix of the stratum corneum (i.e. by enhanced intercellular matrix diffusion).

"Lactam compound", as used herein, is an organic compound containing the -NR-CO-group in a ring. For use in the invention, the R group of this compound should be free from the lactam ring, unlike the penicillins and cephalosporins wherein the R group is bonded to another portion of the lactam ring to form a bucolic compound. Lactam compounds useful in the invention have the
following typical structural formula:

\[
\text{CO} \quad \text{(CH}_2\text{)}_m \text{N--R}
\]

wherein \( R \) is an alkyl, aryl, or phenyl group. These compounds, as disclosed in U.S. Patent Nos. 3,989,816 and 4,316,893 enhance the penetration of chemical agents through the skin. Similar permeation enhancing compounds are disclosed in U.S. Patent Nos. 4,405,616; 4,415,563; 4,423,040; 4,424,210; 4,444,762; 4,525,199; 4,562,075; 4,801,586; and 4,806,341, the contents of which are incorporated by this reference. Concentrations of these agents effective to enhance the skin's permeability to drugs are disclosed throughout these patent references. A synergistic permeation enhancement effect is achieved when a potentiating amount of these compounds is in admixture with an enzyme.

The percutaneous permeation enhancers of the present invention are used as an aid in delivering a drug percutaneously to an animal, including humans. The percutaneous permeation enhancer is first applied to a localized area of the skin in a sufficient quantity and concentration to enhance eventually the permeability of the skin to a drug. The enhancer is kept in contact with the skin for a sufficient amount of time to enhance the skin's permeability to the drug. The area of skin may optionally be occluded during the time in which the enhancer is in contact with the skin. The drug is then applied to the localized area of skin and permeates through the skin to be delivered to the animal.

The invention further includes a drug delivery system. This drug delivery system includes an enzyme preparation, means for applying the enzyme preparation to an area of skin, a membrane for occluding the area of skin after the enzyme preparation has been applied; a drug; and means for applying the drug to the area of skin.
The drug delivery system can be a dual patch percutaneous drug delivery device which includes: a first patch sized and adapted to contact a localized area of skin. This first patch contains, in a reservoir, an effective amount of a proteolytic enzyme. The drug delivery device further includes a second patch attached to the first patch in a common structure. This second patch is also adapted to contact the localized area of skin after the first patch has been removed from the skin. This second patch contains an effective amount of a preselected drug in liquid form for administration percutaneously.

The drug delivery system of the present invention can also be a dual-patch, percutaneous drug delivery kit. This kit includes: 1) a first patch containing enzyme, enzymes or a permeation enhancer preparation; 2) a second patch containing drug or drugs, and 3) structure adapted to receive the first and second patches serially, i.e. sequentially, so that the second patch is removable from the structure only after the first patch removed.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph comparing the percentage urinary recovery of $^{14}$C-TEAB with papain treatment, seven days after papain treatment, and without papain pretreatment per Example 1.

FIG. 2 is a graph comparing the percentage urinary recovery of $^{14}$C-Citric acid with papain treatment, seven days after papain treatment, and without papain pretreatment per Example 1.

FIG. 3 is a graph comparing the percentage urinary recovery of $^{14}$C-glucose with papain treatment; five days after papain treatment; seven days after papain treatment; and without papain pretreatment per Example 1.

FIG. 4 is a graph comparing the percentage urinary recovery of the $^{14}$C-protein lysozyme with papain treatment, and seven days after treatment, as per Example 1.
FIG. 5 is a graph comparing the percentage urinary recovery of the $^{14}$C-protein insulin with papain treatment and seven days after treatment per Example 1.

FIG. 6 is a graph comparing the percentage urinary recovery of $^{14}$C-protein albumin with papain treatment and seven days after treatment per Example 1.

FIG. 7 is a graph comparing the percentage urinary recovery of $^3$H-Hydrocortisone with papain treatment, seven days after papain treatment, and without papain treatment as per Example 1.

FIG. 8 shows the saturation coefficient of citric acid, and the flux, permeability coefficient, and graph for citric acid first day (immediately following 24-hour exposure to papain), second day (24 hours following 24-hour exposure to papain) and third day (48 hours following 24-hour exposure to papain) as per Example 2.

FIG. 9 shows the saturation coefficient of hydrocortisone, and the flux, permeability coefficient and graph for hydrocortisone first day (immediately following 24-hour exposure to papain), second day (24 hours following 24-hour exposure to papain), and third day (48 hours following 24-hour exposure to papain) as per Example 2.

FIG. 10 shows the saturation coefficient of TEAB and the flux, permeability coefficient, and graph for TEAB first day (immediately following 24-hour exposure to papain), second day (24 hours following 24-hour exposure to papain), and third day (48 hours following 24-hour exposure to papain) as per Example 2.

FIG. 11 shows the saturation coefficient of glucose, and the flux, permeability coefficient, and graph for glucose first day (immediately following 24-hours exposure to papain), and third day (48 hours following 24-hour exposure to papain) as per Example 2.

FIG. 12 stylistically depicts a side view of a prior art patch useful in the practice of the invention.

FIG. 13 stylistically depicts the side view of a dual patch drug delivery device according to the
invention.

FIG. 14 stylistically depicts the side view of one embodiment of a dual patch percutaneous drug delivery kit according to the invention.

FIG. 15 depicts the side view of a patch useful in the practice of the invention.

FIG. 16 depicts the side view of a patch useful in the practice of the invention.

FIG. 17 depicts the enhancement of tetraethylammonium bromide ("TEAB"), a cationic substance, through mouse skin by the use of an enzyme preparation containing papain and an activating agent as per Example 7A.

FIG. 18 depicts the enhancement of TEAB penetration through mouse skin by the use of an enzyme preparation containing papain at varying levels and an activating agent as per Example 7B.

FIG. 19 depicts the enhancement of TEAB penetration through mouse skin by the use of an enzyme preparation containing papain at varying levels and with and without an activating agent per Example 7C.

FIG. 20 depicts the percentage of drug (citric acid, an anionic substance) which penetrated through mouse skin at varying times and using various sources of papain in the enzyme preparation as per Example 8.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Enzymes useful in the enzyme preparations, and in the percutaneous permeation enhancers ("enhancers") of the alternate embodiment, are enzymes capable of altering structures of the skin of the particular animal to be treated so as to enhance the skin's permeability to a selected drug or drugs. Enzymes preferred for these purposes do not substantially react detrimentally with the selected drug or non-enzymatic penetration enhancer, do not degrade substantially to an inactive state at body temperature or in solution; do not cause unacceptable discoloration or scarring of the skin, and react in
sufficiently small concentrations to be useful over a relatively small area of skin. A preferred enzyme is papain, which is readily available from Sigma Chemical Company of St. Louis, Missouri or other sources. Other enzymes useful in the practice of the invention include pancreatin (actually a mixture of enzymes), ficin, bromelain, elastase, and pepsin. Further useful enzymes include hyaluronidase, streptokinase, streptodornase, trypsin, chymotrypsin, α-chymotrypsin, α-amylase, deoxyribonuclease, collagenase, sutilain, and other specific and non-specific proteolytic enzymes. For purposes of this disclosure, a non-specific proteolytic enzyme is one that alters a protein's structure at nearly any point or level, including breaking down disulfide bonds, which can result in complete denaturation of the skin protein. A specific proteolytic enzyme, however, is one that merely alters the protein under conditions not sufficiently severe to alter protomer configuration. Papain and pancreatin, are preferred in the practice of this invention; although other proteolytic enzymes, such as bromelain and ficin, will also work.

Some enzyme preparations will also include "activating agents." These activating agents enhance the percutaneous delivery of the drug(s) through the skin. For example, papain from Sigma Chemical may be activated with the chelating agent ethylene-diaminetetraacetic acid ("EDTA") in admixture with cysteine. Such activation of papain increases the penetration of tetraethylammonium bromide (TEAB) through the skin in comparison to use of the papain without such activation. Other possible sources of papain include papain from Allergan. Allergan papain is contained in a contact lens cleaning product (tablets for dissolution in water) wherein activators are included. Amounts of EDTA in a papain enzyme preparation will vary from 2 mg/ml to about 20 mg/ml.

An especially preferred activating agent for use with papain is 0.10 molar cysteine in combination with
0.0375 molar EDTA which activates the papain to achieve
greater penetration of drug through the skin.

Whatever the enzyme or enzymes selected, each is
typically admixed with a liquid, such as water or ethanol
to form an enzyme preparation. The concentration of
enzyme(s) in liquid will be of a sufficient quantity to
increase the skin's permeability to selected drugs over a
predetermined time period. Preferably, the concentration
of enzyme(s) in the enzyme preparation will be great
enough to alter the skin's permeability to the selected
drug in less than 24 hours for practical reasons.
Concentrations of papain in water will generally be
greater than about 0.019 milligrams/milliliter (mg/ml).
Preferred concentrations of papain in water will be less
than about 0.093 mg/ml. Pretreatment of skin samples
with papain concentrations greater than 0.093 mg/ml at pH
7.4 leads to greater drug penetration, but also damages
the skin, possibly leading to bleeding.

Ideally, the enzyme preparations are compounded
to retain stability for long periods of time. Various
factors, such as composition, pH and ionic strength of
the solution, and the chosen solvent, influence the
stability of the enzyme preparations. For maximum
stability, when enzymes such as papain and bromelain are
used, the enzyme preparation should be dissolved in
water, and have a pH of from about 3 to about 10.

Although application of an enzyme alone is
effective, increased concentrations of papain lead to
increased damage to the skin and can cause bleeding.
Further studies have indicated, however, that when a
proteolytic enzyme is admixed with a non-enzymatic
substance there is a further significant increase in
penetration of the drug into the skin. Therefore, lower
concentrations of papain can be used with an increase in
drug absorption. Thus, an alternative embodiment of the
invention includes the admixture of enzyme and non-enzyme
components to enhance permeation of the skin in
anticipation of application of a drug.
Whatever the enzyme or enzymes selected for use in the permeation enhancer embodiment, each is typically admixed with a non-enzyme penetration enhancer such as a lactam compound, (e.g. Azone™), and a liquid, such as propylene glycol, to form an enhancer. The concentration of enzymes(s) in liquid will be of a sufficient quantity to increase the skin's permeability to selected drugs over a predetermined time period.

As used herein, "Azone" is 1-N-dodecylaza-cycloheptan-2-one, available from Nelson Research & Development Co. of Irvine, California. A method of preparing this compound is disclosed in U.S. Patent No. 4,316,893 to Rajadhyaksha in Example 16 (column 11, lines 36-51), Example 10 (column 10, lines 1-19) and Example 8 (column 9, lines 10-49) the contents of which are incorporated by this reference. Concentrations of Azone ranging from about 0.5% to about 10% are useful, although 2% is preferred. All references herein to percentages in a composition are to weight percentages unless indicated otherwise.

Several other lactams which are chemically related to Azone™ may be useful in the practice of this invention. Enzymes are believed to potentiate the ability of lactam compounds to enhance percutaneous penetration of chemical agents. Lactam compounds useful in the instant invention are disclosed in the aforementioned U.S. patents especially U.S. Patent No., 4,316,893 and 3,989,816 to Rajadhyaksha, the contents of which are incorporated by this reference. The preparation of some of these related lactams is disclosed in U.S. Patent No. 4,316,893, Examples 1-23 therein.

The enzyme and lactam compound act to potentiate one another forming a synergistic effect. That is, the permeation enhancement is greater than would be anticipated from the combined individual effect of each agent (see Example 10). Other non-enzyme penetration enhancers which can be used in the admixture include
various alcohols, polyols, sulfoxides, esters, ketones, amides, oleates, various surfactants, alkanoic acids, and alkanols which demonstrate a capability for enhancing permeation. More than one non-enzyme enhancer may be used in combination as a penetration enhancer. As shown by Example 11, studies indicate that these other non-enzyme compounds, in admixture with an enzyme such as papain, can be very effective in increasing permeation of the skin. Those non-enzyme compounds which are water-soluble tend to be more effective in permeation potentiation than non-enzyme compounds which are emulsions. (See Example 11 (8a) and (8b).) The enhancement effect of the non-enzyme component on percutaneous penetration of a drug would be expected to be merely additive. However, as illustrated by Example 11, the enhancement effect of the enzyme and non-enzyme combination causes a dramatic increase in penetration over a mere additive effective.

Preferably, the concentration of enzyme(s) in the enhancer will be great enough to increase the skin's permeability to the selected drug in less than 24 hours, for practical reasons. Concentrations of papain in water will generally be greater than about 0.019 milligrams/milliliter (mg/ml). Preferred concentrations of papain in water will be less than about 0.093 mg/ml. Pretreatment of skin samples with papain concentrations greater than 0.093 mg/ml at pH 7.4 lead to greater drug penetration, but also damage the skin, possibly leading to bleeding.

In addition to the aforementioned non-proteolytic permeation enhancers, other chemical agents which enhance permeation may also be added to the permeation enhancer admixture. Propylene glycol, for example, is a particularly preferred agent since it may also assist in solubilizing the lactam compound(s) in addition to enhancing the skin's permeability to certain drugs.

Solubilizers, such as Spans and Tweens increase the solubility of various components of the enhancers.
into the liquid solvent.

As with preparations of enzyme alone, the percutaneous permeation enhancers are compounded to retain stability for long periods of time. Again, various factors, such as composition, pH of the solution, and choice of solvent, influence the stability of an enzyme preparation. The enzyme preparation should be dissolved in water when papain or bromelain are used, and should have a pH of from about 3 to about 10. The enhancers are also preferably refrigerated before use and kept at temperatures of 3-5°C.

The enzyme or drug preparation may also be stabilized with various preservatives. Considerations in selecting an agent for use as a preservative in the system, as with most pharmaceutical preparations, include: a) the agent's spectrum of activity; b) the agent's stability over time; c) the agent's relative toxicity; d) the agent's allergenic potential; e) the agent's compatibility with the other constituents of the preparation, and f) the agent's odor. Various alcohols, e.g. ethanol or isopropanol, quaternary ammonium surfactants, and other compounds well known to those skilled in the art may be used as a preservative. The United States Pharmacopeia XXXII, page 198; contains a section on stability considerations in compounding at pages 1345 through 1347, the contents of which are incorporated by this reference.

For maximum sustained penetration of drug(s) through treated skin, the pH of the percutaneous permeation enhancer will vary from 5.8 to 8.0, with the preferred pH being about 7.4. (See Example 6)

Although the concentrations of either proteolytic enzyme or enhancers may be decreased if contact time is to be increased, it is generally preferred that certain concentrations be used inasmuch as treatment time may be minimized by use of such concentrations. Long treatment times may be annoying to patients and discourage them from employing a therapeutically necessary treatment.
The preparation of enzyme alone or enhancer preparation can be applied using any of several well-known techniques for applying a liquid to a surface. For example, a brush, swab, or spray container containing the enhancer preparation may be used to apply the preparation to the skin.

In one embodiment of the invention, the enhancer preparation is an admixture of a non-enzymatic compound(s) and proteolytic enzyme(s), and the enhancer preparation is applied directly to the skin surface. In an alternative embodiment, first a non-enzymatic compound dissolved in a solvent is applied, then an enzyme preparation is applied. The order of application of non-enzymatic and enzymatic compounds can be reversed, it only being important that both the compound(s) be in contact with the same area of skin at the same time.

In a particularly preferred embodiment, the enzyme alone or the enhancement preparation is contained within a reservoir of a "patch" which is placed on the animal's skin. As shown in FIG. 12, which is a side view of a typical patch, the patch would include a backing 20, a reservoir 22 to contain the enzyme preparation, a membrane 24 to contain and release the contents of the reservoir, and a protective peel strip 26 or strips.

Patches are typically round or oblong when viewed from above, and are preferably sized to affix neatly to the animal's skin. The membrane 24 is typically rate-controlling or semi-permeable to the contents of the reservoir 22. Adhesive 28 is located around the outer circumference of the patch adjacent to the membrane 24, and a separate, removable, protective peel strip 26 is positioned over the adhesive for removal prior to applying the patch so that the patch will adhere to the animal's skin.

Adhesives are preferably hypoallergenic substances which are useful in retaining the protective peel strips to the patch and the patch to the animal's skin. Preferred adhesives include silicone adhesive
formulations and other adhesives well known to those skilled in the art.

A patch is particularly preferred because the occlusion means are included as part of the system, i.e., the backing 20. Other occlusion means include aluminized plastic, plastic film such as Saran Wrap™, and Biocclusive™ tape. Important factors to consider when selecting occlusion means are a) non-reaction with the contents of the reservoir, b) adequate containment of the contents to the desired localized area of skin, and c) non-reaction with the skin to a detrimental degree. In the case of use with humans, the backing is preferably skin-colored for cosmetic reasons.

When a patch is used as part of the system, the protective peel strip is removed from the patch, and the patch is applied to the skin. The enzyme preparation or the enhancement preparation permeates through the membrane, and contacts the skin. After the skin's structure has been sufficiently altered to allow enhanced passage of the selected drug through the skin, the patch containing the enhancement preparation or the enzyme preparation is removed.

The selected drug or drug preparation, also preferably in a liquid form, is then applied to the skin. The drug or drug preparation is then applied in a similar way as previously described for application of the enzyme or the enhancement preparation. For example, a drug may be contained within the reservoir 22 of a patch which is then applied to the same localized area of skin previously treated with the enzyme preparation or enhancement preparation. Such a reservoir would preferably contain the drug in a sufficient concentration to allow therapeutic levels of the drug to pass through the skin to the animal's circulatory system.

Alternatively, as depicted in FIG. 15, an impermeable membrane 40 can be constructed into the reservoir 22 of the patch to divide the reservoir into two distinct compartments 42, 44. One compartment 42
contains the active ingredient in a dry, stable form, while the other compartment 44 contains the solvent with which the active ingredient is to be admixed. Alternatively (FIG. 16), the dry active ingredient or solvent could be contained within a breakable impermeable container 46 such as a capsule, within the reservoir 22, separating it from the other component. The impermeable membrane or capsule wall could then be purposely ruptured, and the dry active ingredient and solvent mixed to form the particular preparation within the reservoir. The patch is applied to the selected localized area of skin or administration of the particular active ingredient.

FIG. 13 depicts an alternate patch for use with the invention. It, like the patch depicted in FIG. 12, has a protective peel strip 26 which is removed to expose a membrane 24 and backing 20 containing an enzyme preparation contained within reservoir 22. Once the protective peel strip 26 has been removed, the patch depicted in FIG. 13 may be applied to the localized area of skin of the animal for the amount of time needed to increase the skin's permeability to the drug preparation contained within the other reservoir 24. Backing 20, which in this embodiment also serves as a protective peel strip for the reservoir 23 containing the drug preparation, is separated from the second backing 21 of the patch, thus exposing the membrane 25 which, along with the second backing 21, contains the reservoir 23 for the drug preparation. The membrane 25 and backing are then placed on the skin. Adhesive 28 serves both to keep the entire patch together, and to keep the respective portions of the patch attached to the patient's skin.

Drug contained within the drug preparation in reservoir 23 passes through the membrane 25 and onto the patient's enzyme-treated skin. The drug then passes through the skin and into the patient's circulatory system.

Heretofore, those skilled in the art have failed
to realize the full potential of an enzyme preparation's capabilities to enhance the skin's permeability to drugs. Previously, the enzyme preparations and selected drugs were applied simultaneously, or nearly simultaneously and the full enhancement of permeability was not recognized. In contrast, the present system more effectively utilizes the enhancement capabilities of enzymes to allow more precise administration of the drug, thus decreasing a) side effects, b) the amount of drug and enzyme needed, and c) the size of the system. Furthermore, problems of chemical incompatibilities between the drug(s) and the enzyme(s) are lessened so that a potentially greater number of drugs can be administered percutaneously.

Selection of the drug is dependent upon the disease state and animal to be treated. Ideal drugs for percutaneous drug delivery in accordance with the invention include hormones such as progesterone; quaternary compounds such as acetylcholine; and anionic chemicals such as coumadin. Therapeutic classes of drugs for use in the system include anti-hypertensive agents such as beta-blockers, anti-nauseants such as chlorpromazine, and anti-arrythmics, and analgesics.

Polypeptides, such as hormones and the like, are particularly aided in percutaneous delivery by the permeation enhancer invention. Tests performed on pretreated mouse skin indicate that polypeptides of lower molecular weight, ranging from about 800 to about 15,000 in molecular weight, are successfully used in combination with an enzyme/non-enzyme enhancement compound. (See Example 12) There is some indication that polypeptides of higher molecular weight tend to be fractionated when applied to skin pretreated with papain. The exact mechanism of fractionation is unclear, but gel electrophoresis on samples of polypeptides and pure buffered solution diffused through pretreated mouse skin indicated that no endogenous protein is lost from the cells. Examples of polypeptides of lower molecular weight which can be used are insulin and synthetic growth
hormones.

Where active ingredients (i.e., enzymes, lactam compounds or drugs) are not stable in solution for sufficiently long periods of time, the enzyme, enzymes, drug or drugs can be mixed with solvent and then injected with a syringe, or otherwise introduced, into the reservoir of the patch immediately prior to the patch's application to the animal's skin.

The invention allows for the controlled and timed non-invasive release of drugs to the animal. By increasing the concentration of drug in the drug-containing patch, the amount of drug which passes through the skin can be increased and the duration of drug application increased. Furthermore the invention allows for the penetration of larger molecular weight compounds than was previously attainable.

The localized area of the skin to which the enzyme/non-enzyme compounds and drug preparations are applied is preferably a relatively thin layer of skin; other areas of typically thicker skin, such as the sole of the foot, should be avoided. Furthermore, hairy areas of skin should be avoided, or the hair should first be removed.

The system may be present in a kit form.

Included in this kit are means for applying the percutaneous permeation enhancers, occlusion means, and means for applying the selected drug. Means for applying the enhancer and selected drug may be differently marked patches, one patch containing the enzyme preparation and the other patch containing the drug. Different patch marking may include different coloration of the patches and/or the protective peel strips. All components of the kit are contained within a convenient package for use by a medical or veterinary practitioner or patient.

With respect to the preparation of enzyme alone, the kit may include means for mixing the enzyme with solvent such as a small glass container containing a solvent selected for the enzyme; means for mixing the
selected drug(s) with solvent such as a small glass container containing a solvent selected for the drug; and means for introducing active ingredient preparations into the patches such as a needle-bearing syringe. These various means would typically be included with the kit when either or both active ingredients, or constituents thereof, are not stable when premixed in solution for sufficient lengths of time to be useful in a kit.

One embodiment of a percutaneous drug delivery kit is shown in FIG. 14. It includes a first patch 30 containing an enzyme preparation; and a second patch 32 containing a drug selected to be delivered percutaneously, contained within a container 34. Associated with the container are flaps 36A, 36B for retaining the patches 30, 32 within the container 34. The container may contain extension means such as spring 38 to press the patches 30, 32 against the flaps 36A, 36B.

A person using the kit removes the first patch 30 from the container 34 through the flaps 36A, 36B. The flaps 36A, 36B may be made of any material which is flexible, yet strong enough to retain the patches 30, 32 within the container 34 against the force of the spring 38. The first patch is then applied to a localized area of the patient's skin for a predetermined time as previously described. After the predetermined time has passed, the person using the system removes the first patch 30 from the skin. The second patch 32 is then removed from the container 34 and the second patch 32 is then applied to the localized area of the skin for administration of the drug.

As with the kit provided for a preparation of enzyme alone, the kit for the permeation enhancer preparation may include means for mixing the enzyme and non-enzyme components with solvent such as a small glass container containing a solvent selected for optimal use with the enzyme and non-enzyme components; means for mixing the selected drug(s) with solvent such as a small
glass container containing a solvent selected for the drug; and means for introducing active ingredient preparations into the patches such as a needle-bearing syringe. These various means would typically be included in the kit when any, or all, active ingredients or constituents thereof are not stable for sufficient lengths of time when premixed in solution.

While not intending to be bound by one explanation of the invention, the following may be of assistance in understanding the invention.

EXAMPLE I

1A. Enhancement of Model Drugs and Proteins Through Hairless Mouse Skin by a Proteolytic Enzyme (Papain)

Various anionic, cationic, and nonionic chemicals were utilized as model compounds to determine the influence of proteolytic enzymes on the transdermal penetration of these substances. The model chemicals included: $^{14}$C-labeled tetraethylammonium bromide (TEAB), a cationic substance; $^{14}$C-labeled citric acid, an anionic substance; $^{14}$C-labeled glucose, a highly polar nonionic substance; and $^{3}$H-labeled (tritiated) hydrocortisone, a highly nonpolar, nonioinic substance. Additional studies were performed using $^{14}$C-labeled albumin, lysozyme, and insulin to determine the ability of the proteolytic enzymes to enhance the percutaneous absorption of larger molecules.

Enzyme preparations were prepared by dissolving one papain-containing tablet (Allergan, 144 mg) in 2 ml Phosphate Buffer Saline (PBS) solution. Two hundred $\mu$l of this solution was applied to 1 cm$^2$ areas on the back of congenitally athymic hairless mice. The 1 cm$^2$ areas were covered with folded gauze pads to absorb the excess papain solution, then the areas were occluded with a transparent dressing (Biocclusive Tape) and an adhesive
tape. Each mouse was individually housed in separate cages for 24 hours. At the end of this time, residual papain on the surface of the mouse's skin was removed by washing. After cleansing the skin, 150 μl of radio labeled material was applied separately on each marked area of mouse skin and covered with gauze sponge and Biocclusive tape and adhesive tape. Mice were kept in metabolism cages, one animal per cage, and urine samples collected for scintillation counting to determine the quantity of radio labeled materials being excreted. Parallel studies were conducted simultaneously on non-papain treated living mice. These studies served as controls against which the treated animals were measured.

1B. In Vivo Studies on Skin Recovery Following Papain Treatment.

Experiments were conducted to measure the time required for papain-treated mice to return to normal levels of absorption. These experiments were conducted as described in Example 1A, except that following the removal of papain from the mouse's skin, the mice were maintained separately in metabolism cages for varying periods of time prior to the application of the labeled model compounds. After various time intervals, 150 μl of radio labeled chemicals were applied separately on the 1 cm² marked areas on the backs of the mice where papain had previously been applied. The mice were then kept in separate metabolism cages, and at time intervals of 17, 41, and 65 hours, urine was collected and counted using a scintillation counter.

The results of the experiments described in Examples 1A and 1B are presented in the following Table and in FIGS. 1-7.
<table>
<thead>
<tr>
<th>Substance</th>
<th>Time</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric Acid</td>
<td>Immediate</td>
<td>4.3%</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>7 Days</td>
<td>0.2%</td>
</tr>
<tr>
<td>Citric Acid Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25%</td>
<td>Immediate</td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Immediate</td>
<td>1.8%</td>
</tr>
<tr>
<td>Glucose</td>
<td>7 days</td>
<td>0.1%</td>
</tr>
<tr>
<td>Glucose Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td>Immediate</td>
<td>0.07%</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>Immediate</td>
<td>24.7%</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>7 days</td>
<td>0.6%</td>
</tr>
<tr>
<td>Hydrocortisone Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td>Immediate</td>
<td>2.5%</td>
</tr>
<tr>
<td>TEAB</td>
<td>Immediate</td>
<td>35.4%</td>
</tr>
<tr>
<td>TEAB</td>
<td>7 days</td>
<td>5.3%</td>
</tr>
<tr>
<td>TEAB Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td>Immediate</td>
<td>6.2%</td>
</tr>
<tr>
<td>Albumin</td>
<td>Immediate</td>
<td>2.7%</td>
</tr>
<tr>
<td>Albumin</td>
<td>7 days</td>
<td>0.0%</td>
</tr>
<tr>
<td>Albumin Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td>Immediate</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Immediate</td>
<td>16.0%</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>7 days</td>
<td>0.0%</td>
</tr>
<tr>
<td>Lysozyme Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No Papain Treatment)</td>
<td>Immediate</td>
<td>0.0%</td>
</tr>
<tr>
<td>Insulin</td>
<td>Immediate</td>
<td>6.0%</td>
</tr>
<tr>
<td>Insulin</td>
<td>7 days</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
 EXAMPLE 2

In Vivo Studies Using Papain-Treated Athymic Nude Mice Skin Immediately and After One or Two Days Recovery

Because of some complications encountered during studies utilizing papain treatment by in vivo method (e.g. extended excretion due to storage in various body compartments, delayed response time, etc.) it was decided that additional studies should be conducted wherein the mouse skin was allowed to recover in situ, then excised after various time intervals and evaluated by in vitro permeation methods.

These experiments were conducted by preparing, applying, and occluding papain solutions onto the backs of congenitally athymic hairless mice in the same manner as described in Example 1A. At the end of the 24-hour papain treatment, the skin was again cleansed of excess solution. Skin was then excised from part of the animals. The remainder of the animals were housed individually in regular cages for various periods of time (24 hours and 48 hours), after which the treated areas were excised. Immediately following excision of the treated areas, the skins were mounted over Franz-Type, Single-Compartment Diffusion Cells. Four hundred μl of saturated solutions of radio labeled citric acid, TEAB, glucose, and hydrocortisone were applied, individually, to the exposed surface of the mounted skins on the donor chamber. Saturated solutions of each of these substances were used in order to ensure maximum availability of labeled substances. At varying time intervals, 100 μl samples were removed from the receiver chambers for scintillation counting. All experiments were conducted at 25°C.

FIGS. 8-11 show the saturation concentration of each substance tested, and the flux, permeability
coefficient, and graph for each of the substances at the first day (immediately following 24 hours exposure to papain), second day (24 hours following 24-hour exposure to papain), and third day (48 hours following 24-hour exposure to papain).

It can readily be observed that upon only 7 hours exposure, the transdermal penetration of citric acid is about 55 mg from the 1 cm² section of skin; TEAB is nearly 19 mg, glucose is about 22 mg, and hydrocortisone about 125 μg. Experiments performed after a 48-hour recovery period (3rd day experiments) indicated almost complete skin recovery occurs to pretreatment permeability, as judged by percutaneous data. The low absolute quantity of hydrocortisone (125 μg) is probably due to the low solubility of hydrocortisone, even in ethanol, which is the solvent used for hydrocortisone in these examples. All other substances were dissolved in water.

EXAMPLE 3

Hairless mouse skin was pretreated with papain at a concentration of 0.093 mg/ml at pH 7.4 for 24 hours. Afterwards, TEAB was applied. The pretreated skin demonstrated a 33-fold increase in absorption of the TEAB in comparison to normal, untreated mouse skin.

EXAMPLE 4

An enzyme preparation especially useful with ionic drugs (e.g. quaternaries such as acetylcholine chloride; cationics such as alprenolol; and anionics such as warfarin) has the following composition, with a pH adjusted to 7.4:

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain (sigma)</td>
<td>0.093 mg/ml</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.10 M</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.0375 M</td>
</tr>
<tr>
<td>Water</td>
<td>qs</td>
</tr>
</tbody>
</table>

SUBSTITUTE SHEET
EXAMPLE 5

The absolute amounts and relative concentrations of an enzyme preparation containing papain to determine skin damage as measured by bleeding of mouse skin was determined.

A. Absolute amount of papain in water and time until bleeding observed

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Time of Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.070 mg/0.250 ml</td>
<td>Observe bleeding at about 0.5 hrs.</td>
</tr>
<tr>
<td>0.050 mg/0.180 ml</td>
<td>Observe bleeding at about 1.5 hrs.</td>
</tr>
<tr>
<td>0.030 mg/0.108 ml</td>
<td>Observe bleeding at about 5.0 hrs.</td>
</tr>
<tr>
<td>0.010 mg/0.108 ml</td>
<td>Do not observe any bleeding for 24 hrs.</td>
</tr>
</tbody>
</table>

B. Concentration of papain in water and time until bleeding observed.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Time of Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.010 mg/0.108 ml</td>
<td>Do not observe bleeding for 24 hrs.</td>
</tr>
<tr>
<td>0.020 mg/0.108 ml</td>
<td>Do not observe bleeding for 24 hrs.</td>
</tr>
<tr>
<td>0.030 mg/0.108 ml</td>
<td>Bleeding more or less after 24 hrs.</td>
</tr>
<tr>
<td>0.040 mg/0.108 ml</td>
<td>Bleeding more or less after 24 hrs.</td>
</tr>
</tbody>
</table>

EXAMPLE 6

A. Effect of pH on drug penetration through normal untreated hairless mouse skin.

Drug penetration through a skin barrier is thought to be a process of passive diffusion and may be described by Fick's First Law:

\[ J = -D \frac{dC}{dx} \]

where \( J \) is the flux of a drug through a skin barrier, \( D \) is the diffusion constant of a drug in the skin barrier, and \( dC/dX \) is the concentration gradient of a drug between the vehicle and the skin barrier.

For steady state diffusion:

\[ J = \frac{DK}{h} C_V = PC_V \]

where \( K \) is the partition coefficient of drug between a skin barrier and the vehicle, \( C_V \) is the concentration of
drug dissolved in the vehicle, \( h \) is the thickness of the skin barrier, and \( P \) is the permeability of a drug through the skin barrier.

5

Enhancement of TEAB Penetration Through Hairless Mouse Skin Using Enzyme Component Alone

1. Without papain pretreatment
   \[ J = 2.72 \times 10^{-5} \text{ mg/cm}^2/\text{sec} \]
   \[ P = 2.54 \times 10^{-8} \text{ cm/sec} \]

2. Pretreated with Sigma papain of concentration 0.010 mg/0.108 ml (0.093 mg/ml)
   \[ J = 6.87 \times 10^{-4} \text{ mg/cm}^2/\text{sec} \]
   \[ P = 0.85 \times 10^{-6} \text{ cm/sec} \]

   \[
   \text{Ratio of enhancement} = \frac{P \text{ with enhancer}}{P \text{ without enhancer}} = \frac{0.85 \times 10^{-6}}{2.54 \times 10^{-8}} \times 100\% = 33
   \]

3. Pretreated with Allergan papain of concentration 0.170 mg/0.200 ml (0.850 mg/ml)
   \[ J = 1.04 \times 10^{-3} \text{ mg/cm}^2/\text{sec} \]
   \[ P = 1.29 \times 10^{-6} \text{ cm/sec} \]

   \[
   \text{Ratio of enhancement} = \frac{1.29 \times 10^{-6}}{2.54 \times 10^{-8}} \times 100\% = 5070\%
   \]

4. Pretreated with Sigma papain of concentration 0.170 mg/0.602 ml (0.278 mg/ml)
   \[ J = 1.46 \times 10^{-3} \text{ mg/cm}^2/\text{sec} \]
   \[ P = 1.81 \times 10^{-6} \text{ cm/sec} \]

   \[
   \text{Ratio of enhancement} = \frac{1.81 \times 10^{-6}}{2.54 \times 10^{-8}} = 100\% = 7126\%
   \]
Effect of pH on drug penetration through normal untreated hairless mouse skin.

<table>
<thead>
<tr>
<th>pH</th>
<th>J&amp;P Drug (Class)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>Acetylcholine Chloride (quaternary) J=5.83x10^{-6} mg/cm^2/sec, P=2.91x10^{-8} cm/sec</td>
</tr>
<tr>
<td>6.6</td>
<td>J=5.21x10^{-6} mg/cm^2/sec, P=2.60x10^{-8} cm/sec</td>
</tr>
<tr>
<td>7.4</td>
<td>J=6.41x10^{-6} mg/cm^2/sec, P=3.20x10^{-8} cm/sec</td>
</tr>
<tr>
<td>8.0</td>
<td>J=9.32x10^{-6} mg/cm^2/sec, P=4.66x10^{-8} cm/sec</td>
</tr>
<tr>
<td>10</td>
<td>Progesterone (nonionic) J=2.30x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=2.88x10^{-6} cm/sec</td>
</tr>
<tr>
<td>15</td>
<td>J=2.34x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=2.92x10^{-6} cm/sec</td>
</tr>
<tr>
<td>20</td>
<td>J=2.34x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=2.92x10^{-6} cm/sec</td>
</tr>
<tr>
<td>25</td>
<td>Warfarin (anionic) J=1.66x10^{-8} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=1.66x10^{-7} cm/sec</td>
</tr>
<tr>
<td>30</td>
<td>J=9.16x10^{-9} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=9.16x10^{-8} cm/sec</td>
</tr>
<tr>
<td>35</td>
<td>J=4.68x10^{-9} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=4.68x10^{-8} cm/sec</td>
</tr>
<tr>
<td>40</td>
<td>J=4.73x10^{-9} mg/cm^2/sec</td>
</tr>
</tbody>
</table>

B. Effect of pH on drug penetration through hairless mouse skin pretreated with Sigma papain of concentration 0.09 mg/ml for 24 hours

<table>
<thead>
<tr>
<th>pH</th>
<th>J&amp;P Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.9</td>
<td>Acetylcholine Chloride J=6.00x10^{-4} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=3.00x10^{-6} cm/sec</td>
</tr>
<tr>
<td>6.6</td>
<td>J=7.77x10^{-4} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=3.88x10^{-6} cm/sec</td>
</tr>
<tr>
<td>7.4</td>
<td>J=6.46x10^{-4} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=3.23x10^{-6} cm/sec</td>
</tr>
<tr>
<td>8.0</td>
<td>J=6.28x10^{-4} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=3.14x10^{-6} cm/sec</td>
</tr>
<tr>
<td>45</td>
<td>Progesterone J=2.88x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>J=3.51x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td>50</td>
<td>J=2.73x10^{-7} mg/cm^2/sec</td>
</tr>
<tr>
<td></td>
<td>P=2.89x10^{-7} cm/sec</td>
</tr>
</tbody>
</table>

E = 103.09  E = 149.23  E = 100.94  E = 67.38

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EXAMPLE 7

Several experiments were performed to determine the enhancement of TEAB penetration through mouse skin with the use of an enzyme preparation containing papain.

A. As shown in FIG. 17, the amount of drug penetration through mouse skin in the depicted case TEAB, varies directly with the amount of papain used. In this experiment, varying amounts of Sigma papain, activated with cysteine and EDTA, were used. The percent of TEAB penetration through the skin increased as the amount of activated papain present in the pretreatment enzyme preparation increased.

B. As shown in FIG. 18 TEAB penetration through mouse skin increases as the amount of papain in the enzyme preparation used to pretreat the skin increases. FIG. 18 also shows that very small amounts of an enzyme in the enzyme preparation are useful in enhancing drug penetration through the skin, and that without the enzyme, drug penetration is not enhanced.

C. As shown in FIG. 19, TEAB penetration is further enhanced by even greater levels of papain in the
enzyme preparation pretreatment solution. In the depicted case, Sigma papain of 0.170 mg, 0.113 mg, and 0.057 mg in 0.4–0.6 ml of water were first applied to mouse skin, each with cysteine and EDTA as activating agents.

This example also shows that certain enzyme preparations, such as those containing papain from the Sigma Chemical Company of St. Louis, Missouri preferably include an activating agent such as EDTA and/or cysteine.

EXAMPLE 8

As shown in FIG. 20, two different sources of papain were used in enhancing the penetration of an anionic chemical, citric acid, through mouse skin. Each enzyme preparation contained 0.170 mg of papain dissolved in 0.1 ml of water. One sample of papain was obtained from Allergan. The other was obtained from Sigma Chemical. Penetration of the anionic chemical was enhanced to a greater extent by the Allergan papain than that of Sigma Chemical. The Sigma Chemical papain was not activated in this experiment.

EXAMPLE 9

Preparation of Permeation Enhancer

A percutaneous permeation enhancer having the following formula was prepared:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain (Allergan)</td>
<td>0.0465 mg/ml</td>
</tr>
<tr>
<td>Azone™</td>
<td>2%</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>15%</td>
</tr>
<tr>
<td>Tween 20</td>
<td>qs to solubilize Azone™</td>
</tr>
<tr>
<td>Normal Saline Solution</td>
<td>gs ad 20 ml</td>
</tr>
</tbody>
</table>

The Tween 20 was used to solubilize the Azone™ in the admixture.

EXAMPLE 10

The effect of the percutaneous enhancer, as prepared in...
Example II above, was measured on the penetration of the quaternary compound acetylcholine chloride through hairless mouse skin at pH 7.4. The addition of Azone allowed the concentration of papain in the enhancer to be halved which minimized papain's irritating effect on the skin. The results showed that while an eighty-eight fold increase in penetration of acetylcholine chloride was obtained by papain pretreatment alone, and a seven fold increase by Azone™ and propylene glycol alone, a 110 fold increase was achieved by the use of a combination of Azone™ propylene glycol and papain. The data below indicates a potentiating or synergistic effect in combining the agents for use as an enhancer.

Enhancement effect of 0.0465 mg/ml Sigma papain alone and in combination with 2% Azone™ and 15% propylene glycol on the penetration of acetylcholine chloride through hairless mouse skin at pH 7.4.

1. Pretreated with 0.0465 mg/ml papain alone.
   \[ J = 5.67 \times 10^{-4} \text{mg/cm}^2/\text{sec} \]
   \[ P = 2.83 \times 10^{-6} \text{cm/sec} \]
   \[ E = 88.4 \]

2. Pretreated with 2% Azone™ and 15% propylene glycol alone
   \[ J = 4.57 \times 10^{-5} \text{mg/cm}^2/\text{sec} \]
   \[ P = 2.29 \times 10^{-7} \text{cm/sec} \]
   \[ E = 7.2 \]

3. Pretreated with a combination of 0.0465 mg/ml sigma papain and 2% Azone™ and 15% propylene glycol
   \[ J = 7.01 \times 10^{-4} \text{mg/cm}^2/\text{sec} \]
   \[ P = 3.51 \times 10^{-6} \text{cm/sec} \]
   \[ E = 109.7 \]
Effect of Various Non-Enzyme Components in Admixture With Papain on Penetration of Tetraethylammonium bromide

1. Results on the penetration of $^{14}$C-TEAB through normal hairless mouse skin (placebo, n=3)

$$J = (4.02 \pm 2.13) \times 10^{-6} \text{ mg cm}^{-2} \text{s}^{-1}$$

$$P = (4.95 \pm 2.62) \times 10^{-9} \text{ cm s}^{-1}$$

2. Results on the penetration of $^{14}$C-TEAB through hairless mouse skin pretreated with 0.0465 mg/ml papain for 24 hours (n=3)

$$J = (1.33 \pm 0.48) \times 10^{-4} \text{ mg cm}^{-2} \text{s}^{-1}$$

$$P = (1.63 \pm 0.59) \times 10^{-7} \text{ cm s}^{-1}$$

$$E = 32.93$$

3a. Results on the enhancement effect of 5% Brij 96 alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

$$J = 3.10 \times 10^{-5} \text{ mg cm}^{-2} \text{s}^{-1}$$

$$P = 3.81 \times 10^{-8} \text{ cm s}^{-1}$$

$$E = 7.70$$

3b. Results on the enhancement effect of 5% Brij 96/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

$$J = (4.14 \pm 0.65) \times 10^{-4} \text{ mg cm}^{-2} \text{s}^{-1}$$

$$P = (5.09 \pm 0.81) \times 10^{-7} \text{ cm s}^{-1}$$

$$E = 102.83$$

4a. Results on the enhancement effect of 25% EtAc/25% EtOH alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

$$J = 3.68 \times 10^{-6} \text{ mg cm}^{-2} \text{s}^{-1}$$

$$P = 4.54 \times 10^{-9} \text{ cm s}^{-1}$$

$$E = 0.92$$
4b. Results on the enhancement effect of 25% EtAc/25% EtOH/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n = 2)

\[ J = (4.34 \pm 0.01) \times 10^{-4} \text{ mg cm}^{-2}\text{s}^{-1} \]

\[ P = (5.34 \pm 0.01) \times 10^{-7} \text{ cm s}^{-1} \]

\[ E = 107.88 \]

5a. Results on the enhancement effect of 40% EtOH/10% PG alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

\[ J = 1.54 \times 10^{-5} \text{ mg cm}^{-2}\text{s}^{-1} \]

\[ P = 1.90 \times 10^{-8} \text{ cm s}^{-1} \]

\[ E = 3.84 \]

5b. Results on the enhancement effect of 40% EtOH/10% PG/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

\[ J = (2.13 \pm 0.82) \times 10^{-4} \text{ mg cm}^{-2}\text{s}^{-1} \]

\[ P = (2.63 \pm 1.01) \times 10^{-7} \text{ cm s}^{-1} \]

\[ E = 53.13 \]

6a. Results on the enhancement effect of 2% dioxolane alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n = 1)

\[ J = 4.23 \times 10^{-6} \text{ mg cm}^{-2}\text{s}^{-1} \]

\[ P = 5.21 \times 10^{-9} \text{ cm s}^{-1} \]

\[ E = 1.05 \]

6b. Results on the enhancement effect of 2% dioxolane/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

\[ J = (1.65 \pm 1.00) \times 10^{-4} \text{ mg cm}^{-2}\text{s}^{-1} \]

\[ P = (2.03 \pm 1.23) \times 10^{-7} \text{ cm s}^{-1} \]

\[ E = 41.01 \]
7a. Results on the enhancement effect of 10% caprylic/capric triglyceride alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

\[
J = 1.11 \times 10^{-5} \text{ mg cm}^{-2} \text{s}^{-1}
\]

\[
P = 1.37 \times 10^{-8} \text{ cm s}^{-1}
\]

\[
E = 2.77
\]

7b. Results on the enhancement effect of 10% caprylic/capric triglyceride/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

\[
J = (1.00 \pm 0.28) \times 10^{-4} \text{ mg cm}^{-2} \text{s}^{-1}
\]

\[
P = (1.23 \pm 0.35) \times 10^{-7} \text{ cm s}^{-1}
\]

\[
E = 24.85
\]

8a. Results on the enhancement effect of 10% oleyl alcohol alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

\[
J = 1.49 \times 10^{-4} \text{ mg cm}^{-2} \text{s}^{-1}
\]

\[
P = 1.83 \times 10^{-7} \text{ cm s}^{-1}
\]

\[
E = 36.97
\]

8b. Results on the enhancement effect of 10% oleyl alcohol/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

\[
J = (1.46 \pm 0.44) \times 10^{-4} \text{ mg cm}^{-2} \text{s}^{-1}
\]

\[
P = (1.80 \pm 0.54) \times 10^{-7} \text{ cm s}^{-1}
\]

\[
E = 36.36
\]

9a. Results on the enhancement effect of 1% n-decyl methyl sulfoxide alone on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=1)

\[
J = 4.35 \times 10^{-6} \text{ mg cm}^{-2} \text{s}^{-1}
\]

\[
P = 5.35 \times 10^{-9} \text{ cm s}^{-1}
\]

\[
E = 1.08
\]
9b. Results on the enhancement effect of 1% n-decyl methyl sulfoxide/0.0465 mg/ml papain combination on the penetration of $^{14}$C-TEAB through hairless mouse skin (n=2)

$$J = (1.98 \pm 0.38) \times 10^{-4} \text{ mg cm}^{-2}\text{s}^{-1}$$

$$P = (2.44 \pm 0.46) \times 10^{-7} \text{ cm s}^{-1}$$

$$E = 49.31$$

EXAMPLE 12

Fourier Transform Infrared Spectroscopy Studies

A. Comparisons of normal stratum corneum (from mouse skin) to stratum corneum treated with a 0.09% concentration of papain for 24 hours was performed using Fourier Transform Infrared spectroscopy (FTIR). The results showed significant changes occurring in the amide III, beta sheet, and alpha-helix formations of the stratum corneum cells. It is postulated that a shift from a random form of protein to the more organized beta sheet structure occurs. The alpha-helix structures of skin protein could be fractionated, uncoiled, or both. Removal of papain from the mouse showed partial recovery in 24 hours and nearly complete recovery in 48 hours.

FTIR absorbance spectra of pure cast papain showed slight, but significantly different peak positions than papain treated skin and included a peak at 1516 cm$^{-1}$ which did not appear in the treated stratum corneum. This data seems to indicate a complete removal of the papain from the treated stratum corneum, and the FTIR structural data of the skin represent changes only in the protein of the stratum corneum.

A comparison of symmetric and asymmetric CH stretch bands was then made. These two bands represent lipid structure and would show any changes in the stratum corneum lipids caused by the papain. Peak position of these two bands as well as the length of a horizontal line drawn at 70% of peak height showed that there was no change in lipid structure.

B. Azone$^{TM}$ was tested as a permeation enhancer
with and without the presence of papain. When papain was present the changes in protein FTIR areas were not significantly different than those observed with papain alone. Azone™ by itself did not change protein FTIR areas. When Azone™ was present it apparently caused a lengthening of both symmetric and asymmetric CH stretch lines but did not change their peak positions.

**TABLE I**

Comparisons of untreated, papain-treated, Azone™-treated and papain plus Azone™-treated stratum corneum ("SC") are shown below as peak positions or as length of the 70% line.

<table>
<thead>
<tr>
<th>Absorbance in protein areas (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>1456</td>
</tr>
<tr>
<td>1400.5</td>
</tr>
<tr>
<td>1244</td>
</tr>
<tr>
<td>1267</td>
</tr>
<tr>
<td>1295</td>
</tr>
</tbody>
</table>

Azone™ by itself did not significantly effect the protein absorbance areas.

**TABLE II**

Absorbance in lipid areas (cm⁻۶)

<table>
<thead>
<tr>
<th>Absorbance in lipid areas (cm⁻۶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Symmet CH</td>
</tr>
<tr>
<td>Asymmet CH</td>
</tr>
</tbody>
</table>

No significant difference in peak positions.
**TABLE III**

Length of the 70% line in the lipid areas (cm⁻¹)

<table>
<thead>
<tr>
<th>treated</th>
<th>Untreated</th>
<th>Papain Treated</th>
<th>Azone™ Treated</th>
<th>Papain plus Azone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symmet CH</td>
<td>8.6</td>
<td>9.1</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td>Asymmet CH</td>
<td>17.5</td>
<td>17.5</td>
<td>27.2</td>
<td>21.1</td>
</tr>
</tbody>
</table>

70% line lengthened when Azone™ was present.

**TABLE IV**

Overall comparisons of FTIR spectra in Amide III region of untreated, papain treated, and papain treated recovered Data reported as cm⁻¹.

<table>
<thead>
<tr>
<th>Untreated</th>
<th>Papain treated</th>
<th>24 hr. rec</th>
<th>48 hr rec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significance</td>
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<td></td>
<td>Amide Beta</td>
</tr>
<tr>
<td>III</td>
<td>1244</td>
<td>1236</td>
<td>1242.4</td>
</tr>
<tr>
<td>sheet</td>
<td>1267</td>
<td>1262</td>
<td>1271.8</td>
</tr>
<tr>
<td></td>
<td>1295</td>
<td>1288</td>
<td>1301.7</td>
</tr>
</tbody>
</table>

**TABLE V**

Lipid Correlation Data for Papain

Peak Positions in cm⁻¹

<table>
<thead>
<tr>
<th></th>
<th>Symmetric CH</th>
<th>Asymmetric CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal SC</td>
<td>2848.3</td>
<td>2916.5</td>
</tr>
<tr>
<td>Treated SC</td>
<td>2848.5</td>
<td>2916.3</td>
</tr>
<tr>
<td>48 hr recovery SC</td>
<td>2849.3</td>
<td>2916.5</td>
</tr>
</tbody>
</table>

**SUBSTITUTE SHEET**
TABLE VI

Length of 70% line in cm⁻¹

| Normal SC | 17.5 | 8.6 |
| Treated SC | 17.5 | 9.1 |
| 48 hr recovery SC | 19.3 | 10.5 |

TABLE VII

Data of zone exposed skin in cm⁻¹

<table>
<thead>
<tr>
<th>Symm CH</th>
<th>Asymm CH</th>
<th>Asymm 70%</th>
<th>Symm 70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azone exp SC</td>
<td>2848.3</td>
<td>2916.5</td>
<td>27.2</td>
</tr>
<tr>
<td>Azone plus papain exp SC</td>
<td>2848.3</td>
<td>2916.5</td>
<td>21.1</td>
</tr>
</tbody>
</table>

Peak positions did not change but length of the 70% line did change.

For Asymmetric CH streach 70% line

| Normal SC | 17.5 |
| Azone™ treated SC | 27.2 |
| Azone™ plus papain treated SC | 21.1 |

For symmetric CH streach 70% line

| Normal SC | 8.6 |
| Azone™ treated SC | 11.4 |
| Azone™ plus papain treated SC | 11.4 |

No significant changes occurred in the protein areas when azone was used as the only permeation enhancer.

EXAMPLE 13

Penetration of Polypeptides through Pretreated Skin

After papain skin treatment pursuant to Example III.1 above, polypeptides were diffused through the pretreated mouse skin using the same concentration and pH values for each test. Pure buffer solution was also
diffused through the pretreated skin of the mouse to determine if any endogenous proteins diffused into the diffusate. This showed that the compounds measured originated only from the polypeptides.

Comparisons of solutions of non-diffused polypeptides, diffused polypeptides and diffused buffer solutions were made using gel electrophoresis. The system used for these determinations was sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), a procedure well-known in the art. These comparisons determined if fragmentation of the diffused polypeptide molecules occurred.

A series of polypeptides of various molecular weights were studied.

The following peptides were used:

1. Bovine albumin    MW 69,000
2. Carbonic anhydrase MW 29,000
3. Lysozyme         MW 14,400
4. Insulin          MW 6,000

RESULTS

**BSA (bovine serum albumin)**

The BSA that diffused through the pretreated mouse skin was fractionated to a great extent. The non-diffused BSA showed a distinct stained band within the gel while the diffused BSA showed small extended bands indicating compounds of smaller and varying molecular weights. There were no detectable endogenous compounds that diffused out of the papain-treated skin when buffer solution was used as the only diffusing medium.

**Carbonic Anhydrase**

This molecule fractionated during the permeation process. Here a comparison of a solution of carbonic anhydrase and the same molecule that remained in the donor solution of the diffusion cell was made to determine if exposure of the molecule to the papain treated skin without diffusion would cause any change in the carbonic anhydrase molecule. There appeared to
be no change or breakdown of the molecule due to exposure of the papain treated skin without diffusion.

**Lysozyme**

Comparisons of solutions of non-diffused lysozyme, non-diffused lysozyme from the donor cell and diffused lysozyme showed no significant difference in molecular size. The molecule remained intact and did not break apart.

**Insulin (regular, bovine)**

Electrophoresis of a solution of insulin and the diffused insulin produced extended stained areas in the gel. It would seem that the electrophoretic process caused considerable agglutination and the determination of molecular fragmentation due to the permeation would be very difficult if not impossible.

The preceding examples are exemplary and are not intended to limit the scope of the appended claims which define the invention.
What is Claimed:

1. A method of delivering a drug through the skin of an animal comprising:
   applying an enzyme preparation to a localized area of the skin of said animal for a predetermined amount of time and in a sufficient quantity to alter the structure of the localized area of skin;
   occluding the localized area of skin with occlusion means during said predetermined amount of time;
   removing said occlusion means; and
   applying an effective amount of the drug to the localized area of the skin.

2. The method according to Claim 1 further including the step of occluding the localized area of skin after application of the drug.

3. The method according to Claim 1 wherein said predetermined amount of time exceeds 24 hours.

4. The method according to Claim 3 wherein said enzyme preparation contains papain and a buffered saline solution.

5. The method according to Claim 4 wherein said drug is a cationic chemical.

6. The method according to Claim 4 wherein said drug is an anionic chemical.

7. The method according to Claim 4 wherein said drug is a highly polar nonionic chemical.

8. The method according to Claim 4 wherein said drug is a highly nonpolar, nonionic chemical.
9. The method according to Claim 4 wherein said drug is a proteinaceous chemical.

10. The method according to Claim 1 further including substantially removing the enzyme preparation from the skin before applying the drug to the localized area of skin.

11. A percutaneous drug delivery system comprising:
contact means for contacting an enzyme preparation to a localized area of skin;
means for occluding said localized area of skin in contact with said contact means; and
means for applying a drug to said localized area of skin after said contact means and said occlusion means have been removed from said localized area of skin, wherein said contact means and said means for applying a drug are different patches.

12. The percutaneous drug delivery system of Claim 11 wherein said patches are differently marked.

13. The percutaneous drug delivery system of Claim 11 wherein said patches are removably contained within a container so that the contact means is removed before the means for applying a drug can be removed.

14. A dual-patch, percutaneous drug delivery device comprising:
a first absorbent patch sized and adapted to contact a localized area of skin, said first patch containing an effective amount of a proteolytic enzyme; and
a second absorbent patch substantially of similar size to said first patch and adapted to contact a localized area of skin, said second patch containing an effective amount of a preselected drug, wherein said first and second patches are attached to a
common structure.

15. A dual-patch, percutaneous drug delivery kit comprising:

a first absorbent patch sized and adapted to contact a localized area of skin, said first patch containing an effective amount of a proteolytic enzyme and having a protective membrane over said patch structured to prevent loss of said enzyme during storage;

a second absorbent patch sized and adapted to contact a localized area of skin, said second patch having a reservoir containing drug and having a protective membrane over said reservoir to prevent loss of drug during storage; and

kit structure adapted to receive said first and second patches serially so that said second patch is removable only after removal of said first patch.

16. A method of delivering a drug through the skin of an animal comprising:

applying a percutaneous permeation enhancer comprising effective amounts of a combination of enzymatic and non-enzymatic permeation enhancers to a localized area of the skin of said animal for a predetermined sufficient amount of time and in a sufficient quantity to enhance the permeability of the localized area of skin to the drug;

occluding the localized area of skin with occlusion means during said predetermined amount of time;

removing said occlusion means; and

applying an effective amount of the drug to the localized area of skin so that at least some of the drug permeates through the skin.

17. The method of Claim 16 wherein the effective amounts of enzymatic and non-enzymatic permeation enhancers are in admixture with one another before application to the
localized area of the skin.

18. The method according to Claim 17 further including the step of occluding the localized area of skin after application of the drug.

19. The method according to Claim 17 wherein said predetermined amount of time is less than 24 hours.

20. The method according to Claim 19 wherein said non-enzymatic permeation enhancer is a lactam compound and said enzymatic permeation enhancer contains papain and a buffered saline solution.

21. The method according to Claim 20 wherein said lactam compound is 1-n-Dodecylazacycloheptan-2-one.

22. The method according to Claim 20 wherein said drug is a quaternary compound.

23. The method according to Claim 20 wherein said drug is a highly polar nonionic chemical.

24. The method according to Claim 20 wherein said drug is a highly nonpolar, nonionic chemical.

25. The method according to Claim 24 wherein said lactam compound is a 1-substituted-azacycloheptan-2-one enhancer.

26. The method according to Claim 16 further including substantially removing the percutaneous permeation enhancer from the skin before applying the drug to the localized area of skin.

27. The method of claim 19 wherein said non-enzymatic permeation enhancer is a water-soluble compound and said enzymatic permeation enhancer contains papain and
a buffered saline solution.

28. The method of claim 27 wherein said non-enzymatic permeation enhancer is the surfactant Brij 96.

29. The method of claim 27 wherein said non-enzymatic permeation enhancer is a combination of ethyl acetate and ethyl alcohol.

30. The method of claim 27 wherein said non-enzymatic permeation enhancer is ethyl alcohol.

31. The method of claim 27 wherein said non-enzymatic permeation enhancer is dioxolane.

32. The method of claim 27 wherein said non-enzymatic permeation enhancer is a combination of caprylic acid and capric triglyceride.

33. The method of claim 27 wherein said non-enzymatic permeation enhancer is n-decyl methyl sulfoxide.

34. A percutaneous permeation enhancer comprising: an enzyme preparation containing a proteolytic enzyme in sufficient quantity and concentration to enhance the penetration of a chemical agent through skin in admixture with a potentiating amount of a lactam compound for enhancing the penetration of said chemical agent through said skin.

35. The percutaneous permeation enhancer of Claim 34 wherein said lactam compound is a 1-substituted-azacycloheptan-2-one.

36. The percutaneous permeation enhancer of Claim 34 wherein said lactam compound is 1-n-Dodecylazacycloheptan-2-one.
37. The percutaneous permeation enhancer of Claim 36 further including a solubilizer.

38. The percutaneous permeation enhancer of Claim 37 further including propylene glycol in admixture with said enzyme preparation and lactam compound, said propylene glycol being in a sufficient quantity and concentration to further enhance the penetration of said chemical through said skin.

39. The percutaneous permeation enhancer of Claim 38 wherein said solubilizer is Tween 20.

40. The percutaneous permeation enhancer of Claim 39 wherein said enzyme is papain.

41. The method of claim 16 wherein said drug is a polypeptide.

42. The method of claim 41 wherein said polypeptide has a molecular weight less than 15,000.

43. A method of enhancing a localized area of skin's permeability to a chemical, said method comprising: applying a patch to the localized area of skin for a predetermined amount of time, said patch of the type comprising a backing and a membrane affixed to said backing so as to define a pouch for a reservoir, said reservoir containing a percutaneous permeation enhancer compounded to enhance the skin's permeability to the chemical within the predetermined amount of time.

44. The method of claim 43 wherein said percutaneous permeation enhancer is an admixture of effective amounts of activated papain, propylene glycol, 1-n-dodecylazacyclo-heptan-2-one, Tween 20, and normal saline.
45. The method of claim 44 wherein said activated papain comprises a mixture of papain, a chelating agent, and cysteine.

46. The method of claim 20 wherein said papain is combined with a chelating agent and cysteine.

47. The method of claim 46 wherein said chelating agent is ethylene diaminetetraacetic acid.

48. The method of claim 27 wherein said papain is combined with a chelating agent and cysteine.

49. The method of claim 48 wherein said chelating agent is ethylene diaminetetraacetic acid.

50. The method of claim 20 wherein said papain has a concentration of about 0.019 mg/ml to about 0.093 mg/ml.

51. The method of claim 50 wherein said papain has a concentration of about 0.019 mg/ml to about 0.093 mg/ml.
Fig. 1
URINARY RECOVERY OF $^{14}$C-CITRIC ACID

% RECOVERY

CITRIC ACID WITH PAPAIN TREATMENT

7 DAYS AFTER TREATMENT WITHOUT TREATMENT

TIME (HOURS)

Fig. 2

URINARY RECOVERY OF $^{14}$C-GLUCOSE

% RECOVERY

GLUCOSE, WITH PAPAIN TREATMENT

GLUCOSE, 5 DAYS AFTER TREATMENT

7 DAYS AFTER TREATMENT WITHOUT PAPAIN TREATMENT

TIME (HOURS)

Fig. 3
URINARY RECOVERY OF $^{14}$C-PROTEINS

LYSOZYME, WITH PAPAIN TREATMENT

％RECOVERY

<table>
<thead>
<tr>
<th>TIME (HOURS)</th>
<th>17</th>
<th>41</th>
<th>65</th>
<th>89</th>
<th>17</th>
<th>41</th>
<th>65</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Recovery</td>
<td>9.0</td>
<td>8.0</td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

LYSOZYME, 7 DAYS AFTER TREATMENT

Fig. 4
URINARY RECOVERY OF $^{14}$C-PROTEINS

**Fig. 5**

URINARY RECOVERY OF $^{14}$C-PROTEINS

**Fig. 6**

**SUBSTITUTE SHEET**
URINARY RECOVERY OF $^3$H-HYDROCORTISONE

HYDROCORTISONE, WITH PAPAIN TREATMENT

% RECOVERY

20.0
15.0
10.0
5.0

7 DAYS AFTER TREATMENT

WITHOUT PAPAIN TREATMENT

TIME (HOURS)

17 41 65 89 17 41 65 17 41 65

Fig. 7

SUBSTITUTE SHEET
GLUCOSE

○ 1ST DAY
□ 2ND DAY
△ 3RD DAY
* NORMAL

Fig. II
Fig. 17

Fig. 18

Substitute sheet
Fig. 19

Fig. 20

SUBSTITUTE SHEET
INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/00998

I. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (3) A61M 37/00
U.S.CI. 424/ 443

II. FIELDS SEARCHED

Minimum Documentation Searched

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<td>424/443, 444, 94, 62</td>
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Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, * with indication, where appropriate, of the relevant passages</th>
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<td>P,Y</td>
<td>US, A, 4,867,981 (GROF) 19 September 1989 (see summary of invention)</td>
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<td>Y</td>
<td>US, A, 4,668,228 (BOLTON) 21 May 1987 (see abstract)</td>
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<td>Y</td>
<td>US, A, 4,600,574 (LINDNER et al.) 15 July 1986 (see entire document)</td>
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<td>A</td>
<td>US, A, 2,976,212 (FRIEDRICH et al.) 21 March 1961</td>
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<td>A</td>
<td>US, A, 3,029,187 (STEINHARDT et al.) 10 April 1962</td>
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* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance

“E” earlier document but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

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“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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IV. CERTIFICATION

Date of the Actual Completion of the International Search: 11 APRIL 1990

Date of Mailing of this International Search Report: 16 MAY 1990

International Searching Authority: ISA/US

Signature of Authorised Officer: LEON A. R. HORNE