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# AZONE®: A NEW NON-TOXIC ENHANCER OF CUTANEOUS PENETRATION

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and

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SUMMARY. Azone® (1-dodecylazacycloheptan-2-one) enhances the penetration through the skin of a number of drugs. Penetration of both hydrophobic and hydrophilic molecules is enhanced, although more dramatic enhancements are usually seen with hydrophilic drugs. Molecular weights of the drugs considered seems a relatively minor concern, at least in the range of MW of less than 1000 daltons. Azone need not be in solution to exert its influence; in fact, good enhancements are seen when Azone is employed only as a shaken emulsion in water. The optimum concentration of Azone varies with both the drug and the formulation being examined, and cannot now be predicted.

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In general, however, concentrations of 2-10% appear to be appropriate for most formulations.

INTRODUCTON. Transdermal delivery of drugs is an alluring concept. If successful, this method of delivery should avoid or reduce first-pass effects seen with oral administration; could maintain constant plasma levels of drugs, even those with short half-lives; and might significantly reduce problems of patient compliance, especially in the elderly. Other advantages of transdermal delivery have been considered elsewhere in this volume.

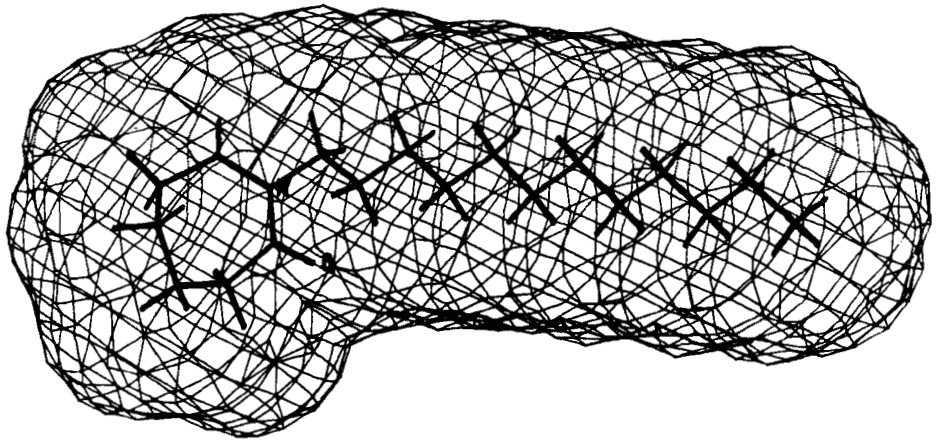
Relatively few drugs can be delivered transdermally (Scheuplein and Blank, 1971; Idson, 1975). Without question, there are examples of agents which penetrate the skin at rates high enough to yield therapeutic levels in the plasma. Scopolamine and nitroglycerin have been thoroughly discussed in this volume. However, the majority of drugs will not penetrate at rates sufficiently high for therapeutic efficacy. This limitation is particularly evident when hydrophilic drugs are considered. In order to allow clinically useful transdermal application of most drugs, some way to enhance their normal rates of penetration must be found.

In this paper, we briefly review data concerning a newly developed enhancer of penetration, Azone. At concentrations of 2-10%, Azone is capable of enhancing penetration of most drugs by factors which may be as much as hundreds of times the control values.

EXPERIMENTAL. Most of the specific experimental details are given in Stoughton (1982a,b) and references therein. Where necessary, details are given in captions.

RESULTS.

Properties. The structure of Azone is presented in Figure 1. At room temperature Azone is a clear colorless liquid. Azone has a freezing point of -7° and a boiling point of 160° at 50 u Hg. It has a density of 0.91, a viscosity of 45.2 cp, and a refractive index of 1.4701. Azone is insoluble in water, but is freely soluble in most organic solvents, including the lower alkanols. It is stable for at least six years when stored at room



1. Structure of Azone. Contour lines are presented to outline the space-filling structure, at approximately Van der Waal's radii. The bold lines represent the center-to-center covalent bond structure of Azone. Heteroatoms are located by abbreviations (N,O). Both hydrogen atoms on C6 lie in a plane nearly perpendicular to the page.

temperature in a brown glass bottle exposed to normal fluorescent lighting.

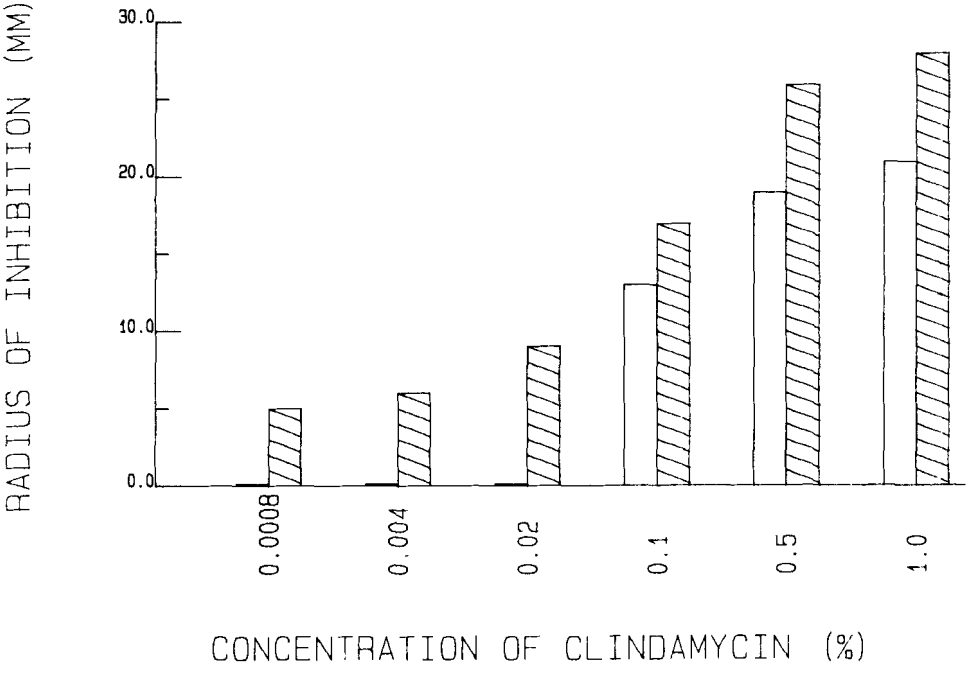
Azone has a smooth, oily but not greasy feel. When incorporated into creams and lotions, Azone imparts a pleasant emolliency to the product.

Azone is capable of forming salts, and can be converted to the hydrobromide by treatment with anhydrous HBr. In the formulations laboratory this reaction has not proven a problem, but the possibility of formation of salts with strongly acidic drugs should be kept in mind. Azone has, in common with most of the N-alkyl lactams, the ability to form non-covalent complexes with certain organic molecules. These complexes can be detected by either infrared or nuclear magnetic resonance spectroscopy. If formed, their effect upon the biological activity of a drug is not predictable. We have observed cases in which complexes were formed, but the enhancement of penetration was excellent and good activity of the drug was maintained. Again, the possibility of formation of complexes should be kept in mind, even though their presence is not necessarily contraindicative of successful enhancement of activity.

Toxicity. Azone has an acute LD50 of 8 gm/kg in the rat and mouse. Similar values are obtained using administration by ip, topical, or iv routes. Azone appears to be a very safe compound, with a toxicity typical of that observed with nutritional components.

Penetration of antibiotics. Several antibiotics show enhanced penetration when applied in formulations containing Azone.

Clindamycin has been examined at a variety of concentrations using a fixed level of 8% Azone in a simple solution of isopropanol (Fig 2). At low concentrations of drug, no



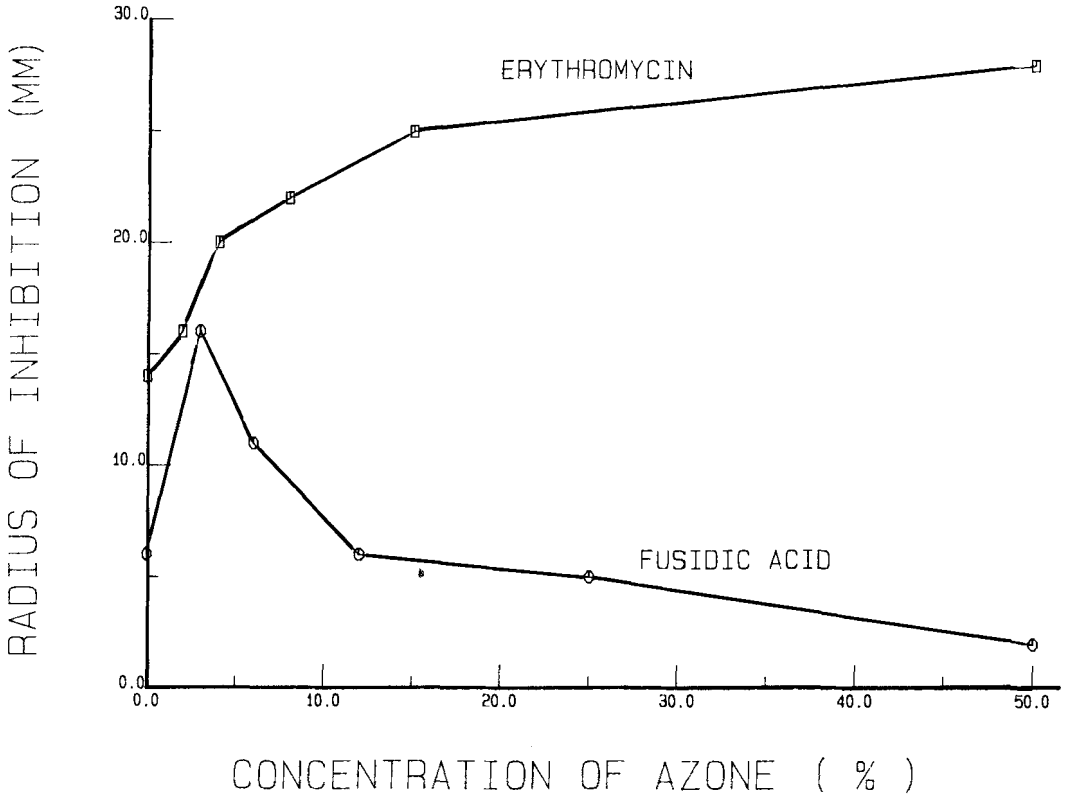

2. Inhibition of the growth of P. acnes by various concentrations of clindamycin in the presence and absence of 8% Azone. Clindamycin was allowed to diffuse into samples of hairless mouse skin for 16 hours, after which 6 mm discs of the corium were placed on media inoculated with Propionibacterium acnes. Readings of the radius of inhibition around the disc were taken after 5 days of incubation. Ten samples were examined at each point. For details see Stoughton (1970, 1982b).

detectable penetration into hairless mouse skin is seen without Azone (open bars). Addition of Azone enhances penetration, yielding significant amounts of clindamycin in the skin (hatched bars). As the concentration of clindamycin is increased, some of the drug enters the skin even without Azone. While the addition of Azone to these samples still enhances penetration, the relative magnitude of the effect is less.

Azone can enhance penetration enough to allow a significant reduction in the amount of drug needed to achieve a given effect. For example, using 0.02% clindamycin in the presence of Azone, one finds nearly as much drug in the corium as with 0.1% in the absence of Azone. In cases of rare or expensive drugs, this aspect of the use of Azone may be of value.

Azone enhances the penetration of sodium fusidate and of erythromycin. In experiments to examine this point, the concentrations of both drugs were maintained constant, and the concentration of Azone was varied (Fig. 3). The penetration of erythromycin is enhanced by Azone in a monotonic fashion, rising to an apparent plateau at concentrations of Azone above 30-40%. The concentration of Azone required to produce half-maximal enhancement is about 6%.

The effect of Azone on penetration of sodium fusidate is not as simple. In this case a low concentration of Azone produces an 2-3 fold enhancement, but greater concentrations show less enhancement. We have observed a similar bell-shaped curve with several drugs, but not with all.




3. Effect of Azone upon the inhibition of growth of P. acnes by erythromycin and sodium fusidate. Solutions of either 1% sodium fusidate or 1% erythromycin containing Azone, propylene glycol (10%) and isopropanol were applied to discs of hairless mouse skin. Ten samples were used for each point with fusidate and 8 with erythromycin. For details see the caption to Fig. 2.

Concentrations in excess of about 15% Azone actually inhibit the activity of fusidic acid, with 50% Azone nearly abolishing activity. The loss of activity at high concentrations of Azone has not been observed with other drugs, and is not now understood. It is possible that Azone and sodium fusidate

interact weakly to form a physiologically inactive complex, but we have no other data to support this suggestion.

Penetration of steroids. More data are now available for this class of drugs than for any other. Azone enhances the penetration of all steroids so far examined.

The effect of varying concentrations of Azone was evaluated in studies which directly measured the penetration of triamcinolone acetonide through hairless mouse skin using in vitro skin cells (Table 1). Solutions containing 2-10% Azone gave optimal penetration, with considerable losses in enhancement at either greater or lesser concentrations. The maximal enhancement observed under these conditions was about 8 fold after seven hours of penetration.

In vivo activity of triamcinolone acetonide has also been evaluated using vasoconstriction in humans. Azone is effective in enhancing penetration in this system (Table 2).

A more extensive range of concentrations of Azone was employed in an in vivo study which utilized a fixed concentration of desonide (Table 3). Optimal enhancement is again seen at concentrations of 2-10%, with a gradual decrease in efficacy as the concentration of Azone is raised to 100%.

Several steroids have now been similarly examined. Data summarizing the enhancement produced by Azone at near-optimal concentrations are presented in Table 4. In each case, an

Table 1

Effect of AZONE on penetration of Triamcinolone acetonide through hairless mouse skin.

| Time (hr) | Azone (%) | Penetration (% of applied dose) |
|---|---|---|
| 7 | 0 | 1.4 ± 0.7 |
| | 2 | 7.4 ± 2.3 |
| | 10 | 10.8 ± 1.6 |
| | 40 | 2.7 ± 0.3 |
| 24 | 0 | 3.8 ± 1.2 |
| | 2 | 10.9 ± 2.8 |
| | 10 | 20.9 ± 2.8 |
| | 40 | 9.7 ± 0.8 |

Radioactive steroid was applied in a solution of AZONE in ethanol to hairless mouse skin *in vitro*. Concentration of steroid, 0.1%. For details see caption to Fig. 5 and Stoughton (1982b).

enhancement of 2-4 fold was observed at concentrations of Azone of 1-3%.

*Effect of polarity of drug.* To examine the effect of the polarity of the drug, we can compare the enhancement of

Table 2

Effect of varying concentrations of AZONE on the vasoconstriction produced by Triamcinolone Acetonide

| Azone (%) | Vasoconstriction Scores (obs/max) | (%) |
|---|---|---|
| 0 | 21/72 | 29 |
| 3 | 47/72 | 65 |
| 8 | 42/72 | 58 |

Vasoconstriction was assessed using the methods of McKenzie and Stoughton (1962) and Stoughton (1972). Ethanolic solutions of 0.1% steroid were applied to the skin of the forearm of 24 human volunteers. Effectiveness of the steroid was evaluated from the resultant blanching, using a scale from 0 (no vasoconstriction) to 3 (intense vasoconstriction). For details see Stoughton (1982b).

Table 3

Effect of varying concentrations of AZONE on the vasoconstriction produced by Desonide

| Azone (%) | Vasoconstriction Scores (obs/max) | (%) |
|---|---|---|
| 0 | 14/42 | 33 |
| 2 | 34/42 | 81 |
| 10 | 36/42 | 86 |
| 30 | 30/42 | 71 |
| 60 | 30/42 | 71 |
| 100 | 22/42 | 52 |

0.1% steroid was used in a solution of isopropanol and AZONE. 14 subjects were employed. For details see Table 2 and Stoughton (1982b).

Table 4

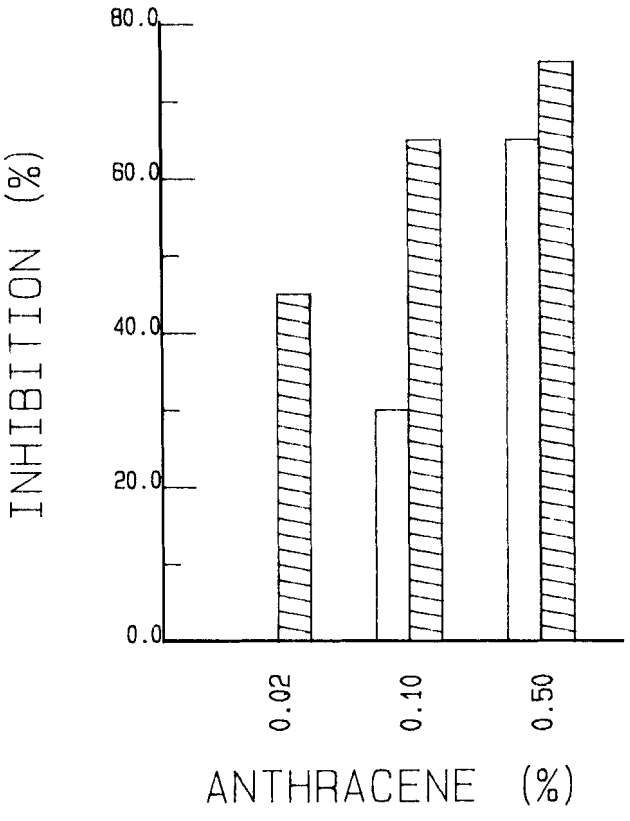
Effect of AZONE on vasoconstriction produced by steroids

| Steroid | Azone (%) | Vasoconstriction Score (obs/max) | (%) | Ratio |
|---|---|---|---|---|
| Triamcinolone acetonide, 0.1% | 0 | 21/72 | 29 | 2.24 |
| | 3 | 47/72 | 65 | |
| Amcinonide, 0.1% | 0 | 13/36 | 36 | 2.25 |
| | 2 | 29/36 | 81 | |
| Desonide, 0.1% | 0 | 14/42 | 33 | 2.45 |
| | 2 | 34/42 | 81 | |
| Desoxymetazone, 0.25% | 0 | 6/30 | 20 | 3.65 |
| | 1 | 22/30 | 73 | |
| Fluocinolone acetonide, 0.025% | 0 | 4/45 | 9 | 4.44 |
| | 2 | 18/45 | 40 | |

For details see Table 2 and Stoughton (1982a, 1982b).

penetration stimulated by Azone using a hydrophobic compound and a hydrophilic one.

Anthracene, a strongly hydrophobic molecule, has been used in the treatment of psoriasis where it acts by inhibiting the synthesis of DNA. Using an *in vivo* model based upon the synthesis of DNA in the skin of mice which are deficient in essential fatty acids, Stoughton (1982a) has shown that Azone significantly enhances the penetration of anthracene (Fig. 4).



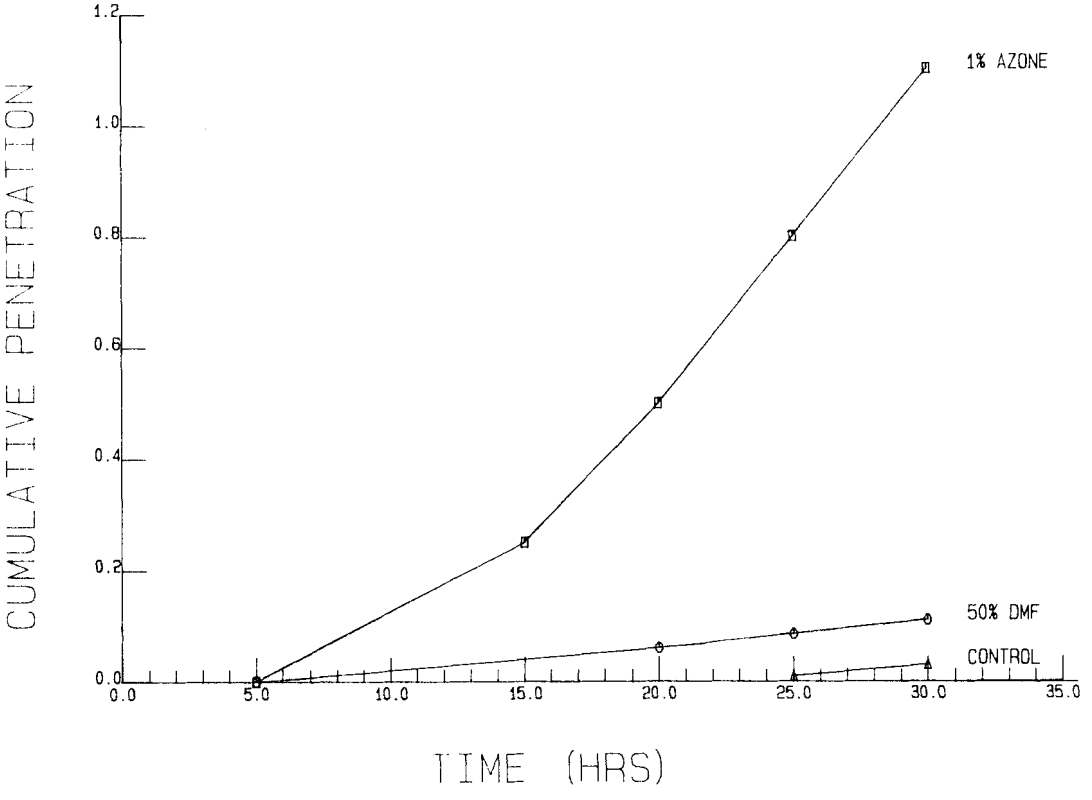

4. Inhibition of the synthesis of DNA by ultraviolet irradiation with varying concentrations of anthracene in the presence and absence of 3% Azone. Ethanolic solutions of anthracene and Azone were applied to skin on the dorsal surface of hairless mice, after which 6 joules of UVA irradiation was given. The animals were then injected with [$^3$H] thymidine and, after one further hour, sacrificed. Discs of epidermis were removed and counted to obtain the level of incorporation of precursor, which under these conditions is largely found in DNA. Ten animals were used in each group. Data are reported as inhibition with respect to the level of incorporation of an unirradiated control patch of skin on the ventral side of the test animal. For details see Otani, et, al. (1980), Walter and DeQuoy (1978), and Stoughton (1982a).

At low concentrations of anthracene, no penetration is observed without Azone (open bars), but good penetration is observed with Azone (hatched bars). As the concentration of anthracene is raised, the effect of Azone is reduced, until high concentrations of the drug show little enhancement. In this case high concentrations of a highly hydrophobic agent can penetrate the skin well enough to negate the advantage of Azone, but examination of lower doses of the drug clearly reveals that Azone is active.

A strongly hydrophilic compound, 8-bromo cyclic adenylic acid, has been examined using Azone (Fig. 5). The steady state rate of penetration of this compound through hairless mouse skin *in vitro* is enhanced by 12 fold when Azone at 1% is compared with control. No systematic attempt has been made to optimize the action of Azone in this system, so this value is probably not optimal.

Comparison of the data for anthracene and 8-bromo cyclic adenylic acid indicates that Azone can effectively enhance the penetration of both hydrophobic and hydrophilic compounds.

*Comparison with other penetrants.* In experiments conducted simultaneously with those described in the preceding section, the enhancing ability of other penetrants has been examined using 8-bromo cyclic adenylate. Dimethylformamide (DMF)at 50% concentration significantly shortens the lag phase of penetration, but has no effect upon the steady-state flux. Azone at 1% is therefore about 12 fold more effective a




5. Effect of Azone and dimethylformamide on the penetration of 8-bromo cyclic adenylic acid through hairless mouse skin. Solutions of 1% 8-bromo cyclic adenylic acid in phosphate buffer containing Azone or DMF were applied to the epidermal side of skin which had been excised from hairless mice and which was maintained under in vitro conditions (Stoughton and Fritsch, 1964). Samples of a recipient solution placed in contact with the dermis were taken at the indicated times and analyzed for 8 bromo cyclic adenylic acid by high performance liquid chromatography. Results are expressed in micromoles penetrating per $cm^2$ of skin. For details see Stoughton (1982a).

penetrant than 50% DMF, if comparisons are based upon steady-state rates. It is also possible to compare the amount of drug which has penetrated after 30 hours. In this case, after correcting for control penetration, 1% Azone is about 13 fold more effective than 50% DMF. In similar experiments DMF was replaced by 50% dimethylacetamide (DMA) or 50% dimethylsulfoxide (DMSO). Data for these two penetrants were essentially the same as those presented for 50% DMF (data not shown). The data indicate that Azone is a much more effective enhancer of penetration than is DMF, DMA, or DMSO.

Penetration of some miscellaneous compounds. Azone can enhance the penetration of nucleosides, as well as of nucleotides. 5-flurouracil has been studied with varying concentrations of Azone, using in vitro penetration of the radiolabelled compound through hairless mouse skin (Table 5). An enhancement of 94 fold is observed with 1.8% Azone after 7 hours, indicating that Azone can effectively enhance the penetration of this antiviral drug.

Effect of formulation. The formulation in which Azone is employed can affect the enhancing activity of the compound. One example will be considered. It is sometimes desired to add Azone to an existing formulation by simply mixing an appropriate amount of the pure penetrant with a sample of formulated product. In general, Azone will exhibit little or no activity under these conditions. For example, addition of 2% Azone to a commercial formulation of fluocinolone acetonide (Synalar) did not increase the vasoconstrictor activity of the preparation. In contrast, activity of the same steroid simply

Table 5

Effect of AZONE on penetration of 5-flurouracil through hairless mouse skin

| Time (hr) | Azone (%) | Penetration |
|---|---|---|
| 7 | 0.0 | 0.7 |
| | 1.8 | 66.1 |
| | 9.0 | 40.7 |
| | 45.0 | 7.0 |
| 24 | 0.0 | 2.5 |
| | 1.8 | 75.7 |
| | 9.0 | 48.5 |
| | 45.0 | 17.0 |

Radioactive drug at 2.4% was applied to hairless mouse skin in vitro in formulations of water and propylene glycol containing varying concentrations of AZONE. For details see the caption to Fig. 5 and Stoughton (1964, 1982b).

dissolved in 2% Azone in ethanol was strongly enhanced, and outperformed the commercial cream (Stoughton, 1982a). In this case components in the formulation must inhibit the activity of Azone. Clearly, the formulation is of importance.

## DISCUSSION.

Azone is an effective enhancer of the penetration of a number of drugs. Several points concerning the use of this new agent may be drawn from the preceding data.

The chemical nature of the drug does not appear to be of great importance: virtually any drug will move through skin more quickly when Azone is present. Hydrophobic and hydrophilic molecules both show enhanced penetration in the presence of Azone. Similarly, the molecular weight of the drug seems not to be a critical variable: the penetration of both small and large molecules is enhanced. It seems clear that some practical limit must exist with respect to the size of the drug. We have not examined this point in a systematic way, but have seen good enhancement of compounds with molecular weights in the range of 300-600. More work will be necessary to define the upper limit to the molecular weight range.

Azone often produces maximal enhancements at low concentrations of drug, and becomes relatively less effective as the concentration of drug is increased. This effect is of practical utility, for the biological effect of low concentrations of drug in the presence of Azone is often equal to that observed with high concentrations of drug without Azone. The use of lower amounts of drugs could result in both reduction in side effects and savings in expense.

The concentration of Azone which is required to produce optimal enhancement varies from one drug to another. If a simple limit on enhancement is seen, such as that observed with erythromycin (Fig. 3), a concentration of Azone which is just sub-maximally active would generally be chosen. In studies with a number of drugs which exhibit saturating behavior, a typical sub-maximal concentration of Azone is 5-10%. A few drugs, of which erythromycin is an example, require higher levels of Azone;

some require less. In contrast to simple saturation, many drugs display a bell-shaped optimization curve when studied with Azone (sodium fusidate, Fig 3). A large fraction of these drugs exhibit optimal penetration at concentrations of Azone in the range of 2-10%. In general, 2-5% Azone seems a good level to use for introductory studies. After the initial work, however, each drug and formulation should be studied over a range of concentrations of Azone in order to optimize penetration.

The formulation in which Azone is used with a given drug is of great importance. One example was mentioned in which the formulation destroyed activity of Azone. The activity of Azone can be inhibited by several common components of pharmaceutical formulations. Petrolatum is especially of particular concern. Even relatively low levels of petrolatum can eleminate an expected increase in penetration due to Azone.

Very simple formulations often give good enhancement with Azone. For example, a shaken emulsion of Azone and an aqueous solution of a drug usually gives excellent enhancement of penetration (see data of Fig. 5 and Table 5). These data also emphasize the point that Azone need not be in solution to be effective. Time spent in optimizing a formulation will be well repaid in greater efficacy in the use of Azone. Some of the concerns of formulation are considered elsewhere in this volume.

With suitable formulations, Azone can provide significant improvement in the penetration of a wide range of drugs. It

should be possible to use Azone to increase substantially the number of drugs which can be employed for clinically effective transdermal delivery.

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