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The Effect of Dimethyl Sulfoxide on Percutaneous Absorption: A Mechanistic Study, Part III

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Synopsis—Additional studies have been carried out to support the suggested swelling role of dimethyl sulfoxide (DMSO) toward hair fibers. Results again indicate that large concentrations of solvent are required before appreciable swelling and greater uptake of DMSO can be realized. The swelling process is easily reversible. Other data indicate that DMSO at low pH values may be responsible for an increase in coulombic repulsions within the fiber which results in greater swelling. With the more complex substrate, skin, the modifying role of DMSO on the skin barrier is more difficult to assign. The DMSO effect has not been shown to be reversible and results indicate that lipid extraction may play a supporting role to protein swelling in this modification process.

Introduction

In two previous papers, the effects of dimethyl sulfoxide (DMSO) upon the *in vitro* percutaneous absorption of picrate ion through excised guinea pig skin and upon some physical properties of several proteins have been reported (1, 2). As a result of studies on bovine serum albumin, β-lactoglobulin, and hair keratin, DMSO was considered to be a protein swelling agent. With a complex substrate such as skin, the role of DMSO is more difficult to assign and further experiments were proposed. The results of these studies are presented in this paper.

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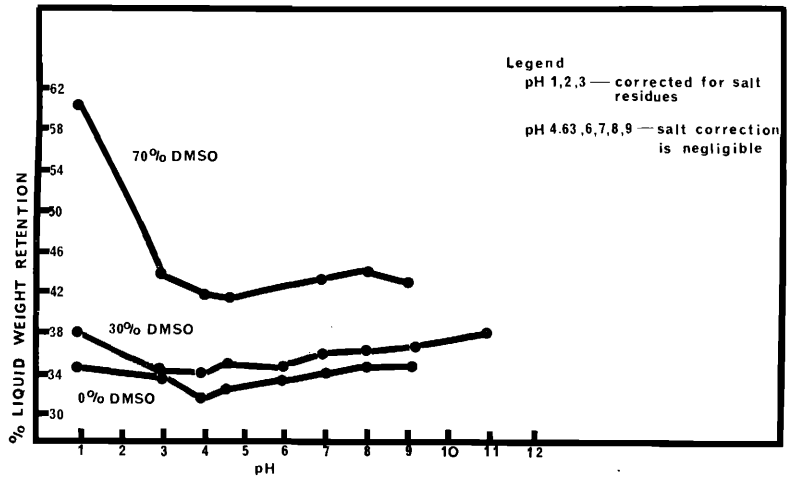

Figure 1. Equilibrium retention by hair fibers as a function of pH

Materials and Methods

All of the materials and chemicals used for these studies were either described in the previous papers or were obtained as pure as possible (1, 2). The methods and techniques are also described in detail in the previous papers (1, 2).

Results and Discussion

Equilibrium Swelling Experiments

It has been reported that the equilibrium retention of liquid by hair fibers from solutions of varying concentrations of DMSO increased very gradually up to a 40–50% initial DMSO bath concentration and then increased markedly beyond this concentration (2). With the use of C^{14}-DMSO, it now has been demonstrated that the mole fraction of DMSO in the liquid retained by hair fibers undergoes a relatively gradual increase up to an initial bath concentration of approximately 40–50% DMSO and then increases sharply. This is the same relationship which is exhibited between mole fraction of DMSO in the initial bath and the volume per cent of DMSO in the initial bath. Thus, it appears that the composition of the retained liquid resembles the concentration of the initial bath solution.

The equilibrium liquid retention of hair in 0, 30 and 70% DMSO solutions as a function of pH was also examined. The results are presented in Fig. 1. Thirty per cent (30%) DMSO is relatively ineffective

in enhancing equilibrium liquid retentions over that of the control at all pH's tested. With 70% DMSO, the enhancement effect is greatest on the acid side of the isoelectric point (*ca.* pH5). These results are in accord with the idea that DMSO promotes greater swelling through the enhancement of electrostatic repulsions within the fiber and with the explanation offered for the effect of dioxane upon the physical properties of bovine serum albumin (3).

The reversibility of DMSO retention by hair fibers was examined in the following manner. Two hair samples were allowed to reach swelling equilibrium independently in pH 7.0 buffer, and were then transferred to 100% DMSO. A new equilibrium was reached in about two days, the degree of swelling at the new equilibrium being approximately twice that observed in pH 7.0 buffer. After about 10 days in the DMSO, the hair samples were rinsed off and transferred back to pH 7.0 buffer. In about one day, the original equilibrium swelling in pH 7.0 buffer was obtained, thus demonstrating the reversibility of the process.

In another experiment, four hair samples were allowed to reach swelling equilibrium in 20% (v/v) DMSO in pH 7.0 buffer. They were then rinsed off, transferred to pH 7.0 buffer, and allowed to re-equilibrate. After this second equilibrium state had been attained, the hair samples were immersed and re-equilibrated in 80% (v/v) DMSO in pH 7.0 buffer. On another set of samples the steps were performed in the reverse direction, starting with equilibration in 80% DMSO. In all cases, the same equilibrium values were attained regardless of the order in which the hair samples were exposed to the different solutions. The details of this experiment are presented in Table I.

Table I
Equilibrium Retention Studies on Hair Fibers at pH 7.0

Procedure	% Liquid Weight Retention (Average plus standard deviation of the mean of four trials)
1A. Hair samples dried	
2A. Immersion in 20% (v/v) DMSO	32.78 ± 0.49%
3A. Transferred to pH 7.0 buffer	32.40 ± 0.78%
4A. Immersion in 80% (v/v) DMSO	48.24 ± 1.41%
1B. Hair samples dried	
2B. Immersion in 80% (v/v) DMSO	48.07 ± 0.82%
3B. Transferred to pH 7.0 buffer	32.46 ± 0.51%
4B. Immersion in 20% (v/v) DMSO	33.23 ± 0.46%
Control sample immersed in pH 7.0 buffer	32.55 ± 0.65%

Table II
Penetration of C^{14}-DMSO Through Excised Guinea Pig Skin as a Function of DMSO Concentration

% DMSO	Absolute Rate Constant
20	3.12×10^{-4} cm hr^{-1}
40	6.46×10^{-4} cm hr^{-1}
60	11.9×10^{-4} cm hr^{-1}
80	357.0×10^{-4} cm hr^{-1}

Diffusion Studies in the Presence of Dimethyl Sulfoxide

In vitro percutaneous absorption studies at 30 ± 0.05 °C and at pH 7.0 were conducted in which excised guinea pig skin was utilized as the membrane between a donor chamber and a receptor chamber. The rate of percutaneous absorption of picrate ion was determined by following spectrophotometrically the transfer of picrate from the epidermal chamber to the dermal chamber. The complete details of these diffusion experiments have been described previously (1).

The diffusion of C^{14}-DMSO as a function of DMSO concentration was studied in the guinea pig skin membrane system in the absence of picrate ion. The results are reported in Table II. The absolute rate constant for the passage of C^{14}-DMSO does not dramatically increase until the concentration of DMSO exceeds 60%. This dependence upon large concentrations of solvent has been reported several times before in connection with this work (1, 2).

When picrate was dissolved in 80% DMSO, substantial diffusion enhancement was observed as expected. Attempts to reverse the DMSO effect by removing the picrate in DMSO from the epidermal chamber and replacing it with picrate in buffer were unsuccessful. In addition to the possibility of the inherent nonreversibility of the system, picrate ion reservoirs within the *Stratum corneum* and bacteriological problems complicated the reversibility attempts (4, 5). In order to avoid the picrate ion reservoir problem, *n*-butanol-C^{14} was used as the burden. A threefold enhancement of the diffusion rate was observed when the *n*-butanol was dissolved in 80% DMSO. Replacement of the *n*-butanol in DMSO by picrate in buffer showed that the barrier to picrate diffusion had been removed and the DMSO effect was not reversible. In another experiment, a pretreatment with 80% DMSO alone for about eight hours destroyed the barrier to picrate diffusion. The controls in this case behaved normally. Thus, it appears that the DMSO is causing either an irreversible protein modification within the *Stratum corneum*, an extrac-

Table III
Reversibility Study on Skin From a Single Animal

	Initial Concentration of *n*-Butanol-C^{14} or Picrate (M)	Absolute Rate Constant kp (cm hr^{-1})
Control-picrate in buffer	4.95×10^{-2}	No penetration after 8 hours
Test *n*-butanol-C^{14} in buffer Diffusion Chamber rinsed and skin membrane frozen overnight	36.7×10^{-2}	24.3×10^{-4}
Picrate in buffer	4.95×10^{-2}	No penetration after 8 hours
80% DMSO pretreatment of epidermal side of membrane for ~8 hours Diffusion Chamber rinsed and skin membrane frozen overnight		
Picrate in buffer	4.95×10^{-2}	43.4×10^{-4}
n-Butanol-C^{14} in 80% DMSO (kinetics followed for ~8 hours) Diffusion Chamber rinsed and skin membrane frozen overnight	46.0×10^{-2}	82.2×10^{-4}
Picrate in buffer	4.95×10^{-2}	57.2×10^{-4}

A male guinea pig was sacrified with a lethal injection of $MgSO_4$. The abdominal skin was immediately clipped, excised, and frozen until use. The length of time between procurement and utilization of the skin was approximately 19 hours.

tion of vital lipids, or, more possibly, some combination of these factors. Previous work with other proteins has suggested a swelling role for DMSO, but, with a complex substrate such as skin, other factors such as lipid extraction may also be of importance. The results of these experiments are presented in Table III.

In order to show that the DMSO-enhanced diffusion process was not specific for picrate ion and *n*-butanol, the passage of the cationic dye, methylene blue, in the presence of 80% DMSO was also observed. At the end of about 24 hours, the dermal side of the skin membrane which had been in contact with the methylene blue-DMSO solution was grossly stained while that exposed to the methylene blue-buffer solution was not stained at all. Small amounts of methylene blue as determined spectrophotometrically had diffused into the dermal chamber in the case of the former but not the latter. Thus, it appears that the solvent-assisted diffusion of substances is a general process with respect to the burden.

Conclusions

Equilibrium retention studies on hair fibers have suggested that DMSO is capable of swelling or unfolding proteins. The ease with which reversal of fiber swelling can be attained adds additional support to the swelling role of DMSO in these cases, as do viscosity studies on bovine serum albumin and β-lactoglobulin.

In addition, Price and Menefee (6), in a recent paper, have presented the following suggestions, based upon stress relaxation measurements:

(a) Large concentrations of DMSO interact reversibly with hair keratin through an initial dehydration effect which is accompanied by a swelling effect, a lowering of the modulus, and a promotion of disulfide interchange.

(b) The relaxation rates of hair fibers incubated in concentrations of DMSO lower than 80% remain essentially unchanged from the rate observed in H_2O. Large concentrations of DMSO are required for appreciable effects to be observed. This concentration dependence is in agreement with the work of others (7) and work reported by the authors of this paper (1, 2).

(c) The effect of DMSO upon hair keratin may also be similar to its effect upon increasing percutaneous absorption through the *Stratum corneum.*

Although the swelling characteristics of hair and the passage of picrate through skin show a similar dependency upon solvent concentration (1, 2), the two processes do not appear to be completely alike. Reversibility studies on diffusion through skin indicate that lipid extractions may be playing a supporting role in the solvent initiated modification of the skin barrier. The lipid role in skin barriers has not been accurately defined (8) and it now appears that DMSO alters the skin barrier through some combination of protein swelling and lipid extraction. It is of interest to note that chloroform-methanol solutions which are capable of both lipid extraction and irreversible protein denaturation within the skin barrier produce a much greater alteration than DMSO under similar conditions (9).

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References

(1) Elfbaum, S. G., and Laden, K., Mechanism of action of dimethyl sulfoxide: Part I, *J. Soc. Cosmetic Chemists*, **19**, 119–27 (1968).
(2) Elfbaum, S. G., and Laden, K., Mechanism of action of dimethyl sulfoxide: Part II, *Ibid.*, **19**, 163–72 (1968).
(3) Van Holde, K. E., and Sun, S. F., Bovine serum albumin in water-dioxane mixtures, *J. Am. Chem. Soc.*, **84**, 66–72 (1962).
(4) Stougton, R. B., Dimethylsulfoxide (DMSO) induction of a steroid reservoir in human skin, *Arch. Dermatol.*, **91**, 657–60 (June 1965).
(5) Stoughton, R. B., Hexachlorophene deposition in human *Stratum corneum*, *Ibid.*, **94**, 646–8 (Nov. 1966).
(6) Price, V. H., and Menefee, E., On the effect of dimethyl sulfoxide on hair keratin, *J. Invest. Dermatol.*, **49**, 297–301 (1967).
(7) Sweeney, T. M., Downes, A. M., and Matoltsy, A. G., The effect of dimethylsulfoxide on the epidermal water barrier, *Ibid.*, **46**, 300 (1966).
(8) Crounse, B. G., The association of lipids with keratinous proteins of human callus, *Ibid.*, **46**, 550–4 (1966).
(9) Elfbaum, S. G., and English, M. Y., unpublished observation.