ENHANCING TISSUE PENETRATION OF PHYSIOLOGICALLY ACTIVE AGENTS WITH DMSO


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ENHANCING TISSUE PENETRATION OF PHYSIOLOGICALLY ACTIVE AGENTS WITH DMSO

A method of enhancing tissue penetration of physiologically active agents, including physiologically active steroids, antineoplastic agents, antigens, anti-ulcerous agents, antimicrobial agents, neurotransmitter agents, antineoplastic agents, anti-inflammatory agents, anticoagulants, vasodilators, ultra-violet screening agents, diagnostic dyes and radiopaque agents and nutrients, by conjointly applying them to the tissue with dimethyl sulfoxide. Penetration of the skin and the mucous membranes of the body cavities by these agents may be enhanced by conjoint application of such agents and dimethyl sulfoxide directly to such membranes. Preferably, for penetration of agents through the skin compositions of DMSO at concentrations of 50% and above are employed and for penetration through mucous membranes, compositions including DMSO at concentrations of 10% and above are employed. Antineoplastic agents, steroids, central nervous system-active agents, local anesthetics, anti-inflammatory agents, diagnostic dyes and radiopaque agents, and vasodilators may be advantageously administered by injection with DMSO in concentrations preferably up to 20% by weight to enhance penetration of internal tissue membrane barriers to achieve better distribution of these agents.

CROSS REFERENCES TO RELATED APPLICATION

This is a continuation in part of co-pending application Ser. No. 329,151, filed Dec. 9, 1963, now abandoned.

BACKGROUND OF THE INVENTION

A predominant and limiting problem in the development and use of physiologically active agents is the inability to administer them as effectively as is desired. In particular, there is often a limitation as to the routes of administration because of the following factors:

1. Some agents are inactivated in the gastrointestinal tract or are absorbed poorly into the body from the tract. Also, undesirable side effects may result which prevent effective oral administration.

2. In cases where injection must be resorted to, there is a risk of needle injury, infection, and other trauma (including the emotional trauma inevitably associated with injections).

3. Few agents are absorbed through the skin or mucous membranes in effective quantities and the rate of absorption is less than would be desirable for those that do.

4. A local concentration for a local effect is often desired but a larger systemic dose must be given to achieve an effective concentration at the local area when the agent can only be injected or given orally, (but not topically). This higher dose often causes undesirable side effects, since dosage related side effects are very prevalent for many agents.

Animal tissues comprise various membranes which are selectively permeable and which allow some substances to pass freely, while rejecting others or permitting only slight passage. Such membranes comprise the body coverings and externally communicating cavities, including the skin and mucous membranes of the body cavities, e.g., alimentary tract, respiratory tract, genitourinary tract, oral cavity, eyes, etc. (collectively defined herein as external membranes). They also include internal membranes such as the linings of the various organs and other internal body structures, e.g., peritoneum and pleura, and the membranes surrounding cellular and intracellular structures. It is desirable in overcoming the aforementioned problems in drug administration, to increase the passage or penetration of agents across such membranes and further to enhance their intercellular and intracellular diffusion in order for them to reach their sites of activity more rapidly to achieve the desired response more quickly and more effectively. It is exceptionally desirable to do this in a reversible manner, by which is meant penetration of the agent into the tissue without damaging or impairing the function or structure of the tissue. It is known that certain substances will penetrate tissue only after the tissue has been irreversibly damaged which is certainly undesirable. Certain agents, such as surfactants, have been known previously for increasing penetration of various agents. However, again, such penetration was effected only through irreversible damage of the tissue.

It has been a major rule in medicine that the "vehicles" or "carriers" have relatively little effect on the penetration rate for a given agent and this rule generally still holds true. Thus, with conventional carriers for medicines, such as alcohol, carbowax, water, etc., few agents will adequately penetrate such formidable external membrane barriers as the intact skin or mucous membrane. It is to be expected that this would be true of all potential "vehicles" or materials combined with physiologically active agents. However, surprisingly, it has been discovered that dimethyl sulfoxide (DMSO) has the unusual ability to greatly enhance the penetration of agents when they are applied to such membrane barriers along with dimethyl sulfoxide. The penetration of agents which previously have not been penetrated to an effective degree may be enhanced sufficiently so that a useful result may be obtained. The penetration of agents which have been known to penetrate to a limited degree in conventional vehicles may be significantly enhanced. New and convenient routes of administration, often with a decrease in side effects of the agents, better localized concentration and a more sustained activity, may thereby be created for many agents.

In my co-pending application (Ser. No. 615,377 filed Feb. 13, 1967) is disclosed my related discovery that DMSO enhances the penetration of plant-active agents (pesticides, dyes, nutrients, hormones, herbicides, and the like) into plant tissue in a highly unusual manner.

Dimethyl sulfoxide is a water-white liquid at room temperature having a freezing point of approximately 18.5° C. and a specific gravity of approximately 1.1. Dimethyl sulfoxide is a well known industrial solvent and it has been available in commercial quantities for at least a decade (from Crown Zellerbach Corporation, San Francisco, Calif.). DMSO was originally synthesized in 1866 and since that time it has been extensively investigated for possible industrial and biological utility and a considerable amount of literature has developed on its properties and uses. Over the last 25 years it has had wide spread use as a solvent in industry and in the laboratory.

DMSO has been investigated in the past for various biochemical uses, for example as a reaction solvent for preparing derivatives of various proteins, and antibiotics, as an extraction solvent for various proteins, as an analytical solvent and as a solvent for various other lab-
oratory uses. It has also been suggested as a solvent for certain pesticides (see, for example, U.S. Pat. No. 3,068,142).

DMSO has been investigated as a preservative agent for in vitro storage of chilled or frozen tissue and it has also been determined to have a protective effect in experimental animals subjected to X-irradiation following injection of DMSO into such animals.

In connection with topical application of the antifungal griseofulvin, DMSO has been listed along with various inert materials as "bland, high boiling fluids" to be used as carriers for the griseofulvin in applying it to the skin to control fungus growth in the skin (see British Pat. No. 810,377). DMSO has been employed as a solvent for preparation of certain injectable formulations, namely chloramphenicol and an anthelminic preparation (see Pat. Nos. 3,044,936 and 3,067,096).

Despite the employment of DMSO as a solvent for these purposes and despite general experimentation with DMSO in the medical field, the unique ability of DMSO to alter membrane permeability and to thereby enhance penetration of physiologically active agents was neither suggested nor discovered. Although DMSO has been a well known and widely investigated solvent for many years, its unique ability to enhance penetration of external and internal membrane barriers is contemplated in the present invention has been totally unrecognized.

SUMMARY OF THE INVENTION

By a mechanism or mechanisms not yet fully understood, DMSO, when applied to animal tissue, increases the permeability of the tissue in a reversible manner to cause a much greater penetration rate for conjointly applied physiologically active agents. Although the mode of activity is still unclear, it is definitely not that of the simple "vehicle" or "carrier" since the effect may be obtained to some extent even when the DMSO is applied to the tissue separately and the enhanced penetrability of the tissue may last for as much as three hours after the DMSO treatment.

When applied to the intact skin along with dimethyl sulfoxide, particularly at a DMSO concentration of 50% by weight and above, or to skin pretreated with the di-methyl sulfoxide, an agent such as a steroid, may penetrate rapidly to and saturate the stratum corneum (the highly resistant "horny layer" of the skin which is the major barrier to penetration). The steroid continues to penetrate through the skin from this "reservoir" in the stratum corneum to the underlying tissue and into the circulatory system.

Similarly, penetration into underlying tissues and into the circulatory system may be obtained from topical application to the mucous membranes of the body cavities as in the case of intraoral, conjunctival sac, rectal, vaginal, and bladder instillation administration, particularly where the DMSO is utilized at a concentration of 10% by weight and above. It is thus seen that a particularly important aspect of this invention is that penetration of agents may be effectively enhanced following topical administration. As used in this connection herein, the term "topical" is intended to include application to all external membrane barriers including the cutaneous or epidermis regions and the mucous membranes including the gastrointestinal tract, the respiratory tract and the genitourinary tract.

Important advantages are also obtained through the injection routes for physiologically active agents. When these agents are injected into the tissues either in a composite cationic agent (dimethyl sulfoxide at DMSO concentrations exceeding 1% and especially in the range of 10-20% by weight) or together with conjoint, but separate application of DMSO to the tissues, the effect is an enhanced and more even distribution thereof into the tissues surrounding the injection site compared with conventional injection techniques. This more even distribution is of considerable advantage for both local and systemic effect for all of the usual injectable routes, e.g. subcutaneous, intramuscular, intraperitoneal, etc.

Where a local effect is desired, the intimate distribution of the agent in the tissue near the injection prolongs and enhances its physiologic activity at this local site. This may permit use of a lower dose to achieve the desired response with a smaller risk of side effects which may result from a higher dose.

For all routes of administration, conjoint application of DMSO along with physiologically active agents having an activity site in the individual cells of the host may additionally result in an enhanced effect of the agent through the ability of DMSO to increase the permeability of such individual cells to such agents.

As previously indicated, the mechanisms of penetration enhancement are as yet not fully elucidated. Accordingly, it is not intended to be bound to one specific theory of operation. However, it is believed that DMSO acts by several mechanisms in enhancing penetration. DMSO is believed to act directly on tissue to alter the general permeability of the tissue membrane. More specifically, DMSO when applied thereto, is believed to decrease the natural resistance of tissue membranes to penetration by foreign agents. DMSO is also believed to promote penetration by a direct transport effect, perhaps by the mechanism of complexing with the agent. This mechanism is believed more applicable to cationic and anionic agents.

GENERAL DESCRIPTION OF THE INVENTION

This invention is applicable to the tissue or organs of all animal phyla, DMSO having differing degrees of influence on penetration of various tissue types of a given animal. Animals of particular importance in the practice of the invention are the mammals, especially man and veterinary animals. However, the invention may also be practiced with other vertebrates, as for example the amphibians, fishes, reptiles, etc. and with the lower species comprising the non-vertebrates.

As indicated previously, a measure of penetration enhancement may be obtained where the tissue is pretreated with DMSO prior to application thereto of the physiologically active agent. The tissue penetrability is thus altered by such pretreatment and this reversible effect gradually diminishes and the tissue returns to its normal permeability state. However, for convenience and optimal effect, it is frequently desirable to administer the DMSO and the agent simultaneously in the same composition.

Penetration enhancement is generally non-selective in terms of the type or physiological effect or effects of agents to be transported across membrane barriers. The extent of penetration enhancement will depend upon many factors, the predominant factors being the relative natural permeability of the particular membrane, the concentration of DMSO applied, the extent of solubility of the agent in DMSO and the chemical and physical properties of the agent.

As a class, cationic agents, means chemical compounds which dissociate into relatively small, mobile anion(s) and much less mobile cation(s) which are considerably larger than the anion(s) (e.g. having a radical weight ratio greater than 1 to 3, but more usually on the order of 1 to 10-100), appears to be the most pronounced penetration enhancement with DMSO. Even penetration of external membrane barriers such as the mucous membranes may be effected with these agents utilizing rather low amounts of DMSO, frequently as low as 10-20% by weight. Following Examples 34, 69, 73 and others illustrate the same. Anionic agents, meaning agents which dissociate into relatively small, mobile cation(s) and large, less mobile anion(s) which are considerably larger than the cation(s) (e.g. having a radical weight ratio greater than three to 1 but more usually on the order of 10-100 to 1) also obtain marked penetration enhancement with DMSO. Although lower concentrations of DMSO, as for example 15% by weight (see following
Example 34), may be effected through various external membrane barriers, higher concentrations of DMSO are frequently required for maximum effect, particularly for epidermal applications. These agents are illustrated by following Examples 34, 38, 51, 70 and others.

Penetration of non-dissociating chemical compounds may also be beneficially enhanced with DMSO. Here again higher concentrations of DMSO, i.e., 50% and above, are particularly desirable for maximum effect, particularly for epidermal applications. Illustrative of this class of chemical agents are following Examples 1, 2, 3, 4, 47, 58, 72 and others.

Penetration of agents which form complexes with DMSO, for example iodine and most metal halides and nitrates, are also beneficially enhanced. Such agents are illustrated by Examples 41, 42, 43 and 74.

The size of the compound obviously may influence to some extent the relative ability of agents to penetrate tissue. However, effective membrane penetration utilizing DMSO has been demonstrated for extremely large compounds, for example compounds having molecular weights exceeding 40,000 (also see Examples 75 and 76 illustrating penetration enhancement of insulin which has a molecular weight of about 6,000). Even for such a formidable membrane barrier as intact human skin, quite large compounds have been demonstrated to be effectively enhanced. As illustrated by following Example 68, heparin, which has a molecular weight of 8,000 and above, can be effectively penetrated through the human epidermis in some cases.

Standard occlusion techniques frequently increase the percutaneous absorption of the larger molecules. In general, at least a limited degree of solubility of the agent in DMSO is desirable to achieve maximum benefit of the present invention. Naturally, the practitioner will select agents, routes of administration and composition forms guided by these well-known principles.

The term "physiologically active" in describing the agents contemplated herein is used in a broad sense to comprehend not only agents having a direct pharmacological effect on the host but also those having an indirect or observable effect which is useful in the medical arts, e.g., the coloring or opacifying of tissue for diagnostic purposes, the screening of U.V. radiation from the tissues, etc. Agents which increase the penetration of which across membrane barriers, particularly external membranes, may be beneficially enhanced upon direct application include: physiologically active steroids, antineoplastic agents, antibiotics, antiseptics, microporous membranes, neuropharmacologic agents, anti-inflammatory agents, anticoagulants, radiopaque agents, and nutrients. Agents, the penetration of which across internal membranes may be particularly beneficial upon injection include antineoplastic agents, steroids, central nervous system-active agents, local anesthetics, anti-inflammatory agents, diagnostic dyes and radiopaque agents and vasodilators.

The concentration of the DMSO applied to enhance penetration may vary over wide limits. The concentration selected is desirable related to the route of administration to be employed. For cutaneous application, compositions including at least about 50% by weight DMSO are preferable in that they have been found to increase by percutaneous penetration in a highly significant manner, with DMSO concentrations closely approaching 100% Maximum cutaneous penetration is generally attained (excluding the agent), but with concentrations much above 50% by weight the incremental increase in penetration rate over that achieved at 50% often is relatively small. On the other hand, above a 90% concentration of dimethyl sulfoxide the side effects of a burning sensation and erythema increase significantly. Accordingly, for topical use, it may be desirable, consistent with physical stability of the composition, to formulate the DMSO in compositions containing a DMSO concentration of between about 50% and 90% by weight and containing water, preferably 10% by weight or greater.

Application to mucous membranes follows generally the procedure for cutaneous administration. However, lower concentrations of DMSO, for example as low as 10% by weight, may be preferred since penetration of mucous membrane is more easily affected. For most indications, preferably lower concentrations of DMSO of about 10% to about 20% by weight are preferably utilized. For some injection routes for example, intra- and articular routes, higher concentrations, say 30-40%, may be preferred.

The amount of the physiologically active agent to be administered will obviously be an effective amount for the desired result expected therefrom. This, of course, will be ascertained by the ordinary skill of the practitioner.

Due to enhanced activity which may be achieved through better penetration, the dosage of agent may often be decreased from that generally applicable. In accordance with the usual prudent formulation practices, the dose near the lower end of the useful range of the particular agent may be employed initially and the dosage increased as indicated from the observed response, as in the routine procedure of the physician.

As previously discussed, the DMSO may advantageously be compounded with the physiologically active agent for concurrent administration. The usual pharmaceutical compounding agents, diluents or carriers may be included in these compositions as desirable for the particular route of administration and dosage form. The amount and type of diluent or carrier used should, of course, be consistent with the compatibility of the agent in DMSO and the diluent. A cosolvent or other standard adjuvant, such as a surfactant, may be called for to maintain the agent in solution or suspension at the desired concentration. Where stability of the agent in the presence of DMSO at the desired concentration is a problem, it may be desirable to prepare the formulation immediately before administration or to administer the DMSO and the agent separately to the tissue.

In selecting the route of administration for a given agent, obviously the known toxicity, side effects and effectiveness for a given route of administration must be taken into account. For example due to skin irritation known to be caused by some agents or due to poor penetration characteristics, some other route than dermal application may be the route of choice for such agents.

Dosage forms for topical application may include solutions (paints), nasal sprays, lotions, ointments (including creams and gels), suppositories and the like. The solutions and nasal sprays may simply comprise the agent dissolved in DMSO, optionally with an amount of water, glycerine or other diluent. For nasal sprays and other mucoadhesive applications isotonic saline may be preferable as a diluent. The DMSO may be present in these forms in various concentrations, say from about 10% to about 75% by weight or higher.

Lotions and gels, ointments or creams, may contain the usual ingredients to provide a base, as for example ceteryl alcohol, an emulsifier such as lauryl sulfate and water. Another base may be formulated by combining equal weight amounts of stearic acid, ceteryl alcohol, triethanolamine and glycerol monostearate with water. Still other bases may utilize polyethylene glycols of different viscosities, depending upon the desired consistency. DMSO may be added to the lotion or ointment base in varying amounts as desired, generally up to around 50% by weight.

A suppository form may be made from a high viscosity polyethylene glycol 4,000, water and DMSO, which may be present in an amount of about 20% by weight.

The concentration of physiologically active agent in the various dosage forms is, of course, commensurate with that normally utilized for the particular agent in conven-
tional formulations for effective results for the intended route. Both the amount of physiologically active agent and the amount of DMSO will be influenced by the type of effect desired. If a more localized effect is required, as for example, in treating a superficial infection with an antibacterial agent, lower amounts of physiologically active agents and lower concentrations of DMSO may be called for. Where deeper penetration is desired, as in the case of local anesthesia, a higher concentration of DMSO may be desirable to promote adequate penetration. Where general systemic concentration of an agent is desired for a topical preparation, generally higher concentrations of DMSO may be used. The amount of agent as, for example, a steroid, may be included in the composition sufficient to provide the blood level desired.

The various pharmaceutical forms are desirable provided in determined amounts, as in containers of a given volume. These amounts may include 100% DMSO concentration containing the desired dose of the agent, or a lesser concentration of DMSO with a diluent and the physiologically active agent dose. Thus, for example, graduated ampules containing, say 5 cc of 100% DMSO with the agent dissolved therein may be provided. The practitioner need only open and dispense all or a determined part to a subject. Nasal spray bottles, aspirators, suppositories, cotton tipped stick applicators, squeeze tubes may all be utilized for topical application.

The following illustrates the practice of the present invention with the various classes of agents.

DESCRIPTION OF PREFERRED EMBODIMENTS

Alteration of membrane permeability

The following example is an invitro demonstration of the effect of DMSO on the penetrability of tissue membranes.

EXAMPLE 1

Penetration of solutes and ions through skin of frogs

The skin of the frog Rana pipiens is often used as a "model membrane" system. The skin is removed and placed as a wall barrier between two glass chambers which are filled with dilute sodium chloride salt solution. The chambers are constructed to permit electrical measurements and addition or sampling of fluid from either side. The fluid in the one chamber bathes the outside of the skin and the fluid in the other chamber bathes the inside of the skin. When water, ions or molecules cross from the inside to the outside solution, the movement is termed an "outflux." A material that would make a membrane more permeable to any substance is said to increase a flux.

Radioactive ions and radioactive labeled compounds were placed in the inside compartment and allowed to cross. The movement was quantified by making serial radioactivity of the bathing fluids. The outflux rate of a given substance was determined during several control periods. Dimethyl sulfoxide was then added to a 2.5% concentration and flux measurements were made for several successive periods. The following table presents sample data to illustrate flux rates.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Ed./15 min.</th>
<th>Mole/15 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.95</td>
<td>0.029</td>
</tr>
<tr>
<td>Cl</td>
<td>0.14</td>
<td>0.009</td>
</tr>
<tr>
<td>Thiorurea</td>
<td>2.27</td>
<td>0.045</td>
</tr>
<tr>
<td>Steroid</td>
<td>3.50</td>
<td>0.084</td>
</tr>
</tbody>
</table>

In a number of similar tests the range of multiple flux increase for these ions and molecules was about 5 to 10.

STEROIDS

Steroids are generally classed as organic molecules which have in common a polyhydrocyclopentaanophenanthrene nucleus and they are so named because they are related to and usually derived from sterols found abundantly in nature in animal and plant fats. Certain steroids are naturally produced in the body and they act as hormones to mediate and control many body functions. These hormones have been isolated or produced synthetically and used in replacement therapy for hormone deficiencies. Additionally and importantly, these steroids have been found to be highly useful in the treatment of a wide range of disease states not primarily due to lack of hormones. In the recent past extensive research effort has resulted in the synthesis of a vast number of new steroid hormone derivatives having biological activity (in excess of 1,500 compounds) and the list is growing rapidly. Such derivatives usually contain modifying groups linked to the steroid nucleus which influenced the activity of the steroids, as by modifying, prolonging, or increasing its activity, increasing stability and/or modifying its solubility characteristics. These modifying groups usually comprise addition salts to influence solubility or a side chain substitution on one or more reactive ring carbon atoms. The side chain may be a direct substitution for a ring hydrogen, an ester formed at a reactive hydroxyl group or an ether group. Many of these steroid derivatives have a higher potency than the naturally occurring steroid hormones, often with a decrease in undesirable side effects which frequently result from administration of the natural steroids. As used herein, the term "steroids" and "steroid drugs" is intended to comprehend both the natural steroids and the biologically active modifications, derivatives and equivalents.

Steroid drugs may have one or more of many types of biological activity such as anabolic, androgenic, glucocorticoid, mineralocorticoid, estrogenic, progestogenic, lipidoaidic (removal of stored fat), circulatory system activity, central nervous system activity, anti-cancer and anti-osteoporotic. Some steroid drugs have the ability to block the activity of other hormones, including the activity of other steroids. Their biological activity may be characterized as antianabolic, antiguocorticoid, antimineralocorticoid (diuretic), antisterogonic and antiprogestogenic. The various activities will be considered hereinafter in connection with specific embodiment of this invention.

GLUCOCORTICOIDS

Hormones produced naturally in the body, by the adrenal cortex are called adrenocortical steroids or corticoids. Some corticoids, predominantly cortisone and hydrocortisone, have the property of influencing the rate of metabolism of glucose. Hence, they, along with their derivatives and modifications, are called glucocorticoids. Glucocorticoids have other predominant physiologic activity which makes them highly useful as drugs. One of the most important activities is the suppression of inflammation, particularly in the treatment of arthritis and rheumatic diseases. Glucocorticoids are also useful in the treatment of dermatoses, drug reactions, bronchial asthma, lupus erythematosus, angioneuroedema and many other disorders. Massive doses of corticosteroids have also been used to induce remissions in leukemia. DMSO may be combined with glucocorticoids to enhance their penetration to the affected tissue for these various disorders. The following examples illustrate compositions and treatments for this purpose utilizing the most prominent and active natural and modified glucocorticoids:

EXAMPLE 2

Penetration of injected corticosteroids

A thirty-two year old white woman was seen with a two days' history of left subdeltoid bursitis. This gave her pain on minimal abduction particularly, but also was present in other movements of the shoulder joints. Physical examination revealed marked tenderness to pressure with obvious protective muscle spasm overlying the joint. Two ml of hydrocortisone was injected into the bursal area. This was associated with three hours relief of pain. The patient was seen again two days later. At this exam-
infection, her pain was just as marked as the first visit. Two ml. of hydrocortisone was injected and 5 cc. of 100% dimethyl sulfoxide was applied liberally to the entire left shoulder area. Within 15 minutes all pain disappeared and the patient reported no return of her symptoms when examined one week later.

**EXAMPLE 3**

**Penetration of injected corticosteroids**

A forty-two year old white male was examined with a one week history of acute subdeltoid bursitis of the right shoulder. Four mg. of Decadron was injected into the right subdeltoid bursa. The patient estimated that about a 20% relief of his discomfort was attained. Full pain returned one day later. At this point, he was re-injected with 4 mg. of Decadron and 4 cc. of 100% dimethyl sulfoxide was placed on the skin over the involved bursa. This time the pain dissipated completely and did not recur.

Both of the above examples show that cortisone injected for subdeltoid bursitis is obviously of benefit, but its relief of symptomatology is enhanced with the simultaneous application of dimethyl sulfoxide to the skin.

**EXAMPLE 4**

**Penetration of corticosteroids**

A twenty-four year old medical student was seen with atopic dermatitis of the right antecubital fossa. Three cc. of 100% dimethyl sulfoxide were applied four times daily for three days. No benefit was noted. One mg. or ¼ cc. of Decadron (dexamethasone 21-phosphate) was applied four times a day for two days without benefit. One mg. of dexamethasone 21-phosphate in 3 cc. of 100% dimethyl sulfoxide was painted onto the involved area four times daily for three days. At the end of this period, evidence of the inflammatory reaction had disappeared.

This example shows an improved action of dexamethasone 21-phosphate when used with dimethyl sulfoxide.

**EXAMPLE 5**

The following lotion formulation may be prepared containing about 0.01 to 1.0%, with preferably 0.1% fluocinolone acetonide:

Fluocinolone acetonide  \( \frac{0.1-1.0}{Gm.} \)

Cetyl alcohol  \( 200 \)

Propylene glycol  \( 100 \)

Sodium lauryl sulfate  \( 15 \)

DMSO  \( 300 \)

Water qs. 1000 cc.

The steroid is dissolved in the DMSO and added to a stirred, cooling melt of the other ingredients. The preparation is particularly useful for the treatment of inflamed dermatoses by topical application to the affected skin area. The amount and frequency of application is in accordance with standard practice for topical application of this steroid. Penetration of the steroid into the inflamed tissue is enhanced and a therapeutic level is achieved more rapidly than when the steroid is applied in conventional formulations.

**EXAMPLE 6**

The following ointment (gel) formulation may be prepared containing about 0.2% to 1.0%, and preferably 0.6% triamcinolone acetonide:

Triamcinolone acetonide  \( 0.2-10 \)

Polyethylene glycol 400  \( 400 \)

Dimethyl sulfoxide  \( 598 \)

Carboxy vinyl polymer powder  \( 1 \)

Triethanolamine  \( 0.4 \)

The corticosteroid is dissolved in a mixture of the first two ingredients, and the carboxy vinyl polymer gelling agent is sprinkled on the surface of the combined liquids and stirred until all the particles have been wetted and dispersed. The triethanolamine is then added dropwise to the mixture until it has gelled, care being taken to minimize the air entrapment. This gel is particularly effective in the treatment of seborrhea and other scalp and hair inflammatory conditions and may be applied in amount and frequency conventionally used for topical application of this steroid. Better penetration and thereby an increased anti-inflammatory activity is obtained for the amount of steroid applied than results from its application in conventional formulations.

**EXAMPLE 7**

The following ointment formulation may be prepared containing about 0.1% to 1.0% prednisone and preferably 0.5%:

Prednisone  \( \frac{0.1-10}{gm.} \)

Glyceryl monostearate, acid type  \( 180 \)

Stearyl alcohol  \( \frac{50}{gm.} \)

Polysorbate 80  \( 20 \)

Water  \( 450 \)

Dimethyl sulfoxide  \( 300 \)

The product is prepared as described in Example 5. The ointment is a valuable base for application of the corticosteroid to inflammatory dermatological areas, particularly when they require injection. Application is in accordance with that usual for topical application of this steroid in conventional bases.

**EXAMPLE 8**

The following cream formulations may be prepared containing about 0.1% to 1% 16a-methyl prednisolone and preferably 0.5%:

16a-methyl prednisolone  \( \frac{0.1-10}{Gm.} \)

Stearic acid  \( 200 \)

Glyceryl monostearate, acid type  \( 200 \)

Sodium lauryl sulfate  \( 20 \)

Dimethyl sulfoxide  \( 200 \)

Water qs. 1000 cc.

As above, the product is prepared as directed in Example 5 and is useful in severe dermatoses requiring injection.

**Mineralocorticoids**

Some natural corticoids have the predominant property of inducing sodium retention (depressing the rate of excretion of sodium salts through the kidneys). Hence, they, along with their derivatives and modifications, are called mineralocorticoids. The principal natural mineralocorticoid is aldosterone, while desoxycorticosterone is the most prominent synthetic mineralocorticoid. Both are useful in treating the mineralocorticoid deficient state in Addison's disease. The following exemplifies the use of DMSO in the administration of mineralocorticoids to enhance penetration:

**EXAMPLE 9**

The following ointment formulation may be prepared containing about 0.5% to 2.5%, preferably 1.0%, desoxycorticosterone acetate:

Desoxycorticosterone acetate  \( \frac{5-25}{gm.} \)

Stearic acid  \( 300 \)

Cetyl alcohol  \( \frac{100}{gm.} \)

Polysorbate 20  \( 20 \)

Sorbitol 70%  \( 100 \)

Dimethyl sulfoxide  \( 300 \)

Water, qs. 1000 cc.

The product is prepared as specified in Example 5. The product may be employed in treatment of pigmentation in Addison's disease by topical application to the affected area. Penetration may be increased sufficiently so that effective results may be obtained. In conventional bases this steroid has had very limited effectiveness topically and injection usually must be resorted to.
Androgens

Androgen is the generic term which comprehends testosterone, the natural male hormone, androstenedione and the modifications and derivatives thereof which have masculinizing activity. Natural and modified androgens are employed in replacement therapy for hypogonadal males. Typical for this purpose is the isobutryrate, decanoate, isocaproate, ethionate, phenylpropionate and cyclopentylpropionate esters of testosterone and 17-methyl testosterone.

However, a much more important drug roll for these steroids is their use as antigestogens in treatment of female genital cancer and as anabolic agents (metabolism stimulation) in triaining debilitated subjects. For these purposes, androgens which have been modified to decrease their unwanted virilizing effects (while retaining their anabolic and antigestogen activity) are generally preferred. The following steroids exemplify these compounds with the commercial source of the compounds indicated:

17α-ethyl-19-nortestosterone (Nilevar)
17α-methyl-19-nortestosterone (Syntex product)
19-nortestosterone phenylpropionate (Durabolin)
9α-fluoro-11β-hydroxy-17α-methyltestosterone
4-chloro-19-nortestosterone acetate (Halotestin)
4-hydroxy-17α-methyl testosterone (Oranabol)
2-hydroxy methyl-17α-methyl dihydrotestosterone (Adrod Parke Davis)
17α-methyl-17β-hydroxyandrostano (3,2-C)-pyrazole (Androstanzone)
1-dehydro-17α-methyltestosterone (Dianabol)

The following examples illustrate the use of DMSO to enhance the penetration of androgens:

EXAMPLE 10

A suppository formulation may be prepared as follows containing about 1 to 5%, preferably 2%, testosterone propionate:

Testosterone propionate .................. gm. 10-50
Polyethylene glycol 400 .................... gm. 400
Propylene glycol monostearate ............... gm. 100
Dimethyl sulfoxide ........................ cc. 500

The solid constituents are melted, added to the solution of the steroid in DMSO and poured into an appropriate mold. The product is recommended for rectal application as replacement therapy.

EXAMPLE 11

A suppository formulation may be prepared as follows containing about 1% to 5%, preferably 2%, 17α-methyl testosterone:

17β-methyl testosterone .................. gm. 10-50
Hydrogenated castor oil .................. gm. 400
Stearic acid ............................. gm. 500

The product is prepared as noted in Example 9 and used in a similar manner.

EXAMPLE 12

A cream formulation may be prepared as follows containing about 1% to 10% preferably 5%, 17α-ethyl-19-nortestosterone:

17α-ethyl-19-nortestosterone ............... gm. 10-100
Cetyl alcohol ................................ gm. 250
Stearyl alcohol ........................... gm. 200
Polysorbate 80 ................................ cc. 20
Water ........................................ cc. 250
Dimethyl sulfoxide, q.s. ................... cc. 1000

This cream may be prepared as noted in Example 5. It may be applied topically for stimulation of epithelialization and connective tissue regeneration.

EXAMPLE 13

A lotion or paint formulation may be prepared as follows containing about 1% to 5%, preferably 2%, testosterone propionate:

Gm.
Testosterone propionate .............................. 10-50
Dimethyl sulfoxide ................................ 890
Water ............................................... 100

The steroid is dissolved in a mixture of the dimethyl sulfoxide and water. The formulation may be applied topically as an anabolic and in the treatment of breast cancer. The enhancement of penetration over that obtained with conventional topical formulations permits more effective topical use of this steroid which previously had to be injected to achieve a response in many cases.

EXAMPLE 14

The following cream may be prepared with the following composition containing about 1% to 10%, and preferably 3%, 2α-methyl-dihydrotestosterone propionate (methylone):

Metholone ........................................... gm. 10-100
Stearic acid ......................................... gm. 500
Glycerol monostearate, acid type ............... gm. 200
Sodium lauryl sulfate .......................... cc. 20
Water .............................................. cc. 400
Dimethyl sulfoxide .............................. cc. 200

The cream is prepared as directed in Example 5. The product is useful in the treatment of muscle wasting and weakness followed breast cancer surgery and may be applied topically to the affected area. Penetration of the steroid is greatly improved over that obtained in conventional formulations.

EXAMPLE 15

The following ointment (gel) may be formulated containing about 1% to 5%, preferably 2%, steroid:

2-hydroxy methyl-17α-methyl dihydrotestosterone ........................................... gm. 10-50
Propylene glycol ................................. cc. 500
Dimethyl sulfoxide .............................. cc. 498
Carboxy vinyl polymer powder ............... gm. 1
Triethanolamine ................................. gm. 0.5

The product is prepared as specified in Example 6. The product is useful in topical anabolic treatment, particularly in preventing thinning of the skin and in inducing blood vessel thickening.

Estrogens and progestins

Estrogen is the generic name for estradiol and its active metabolites estrone and estriol, naturally occurring female sex hormones, and their derivatives and modifications. Estrogens are useful in treating menstruation disorders, infertility, habitual abortions, and endometriosis. Along with progestogens, they are used to control the reproductive cycle in women for contraception. They are also used in replacement therapy, particularly in treating the hormone deficiency states such as in postmenopausal women. Modified estrogens having lower feminizing characteristics are particularly useful for other applications including the treatment of atherosclerosis and osteoporosis. Their antiandrogenic effects are also useful in the treatment of prostatic cancer.

Progesterone is a natural female hormone which plays a primary role in the reproductive cycle of the female mammal, particularly in the menstrual cycle of the primatre. Progesterone and its modifications and derivatives are classified as progestins. Progestins are useful in replacement therapy and in treatment of menstrual disorders and prevention of fetal loss. A number of modified progestogens are useful in contraception. The various modified progestogens include the 19-norprogestogens, the 17α-methyl progestogen derivatives, the 3-enol ethers of progestosterone, and 17-acetoxyprogesterone and the 9-iso-10-iso compounds called retroprogesterones.
The following exemplifies the practice of this invention with estrogenic and progestenic steroids:

**EXAMPLE 16**

The following lotion may be formulated as follows containing about 0.1% to 1.0%, preferably 0.4%, estradiol valerate:

- Estradiol valerate gm 1-10
- Cetyl alcohol gm 200
- Propylene glycol gm 100
- Sodium lauryl sulfate gm 15
- Water cc 400
- Dimethyl sulfoxide cc 300

This product is prepared as noted in Example 11. The product is designed as a means of establishing systemic replacement therapy for estrogens during menopause by simple topical application to the skin or mucous membrane. The DMSO enhances penetration of the estrogen sufficiently to obtain a systemic effect. This has not been possible in conventional formulations.

**EXAMPLE 17**

A suppository may be formulated as follows to contain 0.1 to 1.0%, preferably 0.5%, of 3-methyl ether of ethynylestradiol:

- 3-methyl ether of ethynylestradiol gm 10-100
- Polyethylene glycol 4000 gm 400
- Propylene glycol monostearate gm 100
- Dimethyl sulfoxide (DMSO) cc 500

The suppositories are prepared as noted in Example 11. The product is used in estrogenic replacement therapy and may be used by rectal or vaginal application.

**EXAMPLE 18**

The following ointment (gel) may be formulated containing 0.1% diethylstilbestrol:

- Diethylstilbestrol gm 1
- Propylene glycol cc 500
- Dimethyl sulfoxide cc 498
- Carboxy vinyl polymer powder gm 1
- Triethanolamine gm 0.5

This gel is prepared as detailed in Example 6. The preparation is particularly suitable for topical application in the treatment of adolescent acne.

**EXAMPLE 19**

A cream may be formulated as follows to contain about 0.72% norethynodrel and about 0.0286% mestranol:

- Norethynodrel gm 10.5
- Mestranol gm 0.42
- Cetyl alcohol gm 100
- Stearyl alcohol gm 100
- Polysorbate 80 cc 20
- Water cc 250
- Dimethyl sulfoxide, q.s. cc 1000

This cream is prepared as noted in Example 5. This formulation is to be used as a contraceptive agent applied cutaneously twice monthly at a dosage of 10 grams.

**EXAMPLE 20**

A suppository formulation may be prepared as follows:

- Chlormadinone mg 5
- Stilbestrol mg 1
- Polyethylene glycol 4000 gm 1
- Propylene glycol monostearate gm 400
- Dimethyl sulfoxide cc 100

The suppositories are formed as in Example 11. The product may be employed for treatment of irregular or prolonged bleeding.

**EXAMPLE 21**

The following ophthalmic formulation may be prepared containing about 0.1% to 0.75%, preferably 0.3%, spironolactone:

- Spironolactone gm 1-7.5
- Polyethylene glycol 4000 cc 200
- Dimethyl sulfoxide cc 200
- Water, q.s. 1000 cc

The formulation is prepared by melting the polyethylene glycol 4000, dissolving the steroid in the DMSO, mixing the two liquids together and diluting to volume with water while stirring. The preparation is applied topically to the eye by eye dropper, or similar applicator, for treatment of glaucoma.

**Antineoplastic agents**

Antineoplastic chemical agents are drugs that combat cancerous processes. Antineoplastic agents at their present stage of development are generally only palliative, for in some instances only a temporary alleviation of subjective symptoms is obtained while the malignant process itself advances steadily. Nevertheless, these drugs play an important role in the treatment of malignancies to induce remissions.

A vast number of compounds have been screened for possible anticaner action and screening programs are continuing in an attempt to develop safer and more efficacious antineoplastic agents. Of the screened compounds, only a fraction have proved of clinical experimental interest and a considerably smaller number have survived in practice. Many showing early promise have been discarded because at useful dosage levels they have proved too toxic for practical employment. Others have had only a low rate of successful results, due to the lack of ability to maintain an effective concentration at the site or to penetrate effectively into the neoplastic tissue and into individual neoplastic cells.

These same problems also pose as a serious limitation on the effectiveness of those agents which have proved to be clinically useful. Specifically, in order to reduce and perhaps eliminate neoplastic cells, the antitumor agent must reach these cells in sufficient concentration. This may be hindered or prevented by certain pharmacological "barriers" or membranes. For example, in the case of leukemia, the blood-brain barrier, is an important obstacle to effective treatment with many agents. In most instances the effectiveness of antineoplastic agents is ultimately limited because strains of neoplastic cells eventually develop which are resistant to their action.

The survival of these cells is believed, in at least some instances, to be the result of their natural resistance to the entry of the antineoplastic agent. The proliferation of these resistant cells eventually causes the destruction of the host.

With conventional carriers for medicines, such as alcohol, peanut oil, carbowax, etc., few antineoplastic agents will penetrate such formidable external membrane barriers as the intact skin or mucous membrane to effectively reach the neoplastic tissue. Also, there is little or no evidence that conventional carriers have any ability to alter the permeability of neoplastic tissue or to effectively enhance penetration of antineoplastic agents into
such tissue. Nor do such carriers facilitate penetration of these agents into neoplastic cells.

However, the penetration of antineoplastic agents which previously have not penetrated certain membranes to an effective degree may be enhanced sufficiently with DMSO so that a useful result may be obtained through application to such membranes. Penetration of agents which have been known to penetrate to a limited degree in conventional agents may be significantly enhanced. Better localized distribution, an increased activity and new and convenient routes of administration, often with a decrease in side effects of the agent may thereby be created for many antineoplastic agents. Additionally, DMSO has the ability by some mechanism not yet understood, to overcome the natural resistance of at least some malignant cells to penetration, thereby enhancing penetration of antineoplastic agents into such cells to destroy them.

Penetration into underlying tissues and into the circulatory system may be obtained from topical application to the skin and mucous membranes. This is particularly useful for treatment of superficially located tumors. Also, in treatment of localized tumors by the injection routes, improved localized distribution of antineoplastic agent in the tissue near the site of injection by the use of DMSO may prolong and enhance its physiologic activity at this local site. This may permit using a lower dose of agent to achieve the desired response with a smaller risk of side effect which may result from a higher dose.

The same phenomenon may be of benefit for better penetration of affected tissue in oral administration, in regional therapy by intravenous and intraarterial infusion and injection of antineoplastic agents and in perfusion techniques, such as those following surgical excision of tumors.

Ultimately, for all routes of administration, this enhancement of penetration increases the ability of the antineoplastic agents to cross membrane barriers which impede their effectiveness to come in contact with malignant cells in an effective concentration and to penetrate malignant cells naturally resistant to penetration.

The following illustrates the practice of the present invention with the various classes of antineoplastic agents when the classification "non-estrogenic anti-antineoplastic agents" is utilized, it is intended to mean all of the classes of antineoplastic agents, whether hormonal or non-hormonal, except for estrogenic anti-antineoplastic agents, as for example esterone.

Alkylation agents

Alkylation agents are highly reactive cyclic or unsaturated organic compounds having a denaturing or inactivating action on the malignant cell nucleus. A serious side effect of this group of agents is their tendency to damage normal cells of the body. This causes a depression of the blood-forming action of the bone marrow, and injury to the gastrointestinal mucosa. This limits the time and routes of administration of these agents. Many are even too toxic to permit oral administration and hence regional treatment of the affected site is desirable where it can be done effectively.

The major types of alkylation agents are the nitrogen mustards, the ethyleneimines and the alkyl sulfonates. The more prominent agents include melphalan, phenylalanine mustard, triethylamine melamine (TEM), TEPA, thio-TEPA (tris(1-aziridinyl) phosphine sulfide), chlorambucil, busulfan, CRI-3, (a phosphinic amide mustard) cyclophosphamide and mannolactone mustard. These agents are employed in the treatment of Hodgkin's disease, various forms of leukemia, metastatic cancer, various carcinomas and sarcomas.

The following examples illustrate the practice of this invention in connection with alkylation agents:

**EXAMPLE 22**

Thio-TEPA was administered in a dose of 0.5 mg. per body weight by inclusion in a solution and then direct installation in the freshly evacuated bladder of a dog. The two solutions were:

**Solution 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thio-TEPA</td>
<td>5 mg.</td>
</tr>
<tr>
<td>Isotonic saline, q.s.</td>
<td>5 ml.</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>M 0.05</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>M 0.03</td>
</tr>
<tr>
<td>Dioctyl sodium sulphonate</td>
<td>0.5 mg.</td>
</tr>
</tbody>
</table>

**Solution 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thio-TEPA</td>
<td>5 mg.</td>
</tr>
<tr>
<td>Isotonic saline, q.s.</td>
<td>5 ml.</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>M 0.05</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>M 0.03</td>
</tr>
<tr>
<td>Dioctyl sodium sulphonate</td>
<td>0.5 mg.</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>v/v 15%</td>
</tr>
</tbody>
</table>

Blood counts were made periodically after installation. The white count dropped approximately in half by the sixth day following administration of the composition containing dimethyl sulfoxide and the chemotherapeutic agent. The white count did not substantially drop in the composition containing the chemotherapeutic agent alone.

**EXAMPLE 23**

A lotion formulation may be prepared containing approximately 10% cyclophosphamide and 80% DMSO by blending the following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>10 grams</td>
</tr>
<tr>
<td>Glycerine</td>
<td>10 grams</td>
</tr>
<tr>
<td>Carbowax 4000</td>
<td>10 grams</td>
</tr>
<tr>
<td>DMSO</td>
<td>80 grams</td>
</tr>
</tbody>
</table>

This composition may be applied topically to the skin or mucous membrane adjacent the site of a localized cancer, or the like, typically at a dosage of 6-10 grams.

**Antimetabolites**

Antimetabolites are generally analogues of naturally occurring substances in the body necessary to cell metabolism. These agents destroy cancer cells by interfering with their metabolism. Since antimetabolites tend also to affect metabolism of rapidly multiplying normal cells, gastrointestinal upsets and hematopoietic malfunctions as side effects limit the use and routes of administration of these agents. Regional treatment of affected sites is desirable where effective. Principal antimetabolites are folic acid antagonists such as amethopterin (metothrexate); the purine analogues such as 6-mercaptopurine, azathio- prine, 6-thioguanine and 8-azaguanine; and the pyrimidine analogues such as the uracils (principally the 5- halogen uracils, and 6-azauracil), azauridine and the 5- halogen deoxyuridines.

The following illustrates practices of this invention in connection with antimetabolic agents:

**EXAMPLE 24**

Metothrexate is a known folic acid antagonist used to treat malignancy. Solutions containing this agent were prepared as follows:

**Solution No. 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metothrexate</td>
<td>5 mg.</td>
</tr>
<tr>
<td>Isotonic saline, q.s.</td>
<td>5 ml.</td>
</tr>
<tr>
<td>Evans blue dye</td>
<td>100 mg.</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>M 0.05</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>M 0.3</td>
</tr>
</tbody>
</table>

**Solution No. 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metothrexate</td>
<td>5 mg.</td>
</tr>
<tr>
<td>Isotonic saline, q.s.</td>
<td>42.5 ml.</td>
</tr>
<tr>
<td>Evans blue dye</td>
<td>100 mg.</td>
</tr>
<tr>
<td>Trisodium phosphate</td>
<td>M 0.05</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>M 0.03</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>7.5 ml.</td>
</tr>
</tbody>
</table>
Each of the solutions in an amount of 75 cc. was introduced into the freshly evacuated bladders of dogs of about equal size. Methotrexate is expected to pass the mucosa and bind to the bladder to enter the vascular system, whereupon it is transported to the bone marrow where it will reduce the platelets in whole blood. A blood sample taken from the dog treated with solution No. 2 five to six days after instillation, showed a w.b.c. drop from 600,000 to 100,000 per cc. This was the only test solution which produced a physiological response.

This illustrates the use of DMSO in achieving adequate blood levels of methotrexate for treating leukemia, etc. This formulation may also be utilized for direct infusion into the arterial blood supply for localized neoplasms or for direct injection into accessible localized neoplasms.

EXAMPLE 25

A 5-fluorouracil formulation may be prepared by blending the following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-fluorouracil</td>
<td>10</td>
</tr>
<tr>
<td>DMSO</td>
<td>80</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>0.25</td>
</tr>
</tbody>
</table>

The formulation is particularly useful in topical treatment of skin tumors or other localized superficial tumors. A typical dose is 5 grams. It is also useful in treating viral disorders such as wart.

Vinca alkaloids

A more recent class of antineoplastic agents are the alkaloids extracted from the shrub *Vinca rosea* and their derivatives. The more prominent of these are vinblastine, vinorelbine, vincristine and vinblastine. The mechanism of action of the vinca alkaloids is still not known but they have activity against a wide variety of neoplasms.

The following examples illustrate the practice of this invention in connection with vinca alkaloids:

EXAMPLE 26

Vinblastine sulfate may be administered I.P. to mice at a dosage of 0.15 mg./kg. and concomitantly DMSO is administered I.P. in 40% isotonic solution to a dosage of 2 mg./kg.

This example illustrates the specific use of DMSO, administered separately, to enhance penetration of the antineoplastic agent from the general circulation into the neoplastic tissue.

Hormones

Various hormones, principally steroid hormones and their hormonally active substitutes, have activity against certain types of neoplastic conditions. The corticosteroids, as for example cortisol, hydrocortisone, dexamethasone and prednisone are used in cases of lymphosarcoma, Hodgkin's disease, acute leukemia and mammary cancer metastases to obtain at least temporary remissions. Estrogenic compounds, as for example diethylstilbestrol, estrone, ethinyl estradiol, dienestrol, chloroestrinsane and estradiol propionate, are used primarily in treatment of prostatic carcinoma and to some extent in carcinoma of the breast, chorloepithelioma and bladder cancer. Androgenic steroids, principally testosterone, are employed primarily in treatment of breast cancer.

For specific examples illustrating the practice of this invention in connection with hormonal antineoplastic agents, reference may be made to previous Example 13 and the following example:

EXAMPLE 27

A suppository formulation may be prepared by melting together the following:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol valerate</td>
<td>2.75</td>
</tr>
<tr>
<td>Guaiacol glycerol stearate</td>
<td>100</td>
</tr>
<tr>
<td>DMSO</td>
<td>300</td>
</tr>
<tr>
<td>Diglycol laurate</td>
<td>150</td>
</tr>
</tbody>
</table>

The melted blend is poured into a suppository mold to provide 10 gm. suppositories for treatment of prostatic cancer.

Antigens

Antigens are proteins, protein-polysaccharide complexes and polysaccharide which, when foreign to the blood stream of an animal and upon gaining access to the tissues of such animal, stimulate the formulation of a specific antibody. These antibodies, synthesized by the animal in response to the antigenic stimulus, react with the invading antigen to render it harmless. However, despite this defensive mechanism, antigens can be toxic when they gain access to animal tissue, either through contact from exogenous sources or when produced in the tissue due to microorganism infection.

As a means of diagnosis of antigen sensitivity and as a prophylactic or desensitizing treatment to build up an antibody defense against the invasion of antigens, such antigens, in specially prepared forms, are administered to susceptible subjects.

For example, in diagnosing allergy states, various suspected antigens to which the subject may be allergic are applied intradermally or topically to the scratched skin of the subject. Allergy to a particular antigen is detected from the characteristic wheal and flare response. In treating allergic states, desensitization may be achieved by injection of an extract of the causative antigen in a series of gradually increasing doses.

In prophylactic treatment to immunize subjects to the invasion of antigens of the infective type, toxoids or vaccines are administered to the subject, usually by injection. The antigenic effect of the toxoids and vaccines produces specific antibodies which will then destroy subsequently invading antigens of specific types.

For these purposes, antigens generally must be administered by injection, because, in conventional formulations, they are usually not effectively absorbed through the intact or even abraded skin or mucous membrane. The discomfort, risk of injury and infection and cumbersome procedures involved in such routes of administration are readily apparent.

Antigens may be administered topically with DMSO to the skin or mucous membrane to avoid the disadvantages inherent in subdermal application. Additionally, the DMSO, when combined with the antigen frequently has a denaturing effect upon the antigen to decrease its toxicity to a more acceptable level. This may permit the avoidance of some of the usual extensive procedure required to treat the antigen to detoxify or attenuate it. Where cutaneous application of antigens of a higher molecular weight is desired, they are preferably applied by occlusion techniques and desirably they are applied to the more easily penetrated areas such as the axillary region. However, better results may be obtained with larger proteins and protein-polysaccharides by application to mucous membranes.

The following illustrates the practice of the present invention with various classes of antigens.

Allergens

Certain antigens cause an adverse reaction of the tissues of certain subjects, on exposure thereto (mediated by specific antibody formation), which antigens, in similar amounts, are innocuous to other persons. The condition of sensitivity to such antigens is termed allergy and, accordingly, such antigens are also termed allergens. The chief manifestations of the allergic reaction to allergens are "hay fever," asthma, gastrointestinal disturbance, urticaria, angioneurotic edema, and serum sickness.

There is a wide variety of allergens and their mode of contact is varied. These include such material as vegetable and animal epithelial emanations, various pollens, such as tree pollens, grass pollen, pollen from ragweed, and dust.
As a diagnostic procedure to determine causative allergens, a series of suspected allergens are screened by contacting the tissue of the subject manifesting allergy symptoms with small amounts of the suspected allergens. The patient is then observed for a wheal and flare response at the site of administration. A positive response is indicative of allergy to the tested allergen. An extract of the confirmed allergen is conventionally prepared by solvent extraction from extraneous matter with which it may be associated. The extracted antigen is then detoxified through attenuation by heat and/or dilution with the solvent. The denatured allergen is then administered to the subject in a series of increasing doses to desensitize the patient. In this invention, DMSO is utilized to enhance penetration of these conventional attenuated extracts.

The following examples illustrate compositions and treatments, both diagnostic and desensitizing (hyposensitizing), utilizing a variety of allergens:

**EXAMPLE 28**

A twenty-four year old male subject had a four year history of recurrent hay fever supposedly based on an allergy to tree pollenantigens. He was topically administered an allergenic extract of tree pollen on the upper area of the left forearm. An inch below this area, the extracted allergen in one cc of 100% dimethyl sulfoxide was placed. Below this area was placed one cc of 100% dimethyl sulfoxide. After on hour only the composition containing allergenic extract and dimethyl sulfoxide showed a positive reading. The reading was evaluated as a "marked" positive reaction.

This same subject was later administered increasing doses of diluted tree pollen extract starting with an initial dose of 0.1 cc placed into one cc of 100% dimethyl sulfoxide every seven days. The tree pollen allergenic dose was increased but was always given with one cc of 100% dimethyl sulfoxide. The composition was applied directly onto the skin without resorting to injection by the usual intracutaneous and subcutaneous route. The allergen passed through the skin without a needle as shown by a characteristic reaction which occurred about three hours after each application. There was a marked diminution in the subject's symptomatology after three months of treatment.

**EXAMPLE 29**

Solution A containing 2% of histamine in water was applied to the underside of the forearm. A like concentration of histamine in 98% dimethyl sulfoxide containing 2% water was also applied to the surface of the underside of the forearm. A typical wool was formed from the composition containing dimethyl sulfoxide, whereas no wool was produced with the control histamine solution. This shows that DMSO enhances histamine penetration. While not, itself, an allergen, histamine is useful in hyposensitization therapy in treating allergies.

**Infecetive antigens**

In a wide variety of infective diseases, prophylactic treatment through immunization may be effective. Conventionally, an infective microorganism is administered to the subject to be immunized in order to stimulate the production of antibodies in the subject as a defense against subsequent invasion of the infective microorganism. Usually it is necessary to attenuate the microorganism preliminarily by denaturing it with heat or chemicals or through the attenuating action of successive incubations or infective stages in living tissue. Some infective antigens, such as cowpox, utilized as an antigen for smallpox, produce a low grade infection which produces antibodies specific against a more virulent microorganism, e.g. smallpox.

The vaccine or toxoid thus produced is conventionally administered by injection in a solution or suspension. Examples of conventional toxoids are tetanus and diphtheria toxoids. The various vaccines include those for hoof and mouth disease, tuberculosis, cholera, influenza, typhoid fever, yellow fever, mumps, measles, whooping cough, and pertussis (autogenous vaccines in mycotic infections).

In this invention, DMSO is utilized to enhance penetration of the conventional attenuated antigens. Additionally, DMSO may be used to assist in attenuating the antigens. The following examples illustrate compositions and immunization treatments utilizing a variety of infective antigens:

**EXAMPLE 30**

Immunizing formulations utilizing DMSO for enhancing penetration may be prepared utilizing the following:

calf lymph smallpox vaccine
tetanus toxoid, formaldehyde detoxified
killed typhoid and paratyphoid A+B bacillus

Single immunization doses of each of these vaccines are prepared in a mixture of 0.5 ml. glycerine and 0.5 ml. DMSO. 0.25% hexachlorophene is added as a preservative.

These formulations may be applied topically to the mucous membranes, for example they may be applied as nose drops to the nasal passages. Application cutaneously by air jet injector may also be advantageous.

**EXAMPLE 31**

An influenza virus vaccine may be prepared by combining the following formaldehyde-inactivated virus strains in a mixture of 7 ml. DMSO, 1 ml. glycerine and 2 ml. water:

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taiwan 1/64 (A2)</td>
<td>1000</td>
</tr>
<tr>
<td>Japan 170/62 (A2)</td>
<td>1000</td>
</tr>
<tr>
<td>FP 8 (A)</td>
<td>1000</td>
</tr>
<tr>
<td>Ann Arbor 1/57 (A1)</td>
<td>1000</td>
</tr>
<tr>
<td>Maryland 1/59 (B)</td>
<td>2000</td>
</tr>
</tbody>
</table>

An adult dose of 1–2 ml. of this formulation may be administered twice at a six week interval, either cutaneously with an air jet injector or intranasally as nose drops.

**EXAMPLE 32**

A combination of types 1, 2 and 3 killed poliomyelitis virus strains in a concentration sufficient for one immunizing dose is prepared in 1 ml. of:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>80</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
</tr>
</tbody>
</table>

and protected by addition of 50 mcg. of neomycin base per ml.

This formulation provides an adult dose which may be applied intranasally as nose drops or as a spray. Cutaneous application by air jet injector may also be desired. Serial doses are given in accordance with standard procedure.

**Anti-un cellular microorganism agents**

DMSO is useful in enhancing the penetration and effectiveness of antimicrobial agents having growth-inhibiting properties relative to unicellular microorganisms, i.e., anti-unicular microorganism agents. By "growth-inhibiting" properties it is intended to mean the exhibition of biocidal or biostatic effects toward unicellular microorganisms. The phrase "unicellular microorganism" is intended to include those microorganisms commonly designated by microbiologists as consisting of single cells, such as bacteria, viruses, rickettsiae, yeasts and protozoa, as opposed to multicellular microorganisms such as most fungi. DMSO provides enhanced penetration of the anti-
microbial agent to the locus of the unicellular microorganism. DMSO may additionally provide a lowering of the resistance of the microorganisms to the anti-unicellular microorganism agent, perhaps through action upon the cell wall of the microorganism.

There are many infections in animals caused by bacteria, and there are a good number of agents employed to combat such bacterial action. Following is a discussion of some of the more common antibacterial agents and examples of the use of DMSO to enhance penetration of these agents.

The treatment of tuberculosis caused by the tubercle bacillus Mycobacterium tuberculosis is commonly effected with such drugs as amino salicylic acid derivatives, isoniazid, viomycin, dapsone, and pyrazinamide. The use of DMSO with these or other antituberculosis drugs may be effective in enhancing the penetration of these drugs to the situs of the tubercle bacillus. Additionally, it may render the bacillus more susceptible to the action of the anti-tuberculosis drug.

**EXAMPLE 33**

The following lotion may be formulated, containing about 13% by weight isoniazid:

<table>
<thead>
<tr>
<th>Component</th>
<th>Gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide</td>
<td>80</td>
</tr>
<tr>
<td>Ethanol</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
</tr>
</tbody>
</table>

The above lotion may be employed in the treatment of tuberculosis verrucosus cutis by cutaneous applications of 1 to 2 ml. to the sitiis three times daily. A saran-coated occlusive bandage over the site of application may improve percutaneous absorption.

Urinary tract infections are caused by a variety of bacterial organisms, and these may be treated with synthetic chemotherapeutic agents such as hexamethylene tetramine, orthophenyl phenol and sulphanilamides, and broad spectrum antibiotics such as the tetracyclines and penicillins. Again, the treatment of such infections by a combination of the usual chemotherapeutic agent plus DMSO may potentiate the action of the chemotherapeutic agent upon the bacteria causing the infection by rendering the microorganisms less resistant to the action of the agent in addition to enhancing the penetration of the agent to the situs of the microorganism as in the case of a urinary tract wash.

The following examples illustrate a procedure useful for treatment of urinary tract disorders (as well as other disorders pertinent to the agents employed):

**EXAMPLE 34**

Five female dogs weighing between 11 and 13 kgs. were anesthetized with sodium pentobarbital. Each animal was cattedheterized, its bladder emptied and the test solution instilled through the catheter. The various test solutions were made from the following basic solutions. The gram percent and milligram percent figures relate to the number of such weight units per 100 cc. of a final liquid volume.

<table>
<thead>
<tr>
<th>Component</th>
<th>gm. percent</th>
<th>Gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium salicylate</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>Sodium sulfadiazine</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Evans blue dye</td>
<td>62 1/2</td>
<td></td>
</tr>
<tr>
<td>Sodium heparin (145 units per mg.)</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

Three solutions were prepared from the basic solutions by adding 15% v/v. of dimethyl sulfoxide to one; 15% v/v. acetone to the second; and 15% v/v. of isotonic saline to the third.

A sodium phosphate buffer system was added which was 0.05 M trisacetic sodium phosphate, 0.33 M phosphoric acid, and 0.67 sodium chloride, to make a total of 75 cc. with a pH of 9.

With respect to each of the solutions, 75 cc. of such solution was instilled into the urinary bladders of one of the dogs.

Aminophyllin in 10 gmc./kg. of body weight was inserted into the urinary bladders of the remaining dogs. In one of these 15% dimethyl sulfoxide was added and in the other 15% normal saline. The sodium phosphate buffer was used to bring the volume of both the dimethyl sulfoxide and saline solutions to 75 cc. with a pH of 9.

All five test solutions were allowed to remain in the bladder for four hours. Blood samples were taken from the femoral veins prior to instillation and at 30, 60, 120 and 240 minutes after instillation. With respect to the first three dogs, serum sulfadiazine and salicylate levels were determined on each sample as the diazotized derivative and the ferric salt respectively. Lee and White three tube clotting times were recorded at the same intervals. Gross post mortem inspection was made to detect the presence of the dye.

Referring to the drawings, as seen in FIG. 1, clotting time appeared significantly increased in the dimethyl sulfoxide animals as compared to the saline control animals.

As seen in FIG. 2, a six- to eight-fold increase of serum sulfadiazine was noted in the dimethyl sulfoxide animals beginning at the initial 30-minute period over either the acetone or saline controls.

As seen in FIG. 3, a two- to four-fold increase of serum-salicylate was present in the dimethyl sulfoxide dogs over either the acetone or saline controls paralleling the serum sulfadiazine rise.

Direct inspection of the urinary bladders at the conclusion of the procedure revealed that in both the acetone and saline control animals, the Evans blue dye had penetrated through the mucosa into the muscular layers of the bladder and on opening the peritoneal cavity, the bladder had a faintly bluish tinge. In the dimethyl sulfoxide animals, however, the entire bladder was blue, and in addition, the anterior peritonium overlaying the bladder, the retroperitoneal tissues and the contiguous small and large bowel had bluish tinges.

As to the last two dogs, there was a two-fold increase in the absorption of aminophyllin expressed as milligrams per 100 cc. at 60 minutes after instillation into the bladder.

The dimethyl sulfoxide animal had 4.6 milligrams percent while the saline control was 2.17 milligrams percent at the end of 120 minutes. After 240 minutes, the figures were respectively 5.75 milligrams percent for the dimethyl sulfoxide dog and 3.77 milligrams percent for the saline control.

**EXAMPLE 35**

The following irrigation solution may be formulated containing about 5% by weight of hexamethylene tetramine:

<table>
<thead>
<tr>
<th>Component</th>
<th>Gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide</td>
<td>15</td>
</tr>
<tr>
<td>Water</td>
<td>80</td>
</tr>
<tr>
<td>Sulfoxazole</td>
<td>5</td>
</tr>
</tbody>
</table>

The above formulation may be employed as a twice-daily instillation of 10 to 20 ml. for the treatment of urethritis.

**EXAMPLE 36**

The following antiseptic jelly may be formulated:

<table>
<thead>
<tr>
<th>Component</th>
<th>Gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>70</td>
</tr>
<tr>
<td>Ortho phenyl phenol</td>
<td>0.15</td>
</tr>
<tr>
<td>Phenyl mercuric acetate</td>
<td>0.01</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>1</td>
</tr>
</tbody>
</table>

The foregoing jelly may be packed in 3-gm plastic tubes with a urethral nozzle for treatment of urethritis. Leprosy, like tuberculosis, is caused by an acid fast microorganism, Mycobacterium leprae. Leprosy leads to deformities and paralysis from involvement of the nerves and from injuries of anaesthetic areas of the skin. Various
drugs are employed to treat this disease such as diniido-phenyl sulfone (dapsone), chaulmoogra oil, and diethi- dithiol isothalate (etsuf). Treatment with a leprosy drug plus DMSO has led to some improvement in patients suffering from this disease. An example of a suitable formulation is illustrated in the following example:

**EXAMPLE 37**

The following solution may be formulated containing dapsone as the active ingredient:

<table>
<thead>
<tr>
<th>Gms.</th>
<th>Water</th>
<th>Dapsone</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>20</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Morning and evening cutaneous application of the foregoing solution may be made directly to skin lesions.

Penicillin is the drug of first choice in infections caused by pneumococcus, streptococcus, gonococcus, susceptible strains of Staphylococci, Treponema, Clostridia, B. anthracis and Proteus mirabilis. It is potentially the drug of choice with a secondary indication for use against infections by Bacteroides, Actinomyces, and Salmonella. The use of DMSO with penicillin is illustrated in the following example:

**EXAMPLE 38**

A 20-year old male with bilaterally-infected ingrown toenails was treated with 90% dimethyl sulfoxide applied topically to the right great toe and 90% dimethyl sulfoxide plus 10 cc. of aqueous penicillin (i.e. penicillin G), containing 1 million units applied to the left great toe. After two days, there was no inflammation or infection in the toe treated with the dimethyl sulfoxide and penicillin composition, whereas the toe treated with dimethyl sulfoxide alone had a minimal subsidence of infection. The penicillin in the composition was therefore carried across the skin barrier so that it contacted the infected infection site. These results show that dimethyl sulfoxide enhanced penetration of the antibiotic.

The streptococmyc group of antibiotics which includes streptococmycin, neomyacin, kanamycin, paromomycin, and viomycin are bactericidal for a wide variety of bacteria, including the tubercle bacillus, but must be used with care in view of their renal and central nervous system toxicity. This group of drugs is effective against many penicillin-resistant organisms.

The tetracyclines, particularly tetracycline, oxytetracycline chlortetracycline and demethylchlortetracycline, are the drugs of first choice in infections by Shigella, Brucella, Bacillol, and the Poltischen-LGV-Trachoma viruses. They are possible drugs of choice (secondarily indicated) in Klebsiella infections, hospital-borne coliform infections, and respiratory tract infections.

As previously indicated, DMSO may additionally act to increase the sensitivity of previously resistant strains of microorganisms to antibiotics. Following Examples 39 and 40 illustrate this aspect:

**EXAMPLE 39**

A 36-year old female with an axillary abscess was attended surgically. The abscess was incised and cultured. The findings were: Staphylococcus, coagulase positive, resistant to penicillin, tetracycline and erythromycin, but sensitive to chloromycetin. An application of 50% aqueous dimethyl sulfoxide was made directly into the abscess three times daily. At the end of 48 hours and abscess was recultured and showed staphylococcus, coagulase positive, sensitive to penicillin, tetracycline, erythromycin and chloromycetin. It may be suggested that dimethyl sulfoxide alters sensitivity of the bacteria to antibiotics, perhaps by allowing an increased penetration of the antibiotic into the bacterium.

**EXAMPLE 40**

A 60-year old male subject, with gangrene of the right great toe, secondary to peripheral arteriosclerosis with diabetes, had a culture performed on his toe drainage. The findings were: Staphylococcus, coagulase positive, sensitive to chloromycetin and erythromycin, resistant to penicillin and tetracycline.

Application was made topically with 50% aqueous dimethyl sulfoxide every four hours for 24 hours, and then a reculture of the toe drainage was made. It showed: Staphylococcus, coagulase positive, sensitive also to penicillin and tetracycline.

**EXAMPLE 41**

A 23-year old male with bilaterally infected ingrown toenails on the great toe was treated on the right side with a composition containing 2% iodine, 2.3% sodium iodide and 95.7% dimethyl sulfoxide. The left great toe was treated with a tincture of iodine. A single treatment for two minutes was employed. At the end of four days the dimethyl sulfoxide-iodine composition reduced the infection so that the right great toe was normal, whereas the left great toe was still moderately inflamed. This shows improved germicidal activity of iodine with dimethyl sulf oxide.

**EXAMPLE 42**

The fur was removed from a back area on four rabbits. Three iodine solutions were prepared which contained 2% iodine in 2.3% sodium iodide and 46% aqueous ethanol, and 95.7% dimethyl sulf oxide in water. Each test solution was applied to the exposed area of a different rabbit. Gross observation disclosed that skin discoloration with the dimethyl sulf oxide was transitory relative to the discoloration with the other solutions. The two water and alcohol solutions produced long-lasting surface stains.

Staphylococcus-infected sutures were implant by passing them through the entire skin and subcutaneous portion in the treated area of each rabbit. The iodine sample treated with dimethyl sulf oxide composition applied topically controlled the infection, whereas the aqueous solutions and the ethanol solutions of iodine did not.

**EXAMPLE 43**

The fur was removed from the anterior abdominal area on six rabbits. Two rabbits had 100% dimethyl sulf oxide applied to the areas; two other rabbits had 2% tincture of iodine applied to the areas; and the other two rabbits had a composition applied which contained 2% iodine, 2.3% sodium iodide and 95.7% dimethyl sulf oxide. A full thickness biopsy was taken from the exposed skin areas, the samples transferred to a tube and cultured, and colony counts were taken. The colony counts ranged from 280–100 on the two rabbits which received the tincture of iodine alone. The counts were 20 to 30 on the animals receiving dimethyl sulf oxide alone and the counts were 2 and 3 respectively on the two animals receiving the composition of iodine and dimethyl sulf oxide.

Illustrative of diseases caused by viruses are smallpox, measles, encephalitis, herpes, rabies, etc.

While many drugs are useful in treating viruses in vitro, very few are successful in treating in vivo virus disorders. The most successful of the current antiviral agents are idoxuridine (5-ido-2'-deoxyuridine), N-methylsalin beta thiosemicarbazone, and amantadine. Idoxuridine is useful in treating herpes simplex keratitis, and N-methylsalin beta thiosemicarbazone has proved useful in preventing smallpox after exposure to this disease.

**EXAMPLE 44**

The following lotion may be formulated incorporating idoxuridine at a concentration 0.1 gm./cc. of the following lotion base:

<table>
<thead>
<tr>
<th>Percent</th>
<th>Glycerine</th>
<th>Sodium carboxymethylcellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>14</td>
<td>10</td>
</tr>
</tbody>
</table>

The following formula may be made up in an amount to fill a 100 cc. ampule:

- 20 cc. of 75% Dimethyl sulf oxide
- 14 cc. Water
- 10 cc. Glycerine
- 1 cc. Sodium carboxymethylcellulose
The foregoing lotion may be employed in the treatment of herpes simplex by cutaneous application directly to skin lesions, q.i.d.

The primary disorders caused by protozoa are malaria and amebiasis. The principal drugs employed in the treatment of malaria are quinine, quinacine, quinoline derivatives, and chlorophenyl derivatives.

In the treatment of amebiasis, various drugs are employed such as emetine, various arsenical amebicides, hydroxyquinoline derivatives, antimalarial drugs and antibiotics, such as bacitracin, chlorotetracycline hydrochloride, oxytetracycline hydrochloride, carbomycin, erythromycin, paromomycin, and fumagillin.

EXAMPLE 45

The following vaginal jelly may be prepared and buffered to a pH of about 4.0:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ricinoleic acid</td>
<td>0.8</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.75</td>
</tr>
<tr>
<td>Boric acid</td>
<td>5.0</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>15.0</td>
</tr>
<tr>
<td>Oxyquinoline sulfate</td>
<td>0.03</td>
</tr>
<tr>
<td>Sulfisoxazole (Gantrisin)</td>
<td>8.0</td>
</tr>
<tr>
<td>Traganth</td>
<td></td>
</tr>
<tr>
<td>Acacia</td>
<td></td>
</tr>
<tr>
<td>Methyl paraben</td>
<td></td>
</tr>
<tr>
<td>Potassium hydroxide q.s.</td>
<td></td>
</tr>
<tr>
<td>Potassium bitartrate</td>
<td></td>
</tr>
<tr>
<td>Perfume</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
</tbody>
</table>

The foregoing vaginal jelly may be administered intravaginally as an average dose of 5 cc. in the evening before retiring and again in the morning for the treatment of Trichomonas vaginalis. Various diseases are caused by yeasts. These include moniliasis and North American blastomycosis. Antimicrobial agents employed in treating these diseases include nystatin, amphotericin B, sulfonamides and diamidines.

EXAMPLE 46

The following lotion for topical application to areas infected with yeast organisms may be prepared:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide</td>
<td>80</td>
</tr>
<tr>
<td>Carbowax 1000</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>10</td>
</tr>
<tr>
<td>Nystatin (USP)</td>
<td>100,000</td>
</tr>
</tbody>
</table>

Antihistamines

Antihistamines are agents which antagonize the pharmacological actions of histamine. They are employed in the symptomatic treatment of various allergic states wherein the antihistamine suppresses the symptoms attributable to the actions of histamine released in the body in these disorders. Illustrative of such allergy states are allergies of the respiratory tract, for example, coryza, hay fever and vasomotor rhinitis, allergic dermatoses, angioedema and systemic allergies, for example serum sickness and drug reactions. Additionally, various chemicals and drugs cause histamine release in the body.

Antihistamines also have central nervous system activity as sedatives and hypnotics. They are used in treating nausea and vomiting (antiemetic use), insomnia and symptoms of parkinsonism. They also have local anesthetic activity making them useful in control of itching.

Antihistamines include the ethanolamines, e.g. diphenhydramine, the ethylenediamines, e.g. pyrilamine, the alkylamines, e.g. chlorpheniramine, the pipperazines, e.g. chlorycycline, and the pheonothiazines, e.g. promethazine. In addition, with regard to their use in treating allergy states, antihistamines may be used to counter the side effects due to histamine release caused by the application of DMSO to tissue. Notably, antihistamines may be incorporated into DMSO formulations for topical application (the DMSO being employed either to enhance penetration of some other agent or for its own direct pharmacological effect) to suppress symptoms of histamine release caused by the DMSO (e.g. urtica, burning sensation, etc.). The following examples are illustrative.

EXAMPLE 47

A twenty-two year old white male subject was treated who had a three hour history of a common cold. He had marked nasal congestion and discharge. Two mg. of diphenhydramine hydrochloride (Benadryl) in 0.5 cc. of normal saline was placed into each nostril. The symptoms cleared, and the subject was asymptomatic for one and one-half hours. One half cc. of 50% dimethyl sulfoxide in water was placed in each nostril. The symptoms cleared and remained relieved for three hours.

After the symptoms of nasal congestion and discharge returned, the subject was treated with 0.5 cc. of 50% dimethyl sulfoxide in an aqueous solution containing 2 mgs. of diphenhydramine hydrochloride. The symptoms were quickly relieved and remained absent for twelve hours.

EXAMPLE 48

A cyclizine hydrochloride mist formulation may be prepared and charged into an aerosol container to provide fifty 100 mg., doses which may be administered intranasally for treatment of allergy states and motion sickness, etc. Five grams of cyclizine hydrochloride are incorporated in a halocarbon propellant formulation base containing 4% by volume DMSO.

EXAMPLE 49

A topical ointment formulation of triptelennamine base particularly suitable for treatment of itching dermatoses (applied to the affected area several times daily), may be formulated as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triptelennamine base</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>70</td>
</tr>
<tr>
<td>Sodium carboxymethylcellulose</td>
<td>4</td>
</tr>
<tr>
<td>Water</td>
<td>24</td>
</tr>
</tbody>
</table>

Neuropharmacologic agents

"Neuropharmacologic agents" are those agents having a pharmacologic activity involving the nervous system.

Central nervous system active drugs have their site of activity in the brain and/or spinal cord. Other agents have their activity in the peripheral nervous system. In every case, the rapidity and magnitude of response to a neuropharmacologic agent is dependent upon the speed and extent of conduction or movement of the agent from the site of administration to the site of activity in the nervous system. In many instances, the usefulness of such drugs is limited by the inability of such agents to rapidly penetrate membranes to achieve a useful concentration or distribution at the site of activity in the nervous system. The use of any neuropharmacologic drug must take into account the problem of getting the drug from the periphery to the action site which requires transport across neural barriers, including the blood-brain barrier in the case of CNS agents.

Central nervous system active agents

Central nervous system active agents are those agents which stimulate, depress or otherwise modify central nervous system function to either a selective or non-selective manner. Most of the central nervous system active agents described and illustrated under the following subheadings have useful activity in one or more supramedullary portions of the brain, i.e., those portions lying above the medulla oblongata including the cerebrum, cerebellum, thalamus, and hypothalamus. An example is the amphetamine of Example 34 which is active in the cerebral cortex. Agents which provoke a neuropharmacologic response in the central nervous system generally must pass through the body from the site of administration, through
the blood stream, to the blood/brain barrier. To carry out
their activity, these agents must pass through this
barrier. Relatively little is known regarding passage of
drugs into the brain from the blood stream, but in effect
the "blood-brain barrier" behaves like a lipid membrane.
Most agents pass through this barrier with great difficulty.
The effectiveness of a neuropharmacologic agent on the
central nervous system is dependent upon the rate and
extent of its penetration across this barrier. Therefore, in
addition to enhancing penetration of such agents from the
site of administration into the blood stream, dimethyl
sulfoxide may also play the role of enhancing penetration
of these agents across the blood-brain barrier. Following
are illustrations of the various classes of central nervous
system active agents with specific examples of the use of
dimethyl sulfoxide to enhance penetration of these agents:

Analgesics and antipyretics

Analgesics exert type of depressing action upon the
central nervous system, the result of which is the obtunding
of pain sensations without the loss of consciousness. Some
classes of analgesics, e.g., the salicylates, para-aminophenol
derivatives and the pyrazon derivatives, also are antipyrinics
and their lower abnormal body temperature through
a predominately CNS action. Additionally many
agents in these classes have other actions such as anti-
inflammatory action. The para-aminophenol derivatives
include acetanilid, acetylsalicylic acid and acetylsalicylic
acid. Exemplary of the salicylates are acetylsalicylic acid and
salicylic acid and their lower abnormal body temperature
through a predominately CNS action. Additionally many
agents in these classes have other actions such as anti-

EXAMPLE 50

The following injectable formulation is prepared in 2 cc.
ampules to provide doses of 1–2 cc. for intramuscular
injection (q.i.d. for sustained relief):

Meperidine hydrochloride------------------------mg. 250
DMSO ----------------------------------------gm. 1.5
Water ----------------------------------------gm. 8.5
Sodium acetate -----------------------------gm. 0.5
Sodium formaldehyde sulfoxylate ----------------gm. 0.5

General CNS depressants

General CNS depressants act nonselectively to depress
all excitable tissue, in general, through stabilization of part
or all of the neuronal membrane. They are utilized as
general anesthetics, hypnotics and sedatives. Prominent
among these agents are the barbiturates and related
sedative-hypnotics such as the chloral derivatives, bro-
mides, tertiary acetylcholines, carbamoyl esters of alcohols
and glycols (e.g., ethaminate and mebroba-
mate), monoureides, diureides, benzodiazepines, piperi-
dinedione derivatives, etc. The following examples are
illustrative:

EXAMPLE 51

Three groups of dogs were evaluated. Three dogs in
group No. 1, weighing about 15 to 18 kgs., were given
1 cc. containing 50 mgs. of sodium pentobarbital (Nem-
butal) per 5 pounds of body weight, intravenously. This
produced a profound anaesthetic effect, allowing an ab-
dominally closed procedure to be performed without causing
discomfort to the dog. The last group of three dogs were given 1 cc.
of Nembutal per 10 pounds of body weight. These dogs re-
mained partially awake and surgery could not be per-
formed without causing discomfort to the dogs. The last
group of three dogs were given 1 cc. of Nembutal per 10
pounds of body weight plus 1 gram of dimethyl sulfoxide
per 2 pounds of body weight, instilled orally through a
gastric tube. After one-half hour, surgery could be per-
formed without the animal experiencing any discomfort.

EXAMPLE 52

A chloral hydrate suppository formulation may be pre-
pared by blending together:

Chloral hydrate-------------------------------------Gms. 10
DMSO -------------------------------------------Gms. 4.7
Stearic acid --------------------------------------Gms. 1
Water -----------------------------------------0.5

The melt is poured into a suppository mold and cooled
to form 10 suppositories each supplying a 1 gram dose.
One or two suppositories, as indicated, may be adminis-
tered as a general sedative.

EXAMPLE 53

A unit dose suppository form of imipramine can be prepared by melting together:

Imipramine base -------------------------------mg. 50
DMSO -------------------------------------------Gm. 4.5
Sodium stearate ---------------------------------Gm. 1.0
Glycerine ---------------------------------------Gm. 4.5
Water -----------------------------------------1.0

and cooling the melt in a suppository mold. This dosage
can be administered rectally T.I.D. to relieve depression.

Anticonvulsants and centrally acting muscle relaxants

The various CNS-active agents used to treat convulsive
disorders, particularly the various types of epilepsy, in-
clude the barbiturates, glutamates, hydantoins, acetyl-
ureas, oxazolidinediones and the succinimides. Central-
ally acting muscle relaxants have the ability to diminish
skeletal muscle tone and involuntary movement by action
on the CNS. They are used as an adjuvant in general
anaesthesia and in treatment of alcoholism, parkinsonism
and cerebral palsy.

Such agents include mephenesin, methcarbamol, sty-
rane, chloroazoxane, carbaprodol, metaxazone, tri-
heptyphenylid and benzoprine mesylate. The following
example is illustrative:

EXAMPLE 54

The following parenteral formulation may be prepared and sterilized for treatment of grand mal and psycho-
motor epilepsy to achieve more rapid therapeutic benefit.

Diphenylhydantoin -----------------------------mg. 250
DMSO ----------------------------------------ml. 4
Water ----------------------------------------ml. 1

The dose is 1–3 cc. preferably administered intramus-
cularly.

Psychopharmacological drugs

Agents used to treat psychiatric disorders fall into sev-
eral categories. Drugs used to treat anxiety and neuropo-
logical conditions include the benzodiazepine derivatives, mebro-
bamate and various other non-barbiturate and barbiturate
sedatives which have been treated previously concerning
their employment in the present invention, under the
heading of general CNS depressants. The predominant
drugs employed to treat psychosis are primarily the
phenothiazines and the rauwolfia alkaloids. In treatment of
depression, monoamine oxidase inhibitors and dibenz-
zepine derivatives are the predominant drugs. Pre-

3,551,554
EXAMPLE 55

The following formulation may be melted together, placed in a suppository mold and cooled to form a unit dose for rectal application as a monoamine oxidase inhibitor for treatment of depressive states:

N-benzyl-N-methyl-2-propynylanine \( \text{gm} = 100 \)
DMSO \( \text{gm} = 4 \)
Diglycol laurate \( \text{gm} = 1.5 \)
Carbopol 934 \( \text{gm} = 0.25 \)
Triethanolamine \( \text{gm} = 0.01 \)

Peripheral nervous system active agents

Agents which have activity in the peripheral nervous system include local anesthetics which reversibly block nerve conduction when applied locally to nerve tissue, thereby to relieve pain. They also include agents which have activity at various sites in the autonomic nervous system, including anticholinesterase agents, parasympathomimetic agents, sympathomimetic agents, antimuscarinic agents, adrenergic blocking agents, ganglionic blocking and stimulating agents and neuromuscular blocking agents. Following is a discussion and illustration of the employment of DMSO with these classes of agents in accordance with the invention.

Many compounds which produce local anesthesia have a common fundamental structure, namely, a hydrophilic amino group (a tertiary or secondary amine), connected by an intermediate group through an amide bond or ester linkage to a lipophilic aromatic residue. Representative of these agents are procaine, lidocaine, piperocaine, dibucaine, tetracaine, cyclohexylamine, pramoxine and diphenylamine. Others include benzocaine, orthoform, butyl aminoalcohol, benzyl alcohol, methol, phenol and quinine. Antihistamines have also been employed for this purpose. Because of their low solubility in water and other systems in their active form, local anesthetics are frequently utilized in the form of water soluble salts, rather than as free bases. Advantageously, DMSO may permit use of the free base form when its is employed as a solvent for the local anesthetic in addition to being utilized as a penetrating agent. Employment of local anesthetics with DMSO may provide a special advantage in relieving the burning sensation frequently experienced when DMSO is applied topically for its own pharmaceutical effects or for enhancing the penetration of yet another pharmaceutical agent. The following examples are illustrative:

EXAMPLE 56

A 1% procaine hydrochloride isotonic solution was administered by subcutaneous method in 0.5 mm doses in the human skin. In another human subject 1% procaine in isotonic solution containing 15% v/v of dimethyl sulfoxide was administered by subcutaneous injection in a 0.5 mm dose. The dimethyl sulfoxide composition produced a local anesthetic effect having a prolonged activity of 1.5 times procaine alone.

EXAMPLE 57

Isolated peripheral cutaneous nerves of dogs were stimulated with electrodes to measure impulse conduction. Application of 25% aqueous dimethyl sulfoxide to a first nerve provided a local and reversible block of impulse conduction which lasted twenty minutes. Application of a 2% procaine hydrochloride solution blocked impulse conduction for forty-five minutes. Application of an aqueous solution containing 25% dimethyl sulfoxide and 1% procaine to a third nerve blocked impulse conduction for one and one-half hours. This shows and enhanced effect when procaine is combined with dimethyl sulfoxide.

EXAMPLE 58

The following solutions were prepared:

Solution A

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine hydrochloride</td>
<td>2%</td>
</tr>
<tr>
<td>Trisodium phosphate buffer</td>
<td>2</td>
</tr>
<tr>
<td>Glycerine</td>
<td>2</td>
</tr>
</tbody>
</table>

Solution B

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine hydrochloride</td>
<td>2%</td>
</tr>
<tr>
<td>Trisodium phosphate buffer</td>
<td>2</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>2</td>
</tr>
</tbody>
</table>

Solution A was administered topically to the general area overlying the mental foramen of one human subject, and solution B was also administered topically to the mental foramen of another human subject. The general area overlying the mental foramen is in midline of the exterior surface of the mandible. Solution B produces sufficient local anaesthesia within fifteen minutes to permit a lower bicuspid tooth with carious lesions to be drilled without discomfort. The subject receiving solution A did not develop such a level of anaesthesia within the same time limit.

EXAMPLE 59

A forty year old female subject with pruritis ani was given a topical application of 3 cc of 100% dimethyl sulfoxide to the irritated area. Relief of itching occurred in fifteen minutes and lasted for eight hours. The subject complained of a mild "burning sensation" in the area of application which lasted for ten minutes. The same burning sensation was reported after each of four applications over a two-day period. A 4% xylacaine solution in 100% of DMSO was made. Four cc of the mixture were applied to the same subject who reported similar relief of discomfort with no "burning." This example shows that small amounts of a local anesthetist will lessen the discomfort which may occur with dermal application of high concentrations of dimethyl sulfoxide.

EXAMPLE 60

Two solutions were prepared, one containing 0.44% xylacaine base in 100% dimethyl sulfoxide and the other containing 0.5% menthol in 100% dimethyl sulfoxide. Human subjects who previously reported a "burning sensation" with 100% dimethyl sulfoxide reported absence of such a "burn" when they were given like amounts of the two solutions in a like dermal application.

EXAMPLE 61

Neoagmine, one of the most prominent anticholinesterase agents, may be formulated with DMSO in single dosage form as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoagmine base</td>
<td>0.5</td>
</tr>
<tr>
<td>DMSO</td>
<td>0.7</td>
</tr>
<tr>
<td>Water</td>
<td>0.3</td>
</tr>
<tr>
<td>Methyl propyl paraben</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

This formulation may be packaged in 1 cc ampules each of which is then diluted with 4 cc of distilled water for bladder instillation as a preventative of post-operative distention and urination. The dosage is repeated every 4–6 hours as needed.

EXAMPLE 62

To demonstrate enhanced penetration of the sympathomimetic agent ephrinephrine, ephrinephrine hydrochloride is dissolved in 10% aqueous DMSO to a concentration of 0.05% and applied topically to the stretched ear of a hamster fixed in position for low power microscope viewing of the capillary bed. Whereas ephrinephrine hydrochloride dissolved in water (at the same concentration) applied to the hamster ear produces no visible change
in appearance of the capillary bed, with the DMSO formulation pronounced constriction of the capillary bed being observed by microscope and a general blanching of skin is seen. This effect is transitory. The same DMSO formulation may be used as a nasal spray for relief of asthmatic attacks.

EXAMPLE 63

The sympathomimetic agent, methamphetamine may be formulated in a unit dose with DMSO for intramuscular injection as follows:

- Methamphetamine: 20 mg
- DMSO: 0.3 ml
- USP water: 0.7 ml
- Propyl paraben: 0.0001 gm

The product is packaged in a 1 cc. ampule for administration to maintain blood pressure in hypotensive states as during spinal anesthesia.

EXAMPLE 64

Mecamylamine is representative of agents having a site of activity at the autonomic ganglia (ganglionic stimulating and blocking agents). It may be formulated as follows to provide a single dose in suppository form for rectal application in treatment of hypertension:

- Mecamylamine base: 15 mg
- Propylene glycol stearate: 4.5 gm
- DMSO: 4.5 gm
- Triethanol amine: 0.3 gm
- Stearic acid: 1.5 gm

Melt ingredients together and pour into cooled suppository mold.

EXAMPLE 65

Also exemplary of adrenergic agents is the adrenergic blocking agent dihydroergotamine. A topical lotion may be formulated as follows:

- Dihydroergotamine: 200 mg
- DMSO: 180 ml
- water: 18 ml
- Disodium hydrogen phosphate: 2 gm

Two cc. of this preparation may be applied topically in treatment of herpes zoster for relief of neuritic pain or for peripheral vasodilation in arterial deficiencies.

EXAMPLE 66

Representative of parasympathomimetic agents is pilocarpine. The following eye drop formulation may be prepared:

- Gms:
  - Pilocarpine HCl: 3
  - DMSO: 10
  - Isotonic saline: 86.5
  - Sodium dihydrogen phosphate: 0.5
  - to which is added 0.01% benzalkonium chloride as a preservative.

A typical dose for the treatment of chronic open-angle glaucoma is 1–2 drops in the eye every 4–6 hours.

Anti-inflammatory agents

Anti-inflammatory agents are those which diminish the inflammatory response due to tissue injury. They comprehend the corticosteroids considered previously as well as many of the analgesics and antipyretics discussed under that heading (e.g. the salicylates and pyrazolones). For illustration of use of DMSO to enhance penetration of these agents reference is made to preceding Examples 2, 3, 4 and 34 and the following example:

EXAMPLE 67

The following phenylbutazone topical formulation may be prepared and applied in unit doses of 10 cc. bid topically to the involved area to treat musculoskeletal pain and inflammation:

- Gms.
  - Phenylbutazone: 6
  - DMSO: 90
  - H2O: 10

Anticoagulants

Anticoagulants are agents used primarily in thromboembolic conditions to prevent formation of intra-vascular thrombi and to maintain normal hemostasis. Heparin and its soluble metal salts, the most quick acting anticoagulants, previously required parenteral administration so the employment of DMSO to permit topical application is of considerable advantage. The coumarins and indandione derivatives, e.g. bis-hydroxycoumarin and hennindione, may be administered orally. However, in some circumstances, their topical application may be advantageous. When applied topically with DMSO, these agents may be penetrated into the general circulation to provide the desired blood level. Reference is made to foregoing Example 34 and to the following example for illustration of the use of DMSO to enhance penetration of this class of agents:

EXAMPLE 68

Forty thousand units of heparin (1 cc.) with 1 cc. 100% dimethyl sulfoxide was placed onto human skin (left forearm—thirty nine year old male). A Lee and White 3 tube clotting time was taken prior to application. This was 11 minutes.

Clotting times were taken at 2 hours and 4 hours subsequent to administration. At 2 and 4 hours, the clotting times were 14 and 18 minutes respectively. This example shows that dimethyl sulfoxide was associated with absorption of heparin through human skin.

Vasodilators

Vasodilators are generally used clinically in treatment of ischemic conditions, especially myocardial hypoxia, as in angina pectoris, and to some extent in ischemia of skeletal muscle. Their basic pharmacological action is the relaxation of smooth muscle to effect dilatation. These agents include the nitrates, such as sodium nitrite, glyceryl tri-nitrate and isosorbide di-nitrate, di-pyridamole, cyclandelate, nicotinic acid and aminophylline. DMSO may be utilized to promote penetration for topical application of these agents to provide the desired systemic concentration. The following examples and foregoing example 34 illustrates the use of DMSO to enhance penetration of this class of agents:

EXAMPLE 69

Aminophylline is a known diuretic, central nervous system stimulant, coronary vasodilator and bronchodilator which is normally absorbed across the bladder walls. A test solution of phosphate buffer containing 0.033 M. phosphoric acid/100 ml. and 0.05 M. trisodium phosphate/100 ml., containing 15% dimethyl sulfoxide, was compared with a control solution of the same buffer solution. The pH of both buffer solutions was 9. The control and test solutions were instilled in the freshly evacuated bladders of two dogs in an amount of 75 cc. Blood samples were taken at different time periods for each dog, and analysis for aminophylline by the Brackett and Bradford test was done. The results in the following table showed an increased amount of aminophylline in the blood of the dog receiving the test solution.
### Aminophylline Serum Level in Dogs (Mg. Percent)

<table>
<thead>
<tr>
<th>Time in minutes post injection</th>
<th>Average concentration</th>
<th>Average concentration (15% dimethyl sulfoxide)</th>
<th>Difference in concentration (buffer)</th>
<th>Buffer only</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>1.7</td>
<td>2.6</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>90</td>
<td>2.1</td>
<td>4.6</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>120</td>
<td>4.9</td>
<td>8.9</td>
<td>4.0</td>
<td>4.9</td>
</tr>
<tr>
<td>240</td>
<td>2.7</td>
<td>7.2</td>
<td>4.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

A group of two rats received 100 mg of nicotinic acid in 4 cc of 100% of dimethyl sulfoxide applied to the anterior abdomen. A wet state of application was maintained. A control group of two rats received 100 mg of nicotinic acid in 4 cc of saline. At the end of two hours, the test group showed noticeable vasodilation of the skin with an increase in cutaneous temperature. These observations were absent in the control group. This example shows increased penetration with dimethyl sulfoxide of an agent which is both a vasodilator and a vitamin.

### Example 71

A glycerol trinitrate ointment may be prepared by blending the following ingredients:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol trinitrate</td>
<td>2</td>
</tr>
<tr>
<td>DMSO</td>
<td>10</td>
</tr>
<tr>
<td>Ethanol</td>
<td>8</td>
</tr>
<tr>
<td>Carbowax 1500</td>
<td>20</td>
</tr>
</tbody>
</table>

A typical dosage of 10 mg of glycerol trinitrate is provided with ½ gram of this ointment. The dosage may be applied topically as, for example, to the intact skin of the upper arm, or sublingually.

### Ultraviolet screening agents

Compounds which screen a part or all ultraviolet light from the skin to protect the skin from the damaging effects of over-exposure to sunlight are called ultraviolet absorbers or screening agents. They are applied topically to the skin prior to exposure to absorb the ultraviolet rays to thereby prevent injury to the skin. Examples of such agents are 2-ethoxyethyl p-methoxy cinnamate, diglycolyl triolote, menthol anthranilate, p-amino-benzoic acid, phenyl salicylate, and the benzophenones, e.g., 2,4-dihydroxybenzophenone and 2,2'-dihydroxy-4,4'-dimethoxy benzophenone. DMSO may be used to enhance penetration of these agents into the epidermis, thereby to increase their effectiveness and to increase their lasting power by inhibiting their accidental removal from the skin through bathing, perspiration, etc. The following example is illustrative:

### Example 72

A male subject had a skin markedly sensitive to ultraviolet light. A test solution was made containing 1% ultraviolet absorber in 100% dimethyl sulfoxide. A control solution was made containing the ultraviolet absorber in 100% ethanol, and both solutions were similarly thickened with Carbowax 4000 to provide lotion forms. The ultraviolet absorber was 2,2'-dihydroxy-4,4'-dimethoxy benzophenone. The two lotions were applied to different sides of the subject's face. After one day of severe sun exposure, the subject was examined. The side with the control application showed marked redness. The side with the test application showed only slight redness. Two days later, the control side was blistered, whereas the test side was normal and free of redness.

### Diagnostic dyes and radiopaque media

Tissue compatible cationic dyes are employed in tissue staining both in vivo and in vitro for diagnostic procedures. For use in vivo, this may be for marking or following visually the tissue penetration of another agent or for highlighting or outlining a particular organ or structure for visual diagnosis. For example, sodium indigotindisulfonate is injected for localizing ureteral orifices. Cationic dyes are used for in vitro tissue staining to fix cellular structure and the like for microscopic observations and evaluations. Radiopaque dyes are used for the roentgenography of various body structures, usually administered by injection. Preceding Example 34 and the following examples illustrate the use of DMSO to enhance tissue penetration of diagnostic dyes and radiopaque media:

### Example 73

A solution of 2% basic green MX dye in 100% dimethyl sulfoxide was applied to the freshly washed skin of the forearm. The same dye in a like concentration in water, ethanol, acetone and ethylene glycol, was also applied to freshly washed areas of the forearm. The excess dye was removed with detergent or water, and the extent and depth of the dyeing was determined by stripping away approximately 0.5 mm. thick layer of skin with adhesive tape. The dye and dimethyl sulfoxide was found to be the only composition which carried the dye to the deeper dermal layers. This shows the enhanced dye absorption when administered in combination with dimethyl sulfoxide.

### Example 74

A 25% solution of iodine in aqueous sodium iodide was prepared as a first diagnostic solution, and a 25% solution of iodine in 100% dimethyl sulfoxide was prepared as a second diagnostic solution. Twenty ml. of each solution was injected into the intermedulla of femur bones. The first solution was injected into the right femur, and the second into the left femur of the same subject. X-ray photographs were taken at 5-minute sequences over 240 minutes of both femurs. After 40 minutes, a picture of the left femur, into which was injected the second solution, showed the entire intermedullar space to be radiopaque. At the same time, the first solution in the right femur was rapidly diffusing into the vascular system and being removed from the bone. At 120 minutes, a picture of the left femur was still clearly defined as being radiopaque. At the same time, a picture of the right femur showed no evidence of opacity except for about a one centimeter circle around the injection site.

### Nutrients

As used herein the term nutrients is intended to comprehend the vitamins, carbohydrates, fats, proteins, including proteinaceous hormones and mineral nutrients used by the body to sustain and regulate metabolism and furnish energy. Exemplary of the carbohydrates are glucose (see Example 1) and dextrins, (e.g. maltose dextrins). Metabolizable fats are the glycerol esters of fatty acids, e.g. vegetable oils. The various commercial protein hydrolyzates, e.g. hydrolyzed casein, and insulin are representative of the proteins. The vitamins include the water-soluble vitamins, e.g. vitamins B1, B2, B6, and B12, nicotinic acid, pantothenic acid and p-aminobenzoic acid and the fat soluble vitamins, e.g. vitamins A, D, K and E and folic acid. Mineral nutrients include inorganic salts of fluorine, iodine, manganese, potassium, iron, zinc, copper, magnesium calcium and phosphorous. DMSO is useful in enhancing tissue penetration of nutrients for assimilation by the body particularly by the topical or injection routes. Preceding Examples 1 and 70 and the following additional examples are illustrative:

### Example 75

Two dogs of approximately 15 kgs. of body weight were starved for twenty-four hours and administered three units of crystalline zinc insulin subcutaneously. Another group of two dogs was starved for twenty-four hours and administered three units of insulin subcutaneously in 1 cc of 100% dimethyl sulfoxide. The dogs given the composition including the dimethyl sulfoxide were observed to show signs of severe insulin shock two hours after ad-
administration. The insulin without dimethyl sulfoxide did not produce a pronounced physiological response.

EXAMPLE 76

Four dogs with pancreatectomy were given insulin orally. A group of two dogs were used for controls and were given 15 units of insulin in 3 cc. of isotonic saline, and the other group of two dogs was given 15 units of insulin in 3 cc. of 99% dimethyl sulfoxide. Blood sugars were taken at one, two and four hours. The two dogs in the control group started with blood sugars of 82 mg. percent and 90 mg. percent. At four hours, the blood sugars had risen to 100 and 110 mg. percent.

The two animals in the test group receiving the di- methyl sulfoxide started with blood sugars of 80 mg. percent and 85 mg. percent, and at the end of two hours, one had 60 mg. percent and the other 65 mg. percent. At the end of the four hours, one had 40 mg. percent and the other 35 mg. percent. It is believed that the composition containing dimethyl sulfoxide enhanced penetration of the insulin through the esophageal wall, since insulin is known to be destroyed in the stomach.

EXAMPLE 77

A group of two rats received 0.5 gms. of ammonium fluoride in 4 cc. of 100% dimethyl sulfoxide by applying this composition over the abdomen. Another group of two rats served as controls, receiving 0.5 gms. of ammonium fluoride in 4 cc. of isotonic saline. A wet system was maintained. The test group receiving dimethyl sulfoxide and the ammonium fluoride showed typical epilepticiform convulsions associated with the absorption of large amounts of fluoride. The control group did not show such convulsions. This example shows that dimethyl sulfoxide enhanced absorption of ammonium fluoride through intact skin and therefore may increase fluoride absorption into teeth.

EXAMPLE 78

An ointment base may be prepared from the following:

<table>
<thead>
<tr>
<th>Agents</th>
<th>Parts by wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lanolin</td>
<td>--------------</td>
</tr>
<tr>
<td>DMSO</td>
<td>10</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>5</td>
</tr>
</tbody>
</table>

to which is added 1800 U.S.P. units of Vitamin A and 300 U.S.P. units of Vitamin D₃ per gram of ointment base. This ointment is applied topically for the treatment of burns, skin irritation, diaper rash and pruritus.

EXAMPLE 79

Tests were done with monkey kidney cell tissue cultures containing conventional tissue culture media (containing glucose, minerals and amino acids). Two groups of tests were made with replicates. To one group of tissue culture tests was added 0.003% dimethyl sulfoxide, and the other group was retained as a control. Cell counts were performed after three days, and control groups averaged 1,683,000 per cc., whereas the test group with dimethyl sulfoxide tubes averaged 6,804,000 cells per cc. Enhanced cell proliferation, therefore, occurred in cultures containing dimethyl sulfoxide. This growth is believed to have occurred from greater utilization of the nutrient in the tissue cultures containing dimethyl sulfoxide.

What I claim is:

1. A method of enhancing the penetration into and across an external membrane barrier of a human or animal subject of a chemical agent capable of eliciting a physiological effect upon topical application thereof, said agent being selected from the group consisting of non-estrogenic, antineoplastic agents, antigens, antihistaminic agents, neuropharmacologic agents, antinflammatory agents, anticoagulants, vasodilators, ultra-violet screening agents, diagnostic dyes, diagnostic radiopaque agents and nutrients, which comprise the concurrent topical administration to the external membrane of an amount of said agent effective to produce the desired physiological effect and an amount of DMSO sufficient to effectively enhance penetration of said agent to achieve the desired physiological effect.

2. A method as in claim 1 wherein the said agent is applied to the intact skin in a composition which includes said DMSO and wherein the DMSO in said composition is at least about 50% by weight of the composition.

3. A method as in claim 2 and wherein said agent is an antihistaminic agent.

4. A method as in claim 1 and wherein said agent is applied to a mucous membrane of a body cavity in a composition which includes said DMSO and wherein the DMSO in said composition is at least about 10% by weight of the composition.

5. A method as in claim 1 and whereby said agent is selected from the group of compounds consisting of cationic compounds, anionic compounds and non-dissociating compounds.

6. A method as in claim 5 and wherein the molecular weight of said agent is less than about 8000.

7. A method as in claim 1 and wherein said agent is an antineoplastic agent selected from the group consisting of alkylating agents and antimetabolites.

8. A method as in claim 1 and wherein said agent is applied to said membrane in a composition which includes said DMSO.

9. A method as in claim 8 and wherein said agent is a neuropharmacological agent having useful activity in the central nervous system.

10. A method as in claim 9 and wherein said agent is an analgesic.

11. A method as in claim 8 and wherein said agent is a neuropharmacological agent having activity in the peripheral nervous system.

12. A method as in claim 11 and wherein said agent is a local anaesthetic.

13. A method as in claim 8 and wherein said composition contains a pharmaceutically acceptable thickening agent in an amount sufficient to materially increase the viscosity thereof, whereby to facilitate topical application.

14. A method as in claim 13 wherein said composition is in the form of an ointment.

15. A method as in claim 13 and wherein said composition is in the form of a lotion.

16. A method as in claim 13 and wherein said composition is in the form of a suppository.

17. A method of enhancing the tissue penetration of an injectable chemical agent capable of eliciting a physiological effect in a human or animal subject, said agent being selected from the group consisting of antineoplastic agents, antihistaminic agents, neuropharmacologic agents having a useful activity in a supramedullary portion of the brain, central nervous system depressants, analgesics, local anaesthetics, antinflammatory agents, anticoagulants, vasodilators, diagnostic dyes, diagnostic radiopaque agents and nutrients, which comprises the concurrent administration to said subject of an injected amount of said agent effective to produce the desired physiological effect and an amount of DMSO effective topically or by injection to enhance penetration of said agent into said tissue to achieve the desired physiological effect.

18. A method as in claim 17 and wherein said DMSO is administered by injection in a composition containing at least about 1% by weight of DMSO.

19. A method as in claim 17 and wherein said agent is administered by injection in a composition containing between about 1% and 40% by weight of DMSO.

20. A method as in claim 17 and wherein said agent is selected from the group of compounds consisting of
3,551,554

cationic compounds, anionic compounds and non-dissociating compounds.

21. A method as in claim 17 and wherein the molecular weight of said agent is less than about 40,000.

22. A method of enhancing the penetration of a cationic dye through cell membranes of animal cells for diagnostic purposes which comprise applying to said cell membranes a composition comprising an amount of said dye effective to stain said cells and an amount of DMSO effective to enhance penetration of said dye into said cells.

23. A method of enhancing the penetration of a cationic dye through cell membranes of animal cells for diagnostic purposes which comprise applying to said cell membranes a composition comprising an amount of said dye effective to stain said cells and an amount of DMSO effective to enhance penetration of said dye into said cells.

24. A method as in claim 8 and wherein said agent is an antiinflammatory agent.

25. A method as in claim 8 and wherein said agent is a vasodilator.

26. A method as in claim 8 and wherein said agent is an allergen.

27. A method as in claim 8 and wherein said agent is an infective antigen.

28. A method as in claim 8 and wherein said agent is an antihistamine.

29. A method as in claim 8 and wherein said agent is a nutrient.

30. A method as in claim 29 and wherein said nutrient is a vitamin.

31. A method as in claim 30 and wherein said vitamin is a water soluble vitamin.

32. A method as in claim 9 and wherein said agent is a central nervous system depressant.

33. A method as in claim 9 and wherein said agent has useful activity in a supramedullary portion of the brain.

34. A method as in claim 17 and wherein said agent is a neuropathologic agent having a useful activity in a supramedullary portion of the brain.

35. A method as in claim 17 and wherein said agent is a central nervous system depressant.

36. A method as in claim 17 and wherein said agent is an analgesic.

37. A method as in claim 17 and wherein said agent is an antiinflammatory agent.

38. A method as in claim 37 and wherein said antiinflammatory agent is selected from the group consisting of alkylation agents and antimitabolites.

39. A method as in claim 18 and wherein the agent and said DMSO are injected together in the same composition.

40. A method as in claim 39 and wherein said agent is a vasodilator.

41. A method as in claim 39 and wherein said agent is an anticoagulant.

42. A method as in claim 39 and wherein said agent is an antiinflammatory agent.

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SHEP K. ROSE, Primary Examiner

U.S. Cl. X.R.