Effects of Dimethylsulfoxide on Normal and Burned Dog Skin (34951)

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Dimethylsulfoxide (DMSO), an alkyl sulfoxide, is a powerful solvent derived from a waste product of paper manufacture. Its properties and the clinical problems for which it has been tried have been described in a comprehensive review (1).

Experimentally and clinically DMSO has been reported to penetrate skin rapidly and to cause no permanent tissue damage even in high concentrations (2). Effects on the collagen of the dermis have been claimed in the treatment of several dermatoses—psoriasis, scleroderma, keloids, radiation fibrosis, and eczema (3–5)—suggesting that at least abnormal collagen is affected by DMSO.

Burn therapy with DMSO has received only limited attention. DMSO alone and combined with Sulfamylon was applied intermittently in the treatment of partial and full-thickness burns in rats (6). Penetration of the eschar by DMSO was theoretically demonstrated by the addition of methylene blue. There was no increase in survival rates nor decrease in *Pseudomonas* organisms (7). Rabbit skin placed in DMSO for 24 hr produced skin that could be penetrated easily with a finger, indicating destruction (8). Tensile strength of rat tail tendons treated for 24 hr with varied concentrations of DMSO decreased when a concentration of 95% or higher was used. It was concluded DMSO changed the physical properties of collagen by causing lysis of its intermolecular bonds.

With this background and because dried collagen from tendon swelled markedly in DMSO, we wondered if DMSO could alter the susceptibility of collagen to digestion by proteolytic enzymes that ordinarily did not attack it. By definition, only collagenase digests sclerocollagen while trypsin and similar proteolytic enzymes can digest collagen only after it is denatured. Here we have investigated the effect of DMSO on the tensile strength of normal and burned dog skin and on the capacity of proteolytic enzymes other than collagenase to digest both. Partially denatured beef tendon collagen is also used.

Methods and Materials. Large swatches of fresh skin were excised from the abdomen of a dead dog and were mechanically defatted. Several 12 × 6 cm pieces were cut and burned to full thickness by passing them through a bunsen burner flame for 15 sec with the keratin side toward the flame. Sections of both burned and normal skin were then totally immersed in 50% and 100% DMSO at room temperature for 24 hr. All skin was then fashioned into 8 × 1-cm strips with small triangles cut from the middle of the long sides to leave a central isthmus of 3 mm. Their tensile strengths were tested on a Scott Tester, Model X-3,[1] with special clamps for holding the skin. To correlate anatomical changes, specimens of normal and normal treated, and burned untreated and treated were examined for microscopic differences after coloring with hematoxolin and eosin stain.

The enzyme study was initiated by determining the effect of varied concentrations of DMSO on the activity of elastase and trypsin against substrates that they do attack. One milligram of elastase (Worthington) in 0.1% concentration was incubated with 13 mg of elastin orcein (9), while 0.1% trypsin (Princeton) was incubated with 10 mg of azocol (10). One milliliter of different concentrations (1.0%, 5%, 10%, 25%, 50% and 100%) of DMSO were added while the same

[1] Scott Testers, Inc., Providence, Rhode Island 20901.

TABLE I. Tensile Strengths of Normal and Burned Dog Skin After Treatment with DMSO for 24 hr at Room Temperature.

Type of skin	Tensile strength (lb)					Average	% Loss
Normal (control)	15.0	13.0	13.0	17.0	14.0	14.4	—
Normal and 50% DMSO	13.5	13.0	15.0	12.5	12.0	13.2	8
Normal and 100% DMSO	12.0	12.5	9.0	11.0	10.5	11.0	24
Burned (control)	4.0	4.0	5.0	3.5	6.0	4.5	69
Burned and 50% DMSO	1.5	2.0	2.5	3.0	4.0	2.6	82
Burned and 100% DMSO	2.5	1.5	0.5	0.5	1.0	1.2	91

amount of distilled water was added to the controls. They were incubated for 24 hr at 37°.

Next, enzymatic digestion of normal and burned dog skin was studied before and after pretreatment with DMSO. The dog skin was cut into 1 × 0.5-cm pieces and some pieces were treated with 6 cc 100% DMSO for 8 and 24 hr. After washing three times in distilled water, they were incubated in 0.2% concentrations of trypsin, elastase, protease, and collagenase. To each tube 5 mg of neomycin sulfate was added to inhibit bacterial growth. Cultures were taken after incubation for 24 hr at 37° to be certain of sterility. If contaminated, the tube was discarded. Lysis was judged on a 1–4+ scale. The ease with which the tissue disintegrated on probing was noted. Complete lysis was unattainable because no enzyme was used to dissolve fat.

The effect of DMSO pretreatment on the susceptibility of partially denatured collagen to attack by proteolytic enzymes was studied by putting 15-mg pieces of shredded defatted beef tendon in 1 ml of Tris buffer with 0.5% trypsin (Princeton), elastase (Worthington),

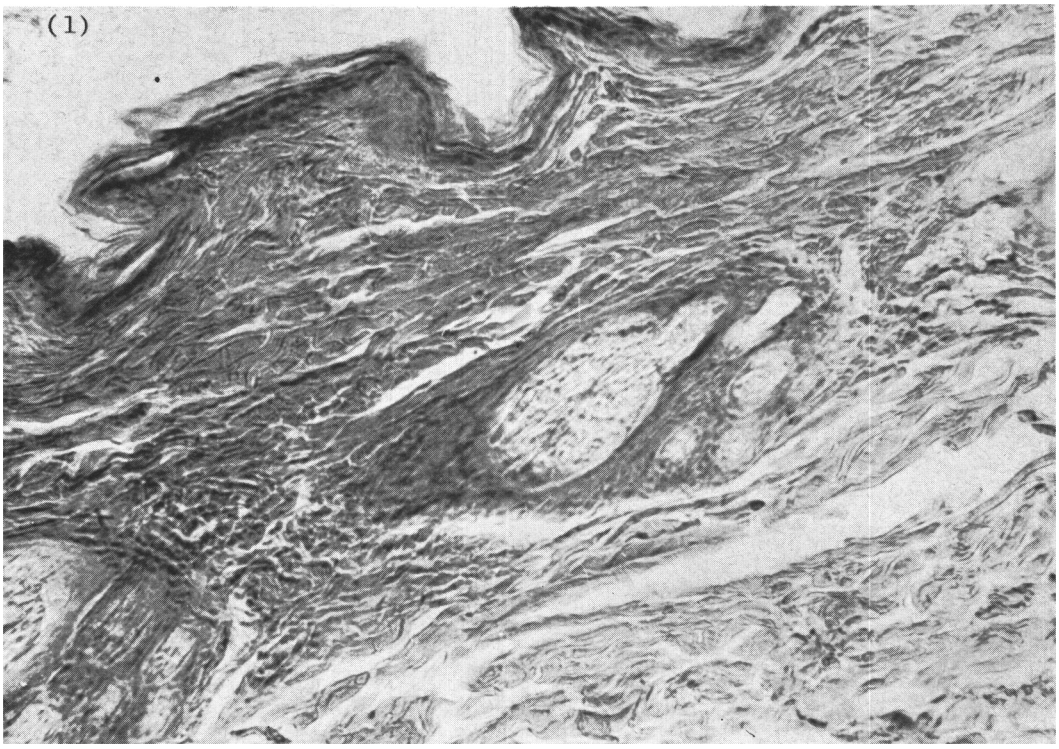

FIG. 1. Darkly stained, compact, uniform collagenous bundles in normal dog skin. H & E stain. 160×.

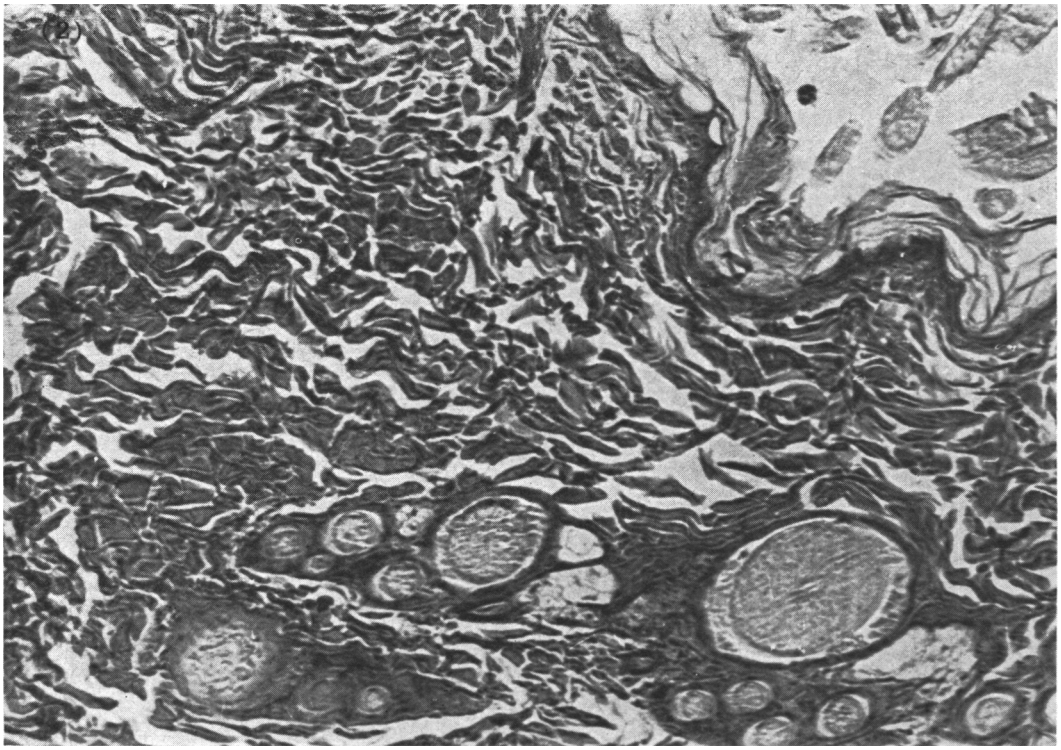

FIG. 2. Swollen and separated collagenous bundles of normal dog skin after soaking in 100% DMSO. H & E stain. 160×.

protease (S.E.A.B.), and collagenase (Worthington). They were incubated for 24 hr at 37°. The collagen was then dried for 21 days and weighed to determine the amount lost. Using a second batch of collagen, 1 ml 100% DMSO was substituted for Tris buffer. In a third experiment pieces of beef tendon collagen were pretreated with 100% DMSO for 24 hr at 37°. Tris buffer, pH 7.2, water, and acetic acid, 3%, were used for controls. This collagen was then washed three times with distilled water and dried for 10 days. At this time, 0.5% concentrations of the same enzymes were added to known amounts which were incubated, dried, and weighed as above to determine if any of the collagen had been made soluble. All samples were subsequently incubated again at 37° for 24 hr in 1 ml of distilled water, rewashed with distilled water, dried for 2 weeks, and weighed.

Results. Normal untreated skin had the greatest tensile strength, average 14.4 lb (Table I). Burned skin treated with 100% DMSO had the least tensile strength, 1.2 lb average. Normal skin pretreated with 50% DMSO had 8% less tensile strength than the average normal skin, whereas with treatment with 100% DMSO there was 24% less strength. Burned normal skin had 69% less tensile strength. Burned skin treated with 50% DMSO had 82% less tensile strength, while that treated with 100% DMSO had 91% less strength than the average normal skin. The data show that burning lowers tensile strength of skin suggesting destruction of contained collagen. DMSO lowers somewhat the strength of normal skin, but not as much as that of burned skin. The amount of lowering is dependent on the strength of the DMSO used and one suspects on the duration of contact.

Microscopic changes in collagen of the dermis are shown in Figs. 1 through 4. The darkly stained, compact, uniform collagenous bundles in normal dog skin are seen in Fig. 1. They become swollen and separate after

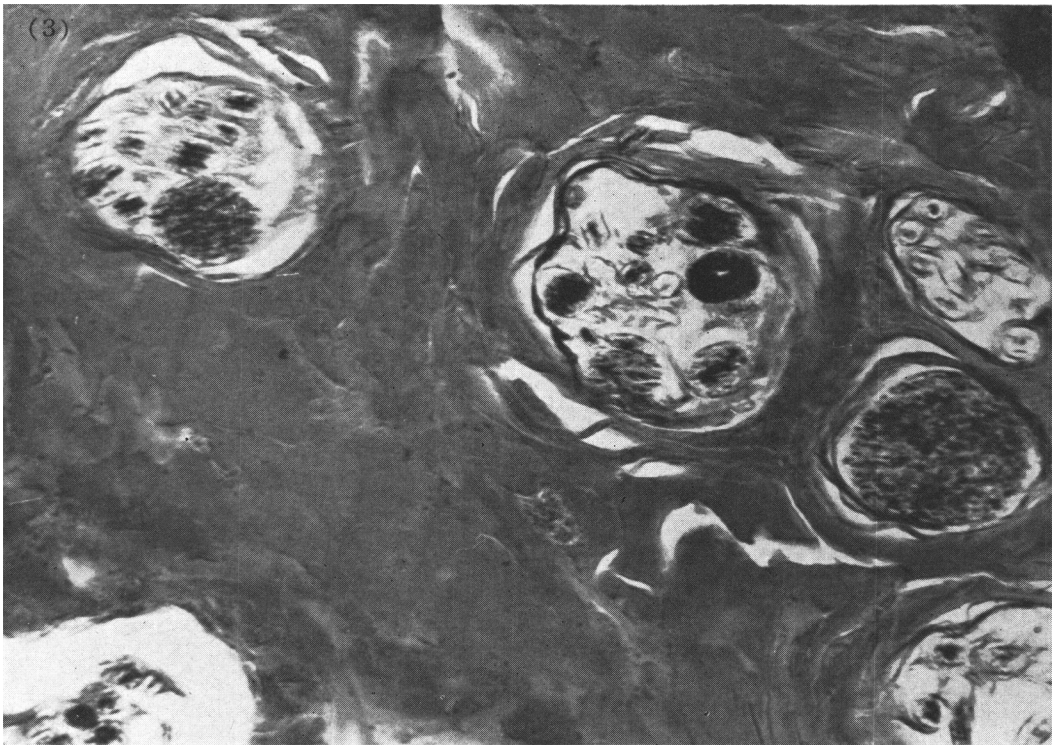

FIG. 3. Collagenous bundles of burned dog skin. Coagulated bundles, loss of identity of fibers, and poor staining. H & E stain. 160×.

soaking in 100% DMSO (Fig. 2). Burning of normal dog skin (Fig. 3) causes coagulation, swelling, and loss of identity of fibers with poor staining characteristics. In addition to these changes, soaking in 100% DMSO results in cleft formation (Fig. 4).

Elastin orcein was completely digested by elastase, while there was 79% digestion in the presence of 10% DMSO. With increased concentrations, elastase activity was totally inhibited. Trypsin digested 83% of azocol without DMSO, but the percentage fell from 78% to 25% as the DMSO concentration was increased from 10% to 100%. Under the circumstances of the experiment, elastase is more affected by DMSO than trypsin.

None of the proteolytic enzymes (Table II) digested normal dog skin except collagenase. All digested, however, a limited amount of burned dog skin. Eight hours of pretreatment of burned skin with 100% DMSO caused no appreciable difference, but after 24 hr of pretreatment there was a marked increase in digestion by elastase, collagenase, and protease, but no change in the action of trypsin. Free DMSO had been removed in these experiments by washing.

Table III shows the effect of DMSO on the proteolytic digestion of partially denatured beef tendon collagen. Five milligrams of trypsin, elastase, protease, and collagenase in 1 ml of Tris buffer digested 75%, 79%, 85%, and 100% of the collagen, respectively. Dissolved in 1 ml of 100% DMSO all the enzymes were completely inhibited except for an insignificant 2% digestion by trypsin. The DMSO control lost 11%. After 100% DMSO pretreatment of this collagen and washing out, all the enzymes caused complete digestion. Interestingly, DMSO-pretreated collagen incubated in Tris buffer (pH 7.2) yielded a loss of 53% on washing, suggesting that the DMSO had caused part of this collagen to become soluble in Tris buffer. With Tris buffer alone only 9% was lost.

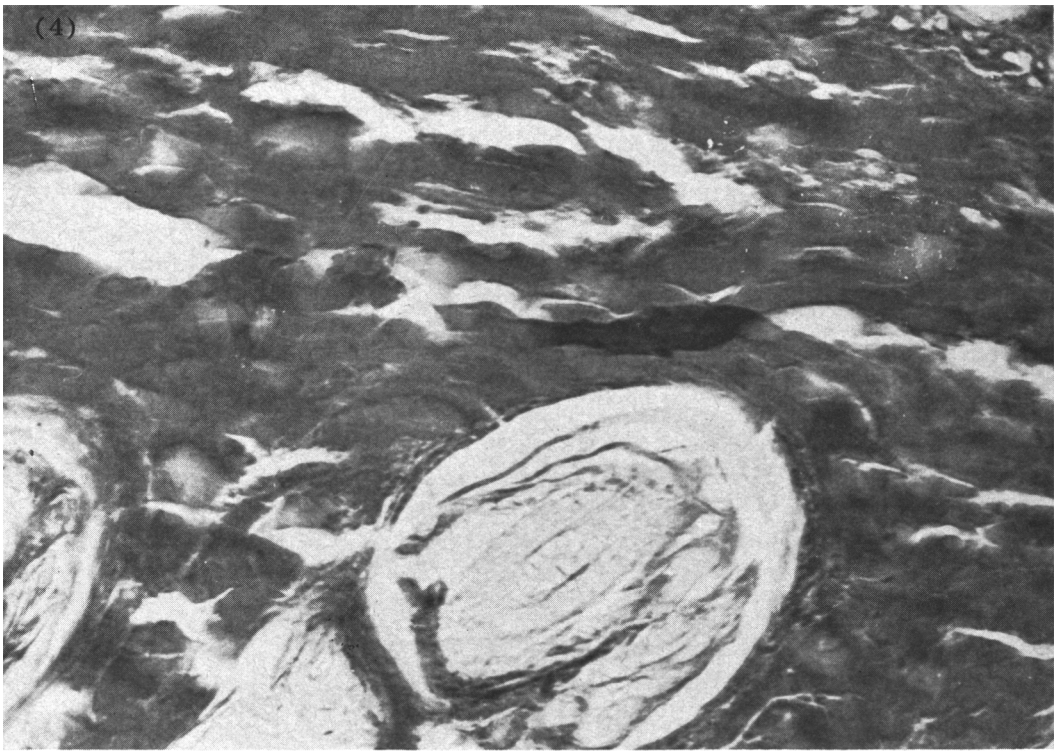

FIG. 4. Collagenous bundles of burned dog skin after soaking in 100% DMSO for 24 hr. Coagulated bundles, loss of identity of fibers, poor staining, swollen hair follicles, and many clefts. H & E stain. 160×.

Incubation of beef tendon collagen in 100% DMSO and 3% acetic acid for 24 hr caused marked swelling and increases in weight (Table IV). At 37° the acetic acid-treated material resisted drying more than the DMSO-treated. When incubated again with water, washed, dried, and weighed, the DMSO-treated sample lost 96% of its weight, while the acetic acid-treated material still weighed three times its original weight. The distilled water-treated sample lost 13%, Tris buffer 15%, and no solution 25%. Thus, while both DMSO and acetic acid caused swelling of partially denatured collagen, the former readily lost its absorbed water on drying at room temperature, while the latter did not, and the secondary solubility of the DMSO-treated collagen in water indicates a chemical change has occurred.

Discussion. DMSO swelled normal and

TABLE II. Effect of DMSO (100%) Pretreatment on Proteolytic Digestion (1–4+) of Washed Normal and Burned Dog Skin.

	No treatment		Treated 8 hr		Treated 24 hr	
Enzyme (0.2%) in distilled water	Normal skin	Burned skin	Normal skin	Burned skin	Normal skin	Burned skin
Trypsin	0	1+	1+	1+	1+	4+
Elastase	0	1+	1+	1+	1+	3+
Protease	0	1+	1+	1+	2+	4+
Collagenase	2+	2+	2+	2+	3+	4+

TABLE III. Influence of DMSO and DMSO Pretreatment on Digestion by Proteolytic Enzymes of Partially Denatured Beef Tendon Collagen (15 mg).

Enzyme (0.5%)	Enzyme in Tris buffer pH 7.2 (% digested)	Enzyme in 100% DMSO (% digested)	In Tris buffer but collagen pretreatment with DMSO (% digested)
Trypsin	75	2	100
Elastase	79	0	98
Protease	85	0	100
Collagenase	100	0	100
Control Tris buffer (no enzyme)	9	—	53
Control 100% DMSO (no enzyme)	—	11	—

burned skin, changed the microscopic appearance of the dermis, and reduced their tensile strengths, particularly burned skin. DMSO pretreatment made skin, particularly burned skin, more susceptible to the action of proteolytic enzymes and to enzymes that ordinarily do not attack collagen. Partially denatured beef tendon collagen not only became greatly swollen with DMSO and more susceptible to the action of enzymes, but it became water and Tris buffer soluble. Lower concentrations of DMSO were required to attack partially denatured collagen or burned skin than was required to attack normal skin.

DMSO in concentrations above 10% interfered with the action of proteolytic enzymes, especially above 25%.

These results find some parallel in the works of others (5). Patients with scleroderma treated topically with high concentrations of DMSO had increased amounts of acid mucopolysaccharides in involved areas, presumably from a breakdown of collagen. But mucopolysaccharides increase locally anyway during healing (11). Postirradiated subcutaneous plaques treated with topical 90% DMSO underwent involution that correlated microscopically with a reduction of both soluble and insoluble fractions of collagen, while mucopolysaccharides remained constant (4). A shift toward degradation in the balance between synthesis and degradation rather than an actual breakdown of collagen was suspected. A reduction in the collagenous fraction extractable with neutral salt solution, but not in insoluble collagen was found when rabbit skin was treated *in vitro* with pure DMSO (8). In *in vivo* studies of rabbit's skin both synthesis and catabolism of collagen were believed to be diminished simultaneously after DMSO treatment because no significant change occurred in the amount of insoluble

TABLE IV. Weight Change of 15 mg of Partially Denatured Beef Tendon Collagen Incubated at 37° for 24 hr in Various Solutions.

Incubating solutions	After 1 week of drying (mg)	After 2 weeks of drying (mg)	After incubating again in distilled water and drying for 2 weeks	% Change in weight after washing and second drying
100% DMSO	62.69	14.72	0.56	—96
3% Acetic acid	655.18	342.33	371.09	+30X
Distilled water	16.89	14.69	13.10	—13
Tris buffer pH 7.2	13.91	13.62	12.79	—15
Control distilled water not incubated	16.52	14.41	11.27	—25

collagen present. No change occurred in the tensile strength of rat tail tendon until a concentration of 95% DMSO was used (8).

Because DMSO inhibited the action of proteolytic enzymes, this inhibition might be expected to occur against naturally occurring enzymes, as white blood cells, as well as in those added externally. In these experiments however, there was a prolonged contact between the DMSO and the enzyme that may not occur *in vivo*. If external enzymes were used after DMSO it should be washed away. A small but constant amount of DMSO has been found still fixed to various tissues after 24 hr of dialysis (12). Others have reported effects of DMSO on enzymes. Increased trypsin-catalyzed hydrolysis of *p*-toluene sulfonyl-L-arginine methyl ester has been found with up to 20% concentration of DMSO, but above 30% there was precipitation of the buffer or substrate (13). With a different substrate, an almost linear decline in trypsin activity was found with increasing concentrations of DMSO starting at 5% (12). Changes in protein configuration may be partially responsible for the effects of DMSO on enzymes. DMSO inhibition of pancreatic DNAase has been demonstrated at pH 6, but at higher pH the action of DNAase was enhanced (14). The marked enhancement of digestion of shredded beef tendon collagen we found with trypsin, elastase, and protease after pretreatment with 100% DMSO and washing away illustrates how the attack on the substrate may be regarded as enhancement of the activity of an enzyme rather than a change to different solubilities. Cutting, tearing, pretreatment with acids, bases, some salts, and heating have been found to make collagen more vulnerable to attack by trypsin (15). DMSO must now be added to this list. Indeed, properly applied, DMSO treatment alone might be all that is needed for the lysis of burned collagen. The effect becomes apparent, however, only after exposure in excess of 8 hr and at high concentrations.

In treating burns in rats it has been suggested that 90% DMSO applied in a spray twice a day might have been more valuable if applied continuously (6). On the other hand, because of water evaporation on application, a concentration of 67% might be most efficacious for topical use because the heat of hydration on application in higher concentrations might increase diffusion rates across the epidermis (16). DMSO hydrate contains 2 moles of water. The absorption of DMSO from the local area must decrease its concentration and affect the duration of action of a higher concentration. The toxicity of a maintained high concentration in the burned individual must be studied separately. DMSO has been shown to be bacteriostatic against *Staphlococcus* and *Pseudomonas* (17).

Summary. 1. Dimethylsulfoxide swells collagen in the dermis and decreases the tensile strength of both normal and full-thickness burned dog skin, especially the latter. These effects are more pronounced with 100% than with 50% DMSO.

2. The digestion of burned dog skin *in vitro* by elastase, collagenase, and protease is increased by pretreatment for 24 hr with 100% DMSO.

3. Pretreatment for 24 hr with 100% DMSO not only increases the degree of digestion of partially denatured beef tendon collagen by enzymes, but also partially converts it to a form soluble in water and Tris buffer at pH 7.2.

4. DMSO appears to be a denaturant of collagen, more active against burned and partially denatured collagen than against sclerocollagen.

5. A continuous application of DMSO at 90%–100% concentrations for at least 24 hr might render full-thickness burn eschar more readily removed by solutes and might increase its susceptibility to enzymatic digestion.

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Received Jan. 23, 1970. P.S.E.B.M., 1970, Vol. 134.