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Effect of Dimethyl Sulfoxide and Other Reagents upon Mechanical Properties of Stratum Corneum Strips

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Synopsis—The mechanical properties of guinea pig stratum corneum strips exposed to DIMETHYL SULFOXIDE (DMSO) and several other reagents have been examined. With DMSO concentrations greater than 50% and after a 24-hour incubation period, a marked reversible strengthening of the SKIN strips relative to their behavior in water was noted. This observation correlates well with other experiments and reports which indicated that the DMSO swelling of skin and hair is accompanied by a stiffening effect.

To further elucidate the proteinaceous nature of the skin barrier, several other reagents were examined for their effects upon the strength of stratum corneum strips. A known cystine bond reducing agent (ammonium thioglycolate) and protein denaturating agents (urea and formic acid) considerably weakened guinea pig stratum corneum. The cross-linking ability of formaldehyde was revealed in its strengthening effect upon skin strips. Trichloroacetic acid (a protein precipitant) and phenol behaved in a similar manner. The reversibility of the mechanical effects caused by these various reagents has been studied and the results are presented.

INTRODUCTION

Previous reports from this laboratory have shown that dimethyl sulfoxide (DMSO) is capable of swelling and unfolding several soluble proteins (1). Other laboratories have demonstrated a swelling of wool fibers caused by large concentrations of DMSO (2, 3). Hair keratin undergoes a similar swelling effect which occurs in a reversible manner (1, 4, 5). However, it is of interest that the mechanical properties of hair

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appreciably swollen in large concentrations of DMSO indicate that the fibers appear stronger than in H_2O even though they have undergone extensive swelling (6).

With skin, the penetrating ability of DMSO has been well documented (7–12). One is tempted to envision this effect in part as an opening up or swelling of the proteinaceous components of the stratum corneum matrix. Indeed, wrinkling and turgid effects have been observed both on the fingers and palms of laboratory personnel and patients who came into contact with DMSO and on whole guinea pig skin membranes which had been exposed to large concentrations of DMSO (1, 8, 9).

Although the swelling characteristics of hair in DMSO and the DMSO-enhanced passage of materials through whole skin show a similar dependence upon DMSO concentration, the two processes may not be completely alike. The nonreversibility of the *in vitro* DMSO-enhanced diffusion of picrate ion through whole intact skin indicates lipid extraction or irreversible protein denaturation may have played supporting roles in the solvent initiated modification of the skin barrier (4).

Since chemical effects upon stratum corneum and the resulting physical responses are relatively unknown, a study of the effect of DMSO and several other reagents on the mechanical properties of isolated strips of stratum corneum has been undertaken. It was hoped that this approach would provide more insight concerning the proteinaceous nature of the stratum corneum, especially since the physical or mechanical responses of tissue strips to chemical reagents reflect in part different degrees of solvation, hydration, and protein denaturation (13).

Experimental

Materials

Analytical grade reagents and solvents were used without further purification.

Ammonium thioglycolate (0.1*M*) was prepared by adjusting 0.1*M* thioglycolic acid to pH 9 with concentrated ammonia solution.

Ammonium chloride (0.1*M*) was adjusted to pH 9 with concentrated ammonia solution.

Guinea pig stratum corneum was isolated by exposing excised intact skin to ammonia vapors and then gently peeling off the stratum corneum (14). The sheets of stratum corneum were stored at −12°C.

Strips of human stratum corneum were obtained from sunburn peelings.

Methods

Sheets of stratum corneum which were conditioned for 24 hours at 21°C and RH 65 ± 2% were cut into strips ($1^1/_4$ in. × $^1/_4$ in.) and attached to small tabs by means of an epoxy resin. Only strips without breaks, tears, or obvious holes were considered suitable for the experiments. The strips of stratum corneum were then immersed for about 24 hours in $CHCl_3$–saturated water (this medium was used in order to prevent microbiological decomposition), and calibrated by stretching them to approximately 5% extension in distilled water on the Instron Extensometer operating at a constant rate of extension (0.1 in./min). Cell A with the high sensitivity setting which gave a full scale load of 2 g was used in most cases.

The strips were relaxed overnight in $CHCl_3$–saturated water and then transferred for an additional 24 hours into the solvent in which they were restretched. It was not determined whether or not this 24-hour solvent incubation time was sufficient for equilibrium to be reached. In order to eliminate the differences between various samples, the same skin sample served as its own control and test. As many experiments as possible were done on the skin of one animal, especially when the effect of the solvent concentration on the work index was examined.

It is relatively difficult to obtain a large and uniform piece of guinea pig stratum corneum and, therefore, it is not practically possible to cut all the strips in the same direction with the ridges and grooves running perpendicular or parallel to the applied force. In order to avoid any artifacts resulting from different orientations of the strips, the direction of the striations was noted before the stratum corneum strips were tested on the Instron. The results indicated that there was no significant difference between strips cut with the striations perpendicular or parallel to the applied force.

The changes in mechanical properties were evaluated by calculating the ratio of the work needed to stretch the skin sample approximately 5% in a given solvent (or a repeat time in water) to that measured during its initial calibration in water (15, 16).

$$\text{Work index} = \frac{\text{work required to stretch strip 5\% in the solvent (or a repeat time in water)}}{\text{work required to stretch strip 5\% in water initially}}$$

In order to calculate the work necessary to stretch the tissues, the

work profiles were cut from the Instron charts and weighed. Thus, the work index was calculated from the ratios of the weights before and after treatment.

The reversibility of the solvent effect was assessed by rinsing the tested strips free of solvent with distilled water, relaxing them overnight in $CHCl_3$–saturated H_2O, and restretching them a third time in water. The work obtained was compared to that observed during the calibration and expressed as the work index.

Slight modifications of the testing procedure were made in the following cases:

(a) During experiments with ammonium thioglycolate the strips were calibrated in water and relaxed in water as usual. Then they were transferred for approximately 15 minutes to the appropriate reagent (ammonium thioglycolate or ammonium chloride) and restretched in distilled water.

(b) In the case of 97% formic acid, the strips were calibrated and relaxed in water as usual, and then transferred to the reagent for 2 minutes and restretched in distilled water.

Results and Discussion

Relaxation of Stratum Corneum Strips after Stretching in Water

In a first series of experiments, the reversibility of the stress-strain characteristics of human and guinea pig stratum corneum as a function of per cent extension was examined. The strips which were prepared in the usual manner were stretched to 5, 10, and 20% extension in distilled water, relaxed in $CHCl_3$–saturated water, and restretched in water (Table I). The human strip stretched for 20% extension seemed to be irreversibly damaged and the repeat load-extension curve showed a marked difference from the untreated one. Guinea pig strips stretched for 20% extension (not reported) also appeared irreversibly changed.

Those human and guinea pig strips stretched up to 10% extension in water relaxed completely and gave identical curves even after immediate recycling.

Guinea pig strips were stretched 5% for three times in water following a stretch-relax-stretch-relax-stretch procedure. The differences in work index which were observed (Table II) were not considered to be significant. Other experiments (not reported) indicated that five re-

Table I

Changes in Work Index of Human and Guinea Pig Stratum Corneum Strips Stretched in Water at Various Extensions

Stratum Corneum	Extension (%)	Work Index $\left(\frac{\text{Final Water}}{\text{Initial Water}}\right)$	Number of Experiments	Comments
Human	10	1.01	2	Reversible system
Human	20	0.82	1	Irreversible system
Guinea pig	5	1.05 ± 0.08[a]	10	Reversible system
Guinea pig	10	1.02	2	Reversible system

[a] Mean and 95% confidence level (17).

Table II

Changes in Work Index of Guinea Pig Stratum Corneum Strips Stretched Three Times in Water (5% Extension)

Work Index $\left(\frac{\text{Second Stretch in Water}}{\text{Initial Stretch in Water}}\right)$	Work Index $\left(\frac{\text{Third Stretch in Water}}{\text{Initial Stretch in Water}}\right)$
0.98	1.15
1.14	1.16
1.28	1.10
1.03	1.08
1.09	1.13
1.05	1.04
1.09 ± 0.12[a]	1.11 ± 0.05[a]

[a] Mean and 95% confidence level (17).

cyclings were possible with no apparent differences occurring in the load-extension curves. To allow for a greater safety margin, 5% extension was adopted as the standard in further experiments.

Effect of Dimethyl Sulfoxide on the Work Index of Stratum Corneum

The effect of a range of aqueous DMSO concentrations on stratum corneum within 24 hours was examined. The results are presented in Table III and Fig. 1. There was relatively little change in the work index at the lower concentrations (20–40%). The work index increased as the concentration of DMSO was changed from 40% to 80%. The critical concentration seemed to be about 60% DMSO although the exact concentration point may differ from animal to animal. However, at an 80% concentration there was a marked increase in the work index.

Table III
Effect of DMSO on Work Index of Guinea Pig Stratum Corneum[a]

DMSO Concentration (%) (v/v)	Work Index $\left(\frac{\text{Solvent}}{\text{Water}}\right)$	No. of Experiments	Work Index $\left(\frac{\text{Final Water}}{\text{Initial Water}}\right)$	No. of Experiments	Comments
20	0.90 ± 0.09[b]	6	1.10 ± 0.22	4[c]	No significant change
40	1.04 ± 0.08	9	1.05 ± 0.09	9	No significant change
60	1.38 ± 0.14	17	1.11 ± 0.19	4	Reversible system
80	1.92 ± 0.22	16	1.13 ± 0.17	12[c]	Reversible system

[a] Mean values from different animals.
[b] Mean and 95% confidence level (17).
[c] Reduction in number of experiments resulted from mechanical breakage of strips.

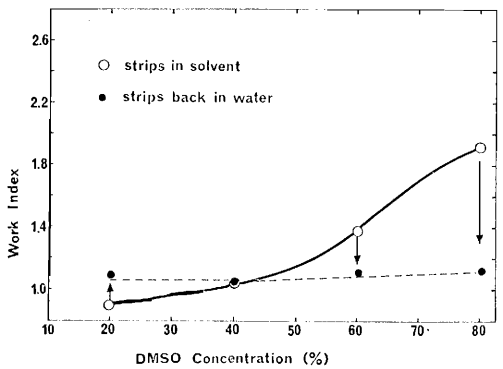

Figure 1. The reversible effect of DMSO concentration on work index of guinea pig stratum corneum

This dependence on a large concentration of the solvent has been observed in detail in other studies on hair swelling and skin permeability (1, 4), where it was suggested that DMSO has the ability to swell or unfold protein chains. In hair, the swelling effect is accompanied by a hardening or loss of elasticity (6). Qualitative observations on skin have also suggested that the DMSO effect is accompanied by a wrinkling or hardening of the skin and the physical studies reported here correlate with those observations.

The increase in work index is reversible and this was revealed by the observations that the mechanical responses of the strips returned to the original values obtained before the treatment in DMSO. Since DMSO is capable of dissolving the lipids of the stratum corneum, and since during the subsequent immersion in water soluble proteins may also be dissolved (18), it appears that DMSO-insoluble keratin elements and not lipids or soluble proteins bear the mechanical stress of the stratum corneum under these conditions. The reversibility of these DMSO strengthening studies on stratum corneum at first does not seem to agree with the nonreversibility experienced with the DMSO skin permeability studies reported earlier (4). However, whereas a lipid role has been established for skin barrier function, the present physical studies with guinea pig stratum corneum exclude it completely. Thus, reversibility might be expected in one case and not in the other.

It was also noticed that there was a visible change in the refractive indices of the stratum corneum strips on immersion in DMSO. The strips became completely transparent. However, after rewetting with water they became opaque again. This change in appearance is most probably correlated with the dehydration-solvation process of the samples which occurs during the initial submerging in DMSO.

Effect of Other Chemical Reagents on the Work Index of Stratum Corneum

Since the above data suggest that the DMSO effect upon the work index is dependent not upon lipids or soluble proteins but upon the insoluble keratin components of the stratum corneum, studies were conducted to characterize the insoluble keratinaceous portions of the stratum corneum which bear the mechanical stress reported here. Thus, the effect of various chemical reagents on the work index was examined.

Phenol, Trichloroacetic Acid, and Formaldehyde

Solutions of phenol (2% and 5%), 5% trichloroacetic acid (TCA), and 10% formaldehyde were examined, and it was found that all of these materials increased the work index of stratum corneum strips.

A series of experiments was undertaken to establish the dependence of the work index on the phenol concentration. The results are presented in Table IV. When 1% phenol was used, there was no significant change in the work index. With 2% and 5% solutions of phenol, the work index increased, although there was no significant change between the 2% and 5% concentrations. At first this effect was rather unexpected

Table IV
Effect of Phenol Concentration on Work Index of Guinea Pig Stratum Corneum[a]

Phenol Concentration (%)	Work Index $\left(\frac{\text{Solvent}}{\text{Water}}\right)$	No. of Experiments	Work Index $\left(\frac{\text{Final Water}}{\text{Initial Water}}\right)$	No. of Experiments	Comments
1	1.10 ± 0.05[b]	9	1.05 ± 0.06	9	No significant change in properties
2	1.65 ± 0.32	6	1.21 ± 0.16	6	Partly reversible
5	1.39 ± 0.12	9	1.11 ± 0.16	6[c]	Partly reversible

[a] All experiments performed on the skin from a single animal.
[b] Mean and 95% confidence level (17).
[c] Reduction in number of experiments resulted from mechanical breakage of strips.

and contrary to that reported on wool. When wool fibers are treated with phenol they swell considerably and become much weaker This weakening effect of phenol in the case of wool is reported to be reversible (19). However, there is literature evidence based on dilatometric and thermodynamic considerations that when phenol absorbs onto hair keratin, extensive aggregation between adjoining phenol molecules occurs (20). This behavior could explain the ability of phenol to precipitate soluble proteins. Stratum corneum has several protein components of varying solubility (18) and phenol may precipitate one or more of these fractions within the skin matrix. A combination of phenol-induced aggregation and protein precipitation could result in a strengthening effect providing other bond-breaking mechanisms are overcome. The composition of wool does not include the variety of protein fractions found in stratum corneum.

With phenol, the increase in work index was only partly reversible. The strips soaked in phenol became icy-white and stiff. Apparently some permanent denaturation of the stratum corneum had taken place.

Trichloroacetic acid (TCA) also strengthened the stratum corneum but the effect appeared to be reversible (Table V). Binding studies have suggested that TCA interacts with protein amide groups to form insoluble precipitates (21). Indeed, TCA is widely used as a means of deproteinizing biological fluids. The combining ability of TCA with peptide

Table V
Effect of Formaldehyde and Trichloroacetic Acid on Work Index of Guinea Pig Stratum Corneum[a]

Reagent	Work Index $\left(\frac{\text{Solvent}}{\text{Water}}\right)$	No. of Experiments	Work Index $\left(\frac{\text{Final Water}}{\text{Initial Water}}\right)$	No. of Experiments	Comments
5% TCA	1.45 ± 0.12[b]	4	1.10 ± 0.26	3[c]	Reversible
10% formaldehyde	1.44 ± 0.39	4	1.56 ± 0.42	4	Irreversible

[a] The skin from one animal was used for the TCA results and the skin from another animal was used for the formaldehyde results.
[b] Mean and 95% confidence level (17).
[c] Reduction in number of experiments resulted from mechanical breakage of strips.

groups may be similar to a mechanism suggested for trifluoroacetic acid (TFA). Near-infrared studies and electrical conductance measurements on model amide compounds and synthetic polypeptides have indicated that TFA is capable of protonating the amide groups with the subsequent formation of ion pairs (22). Aggregation of adjoining ion pairs within the insoluble skin matrix could produce a strengthening effect. In addition, the precipitation of soluble components within the same matrix could also contribute to the increased strength.

An irreversible effect was observed on strips treated with 10% formaldehyde (Table V). Here the cross-linking ability of the reagent is revealed in a very definite manner.

Ammonium Thioglycolate

The effect of ammonium thioglycolate on stratum corneum was examined since this reagent is capable of reducing disulfide bonds in wool and hair keratin.

The maximum potency of this reagent occurs at pH $\geq$ 9. To evaluate the changes in the work index due to the pH, five strips were treated with 0.1*M* ammonium chloride adjusted to pH 9. There was no significant change in the work index in ammonium chloride–treated strips at pH 9. Thus, it appears that the considerable weakening of stratum corneum in 0.1*M* ammonium thioglycolate can be attributed to the action of that reagent on the cystine cross linkages in the stratum corneum. The subsequent 24-hour relaxation process in which no attempt was made to exclude dissolved atmospheric oxygen resulted in a partial reversibility of the cystine reduction process. The results are presented in Table VI.

Table VI

Effect of Ammonium Thioglycolate on Work Index of Guinea Pig Stratum Corneum[a]

Reagent	Work Index (Solvent / Water)	No. of Experiments	Work Index (Final Water / Initial Water)	No. of Experiments	Comments
0.1*M* NH_4Cl pH 9	1.06 ± 0.08[b]	4	1.04 ± 0.29	3[c]	No change in work index
0.1*M* ammonium thioglycolate	0.48 ± 0.12	5	0.85 ± 0.11	4[c]	Partly reversible

[a] All experiments performed on the skin from a single animal.
[b] Mean and 95% confidence level (17).
[c] Reduction in number of experiments resulted from mechanical breakage of strips.

Urea and Formic Acid

The action of two other protein denaturing reagents on this system has also been examined. Both 6*M* urea and 97% formic acid weakened stratum corneum considerably. Formic acid (97%) disrupted and weakened the skin in a very short time (<2 min). Formic acid is known to break both hydrogen and salt linkages. Due to the intense weakening of the sample it was not possible to undertake the reversibility study (Table VII).

The effect of 6*M* urea was not as dramatic and the action was partly reversible. Urea has been implicated by several investigators as capable of weakening hydrogen bonds, hydrophobic bonds, or more possibly some combination of the two (23). The urea results upon stratum corneum are presented in Table VII.

Table VII

Effect of Protein Denaturating Agents on Work Index of Guinea Pig Stratum Corneum

Reagent	Work Index (Solvent / Water)	No. of Experiments	Work Index (Final Water / Initial Water)	No. of Experiments	Comments
6*M* urea[a]	0.73 ± 0.16[b]	7	0.90 ± 0.16	6[c]	Partly reversible
97% formic acid[d]	0.25	2	[d]	..	...

[a] 6*M* urea experiments. Average value from the skin of two animals.
[b] Mean and 95% confidence level (17).
[c] Reduction in number of experiments resulted from mechanical breakage of strips.
[d] 97% formic acid, skin from a single animal; reversibility study not possible.

Conclusions

After examining the effect of DMSO and several other chemical reagents on the mechanical responses of guinea pig stratum corneum the following conclusions can be drawn:

1. The action of DMSO within 24 hours depended very much on the concentration of the solvent. Below 50% DMSO, there was no significant change in the mechanical properties. At solvent concentrations greater than 50%, there was a marked reversible increase in the work index. A skin hardening and wrinkling effect due to DMSO had been qualitatively observed with the skin of humans and on animal skin membranes exposed to DMSO, and it is tempting to speculate that these effects are related to the mechanical responses of the stratum corneum strips to DMSO reported here (1, 8, 9). The physical picture emerging from these studies is one in which the fibrous protein components of the skin barrier are caused to swell within a restraining environment. The action of DMSO upon the keratin in hair compared to the effect in water seems to be similar in that a swelling effect is accompanied by a strengthening phenomenon (6).

2. The nature of the proteinaceous components of guinea pig stratum corneum which underwent the experimental mechanical stress described here is such that a cystine bond reducing agent (ammonium thioglycolate) weakened it in a partially reversible manner. Thus, a structural role for disulfide bonds in stratum corneum has been demonstrated.

Trichloroacetic acid, phenol, and a cross-linking reagent (formaldehyde) have been shown to increase the work index or impart strength to skin strips. With 5% TCA, the increase is reversible; with 2 and 5% solutions of phenol, it is only partly reversible. TCA and phenol may be exerting their strengthening effects partly through the formation, respectively, of salt-like linkages and hydrophobic-like linkages and partly through the precipitation of soluble protein components of the stratum corneum. Ten per cent formaldehyde increases the work index of stratum corneum strips in an irreversible manner, thus demonstrating its cross-linking ability.

Protein denaturating reagents (6*M* urea and 97% formic acid) weakened the skin strips considerably. The action of 6*M* urea was only partly reversible. Guinea pig stratum corneum strips immersed in formic acid were irreversibly damaged. Thus, a role for salt linkages, hydrogen bonds, and hydrophobic bonds has been established as structural components of the stratum corneum.

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