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Dimethyl Sulfoxide

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Volume 1. Basic Concepts of DMSO

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DEDICATION

To the many scientists from all over the world whose names appear on references relating to DMSO and who actually made this text possible.

Preface

Rapid accumulation of scientific articles on the clinical usefulness of DMSO following Jacob and Herschler's original publications led the authors to believe that it might be well to have current basic science thinking of DMSO under one cover.

The editors attempted to choose chapter authors that had qualifications for critical scientific evaluation of the literature in their specialties as well as being able to devote the time necessary to produce such a chapter. We are indeed grateful to those men who dedicated their efforts in the preparation of this book.

This text has been a continuous process for the past 2 years. We have maintained a constant check of the literature and believe that it is essentially current as of May 1969. It is inevitable that with such a massive literature some articles were missed and as these are called to our attention they will be added to subsequent printings.

We are aware of important clinical articles which will be summarized later in a sequel to this volume.

We are particularly indebted to Dr. Chauncey Leake and Dr. D.W.E. Baird, Dean emeritus of the University of Oregon Medical School, who stood steadfastly by us in the darkest hours when world opinion seemed to be against DMSO. We are also grateful to Dr. Gerhard Laudahn, of the Schering A.G. Corporation, who stimulated interest in research of this drug amongst European scientists even when it appeared that the drug might not be of any commercial value because of suspected "toxicity."

We will be ever grateful to the New York Academy of Sciences for its persistence in sponsoring a clinical conference on DMSO in March 1966 when political and social pressures would have made it much simpler for them to have abandoned the project. Instead, they used only

one criterion in deciding to sponsor an international symposium on DMSO after the drug had been declared "toxic" in humans; *let scientific facts speak for themselves.*

Without the help of these men and the Academy, clinical use of DMSO might have died in its infancy.

As the work on this volume draws to a close, we are receiving reports of research on DMSO in iron curtain countries. These studies have not been completely translated yet and will have to await future revisions of this volume. We are also aware of current unpublished research by other scientists throughout the world that therefore cannot be included in the initial publication.

The publishers have been extremely patient with us. We acknowledge with gratitude their foresight and stimulus in encouraging us to proceed with this endeavor.

Finally, our most heartfelt thanks to the many secretaries, typists, librarians, and scientists who so faithfully helped with the preparation of final copy—thank you each one.

S.W.J.
E.E.R.
D.C.W.

Foreword

Dimethyl sulfoxide, DMSO, is a remarkably versatile chemical compound, especially in its wide range of applicability to many major biological problems, involving both plants and animals, and importantly, humans as well. Its basic physico-chemical properties make possible its broad applications. Especially significant are its solvent properties. Its capacity to bind water led to its early use in preserving animal and human tissues and cells. Its radioprotective effect on living material was also quickly noted and its penetrating power led to its agricultural use in control of plant diseases, and in aiding nutritive element transport in plants.

Although prepared by Alexander Saytzeff in 1866, DMSO attracted little biological attention, except as a tissue preservative, until the discovery was reported in early 1964 by Stanley Jacob and Robert Herschler of its wide range of primary pharmacologic actions and unique tissue penetrability in plants and animals. This suggested many possible clinical applications. Among other things it was used to dissolve chemicals for direct application and absorption through the skin. Clinical studies showed that DMSO itself could relieve the pain and inflammation of musculoskeletal injuries. DMSO was made available to drug manufacturers through the Crown Zellerbach Corporation, which holds a process patent on making the compound from lignin. Clinical investigation was progressing routinely when a report was made that DMSO produced disturbances of the lens of the eye in experimental animals. The U.S. Food and Drug Administration then halted further clinical study on the drug.

Following two large conferences, one arranged by the New York Academy of Sciences in New York City, the other by Schering A.G. at the University of Vienna Medical School, it was apparent by 1967 that

allegations of toxicity from clinical use of DMSO were exaggerated. By that time many thousands of patients treated by DMSO for a variety of skin and musculoskeletal conditions had shown enough significant improvements to justify continuing clinical evaluation. Many careful clinical reports now testify to its relatively high clinical effectiveness and safety. Administrative roadblocks in the United States unfortunately still hamper appropriate use of DMSO in our country. On the other hand, it is now employed clinically in Europe, with much apparent satisfaction.

It is appropriate that Stanley Jacob, who so modestly, steadily, and effectively continued to study DMSO after he had first called attention to its potential usefulness in medicine, should bring together and edit for publication with two able colleagues, Edward E. Rosenbaum and Don C. Wood, reliable scientific information on the wide usefulness of this very interesting compound. This monograph should long serve as a basic reference source for information about DMSO. Impressive indeed are the vast numbers of publications on DMSO to which reference is made. It is likely that stimulus to further investigation of the remarkable physico-chemical properties of DMSO will result in ever more beneficial applications. It is hoped that this monograph may provide such stimulus.

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Contents

Chapter 1

Chemistry of DMSO

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I. INTRODUCTION

Excellent reviews dealing with the chemistry of dimethyl sulfoxide (DMSO) have appeared during the past decade *(1–13)*. The extraordinary interest in DMSO can be traced to a combination of the following factors:

(1) DMSO is one of the most prominent members of the family of polar but aprotic solvents *(14–18)* that have become widely available since World War II and which also include *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), *N*-methylpyrrolidone, sulfolane or tetramethylene sulfone (TMS), acetonitrile (AN), ethylene and propylene carbonates, and hexamethylphosphorotriamide (HMPT). Many of the traditional concepts of the role of solvent molecules in chemical processes were evolved by considering the existence of two opposite classes of solvents, the nonpolar and the polar. The latter, historically speaking, always belonged to the protic kind (water, the lower alcohols, acetic acid, and so forth), with the result that subconsciously the behavior of polar solvents became synonymous with that of the protic solvents. With the advent of the polar, aprotic solvents a better understanding was attained of the effects caused by specific solvation of the reacting species, and these specific solvation effects began to be distinguished from effects previously attributed to changes in the dielectric constant of the reaction medium.

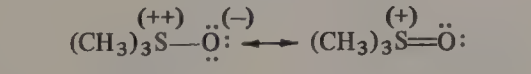
(2) More so than any other member of the polar, aprotic solvent family, DMSO is also an extremely versatile reagent because it can act as a nucleophilic reagent at either the oxygen or the sulfur terminal of the sulfoxide function, behaving therefore either as a "hard" or "soft" base *(19)*, respectively. In addition, it is capable of being either an oxidizing or a reducing agent. Furthermore, the hydrogen atoms are capable of being ionized in the presence of strong bases, thus opening the way to a broad, new field of synthetic applications utilizing the anion of DMSO ("dimsyl"). At the same time, DMSO contains an excellent "leaving" group in the form of the methanesulfinate anion $(CH_3SO)^-$, and this gives rise to a series of interesting consequences in the chemistry of the dimsyl ion.

(3) Last but not least is the discovery of the exceptional biological properties of DMSO that have led to some immediate applications in medicine *(20)* and have aroused worldwide interest in its chemistry.

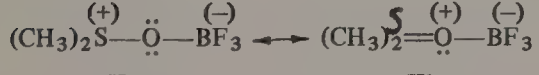
The remaining chapters of this monograph are dedicated to the biological aspects of DMSO and thus it is appropriate that this chapter attempts to bring to light those physical and chemical characteristics of DMSO that may have a direct bearing on its biological properties. The latter hinge, to a great degree, on the unique ability of DMSO to penetrate and diffuse through living tissues, a property most likely related to the structure and thermodynamics of molecular complexes formed by DMSO with water, protein, lipids, salts, and a host of other substances.

II. THE STRUCTURE AND SOME PHYSICAL PROPERTIES OF DMSO

The DMSO molecule can be visualized in terms of a tetrahedron, the center of which is occupied by a sulfur atom; two methyl groups, an oxygen atom, and a nonbonding electron pair are located in the apex positions. There is general agreement in the literature with regard to the bond angles present in DMSO: 96.4° *(21,22)* or 97.4 ± 0.4° *(23)* for the C—S—C, and 106.7 ± 0.4° *(21-23)* for the C—S—O bonds. Less satisfactory is the agreement on the bond distances, especially the length of the S—O bond. The most recent value *(23)* of 1.513 ± 0.005Å (or 1.531 when corrected for the libration motion) is significantly larger than the previously accepted S—O bond distance of 1.47 ± 0.03Å *(21-25)*. This difference has an important bearing on the long-standing controversy *(26-35)* concerning the single bond or double bond nature of the S—O bonds in sulfoxides, sulfones, sulfites, and related structures. If we accept the value of 1.513 for the S—O bond length in DMSO and compare it to the values of 1.45 and 1.517Å reported for the structurally similar trimethylsulfoxonium ion *(36)* and the DMSO-boron trifluoride complex *(37)*, respectively, it follows that the S—O bond order increases when the sulfur atom becomes increasingly electron deficient and, contrarywise, the S—O bond order decreases somewhat when the nonbonding electrons of oxygen are donated to an electron acceptor. Thus, the electron distribution in the trimethylsulfoxonium ion and in the $DMSO \cdot BF_3$ complex are more accurately represented by Ib and IIa, respectively.



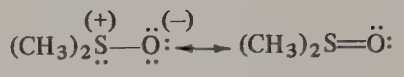
Ia Ib



IIa IIb

The most extensive analysis of S—O bond lengths in a variety of compounds lead to an estimated bond order of 1.56 *(34)* for DMSO on the basis of the interatomic distance of 1.47Å, while acceptance of the bond length value of 1.513Å reduces the bond order to approximately 1.42. In conclusion, we can see that the bond order of the S—O bond in DMSO hovers around the value of 1.5, but if we must choose one of

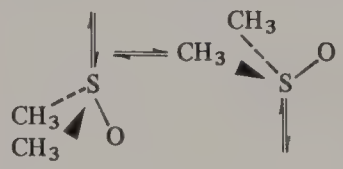
the two extreme electron distributions, the representation IIIa appears to be more appropriate.



IIIa IIIb

The C—S bond length reported originally *(22)* as 1.82° has been reduced to an average value of 1.79 ± 0.01Å in more recent determinations *(23)*. More interestingly, there is a disparity between the two C—S bonds of DMSO in the crystalline state *(23)*. We shall return to this point later when the structure of aggregates of DMSO molecules is discussed.

Even though molecular symmetry eliminates the possibility of stereoisomerism in the case of DMSO, it is of interest to inquire about the molecular vibration in which the pyramidal arrangement of the methyl and oxygen groups becomes inverted (IV).



IVa IVb

Since the stereomutation of unsymmetrical sulfoxides is relatively insensitive to structural changes in the two carbon groups *(38)*, it can be assumed that activation parameters for the inversion of the DMSO structure fall into the range of values observed for a number of sulfoxides, namely, an enthalpy of activation of 35–42 kcal/mole and an entropy of activation of −8 to +4 eu. The thermally induced inversion is a slow process if it is considered that at 210°C the first-order rate constant of racemization of optically active sulfoxides is about 10^{-5} sec^{-1}. This corresponds to a half-life of about 6 hr and explains why a resolved sulfoxide retains its configurational identity under average laboratory conditions. However, the stereomutation of sulfoxides can be facilitated under a variety of catalytic conditions *(38)* and some of these are relevant to the oxygen exchange of DMSO discussed in Section III,B.

The conclusion that DMSO is highly associated in the liquid and solid states is inescapable when certain of its physical properties are considered.

TABLE 1

Selected Physical Properties of DMSO

Property	Value	Ref.
Boiling point at 760 mm Hg, °C	189.0	(*1*)
Vapor pressure at 20°C, mm Hg	0.37, 0.417	(*7*)
Specific heat at 25°C, cal/g	0.4698	(*7*)
Specific heat of vaporization at b.p., cal/g	175	(*7*)
Molar heat of vaporization at b.p., kcal/mole	13.67	(*1*)
Entropy of vaporization at b.p., cal/deg. mole	29.6	(*1*)
Enthalpy of vaporization at 25°C, kcal/mole	12.64	(*6*)
Coefficient of expansion, ml/g. deg	0.00088	(*2*)
Specific gravity at 25°C, g/ml	1.0958	(*6*)
	1.09556	(*253*)
Melting point, °C	18.55	(*6*)
Specific heat of fusion, cal/g	38.8	(*39*)
Molar heat of fusion, kcal/mole	3.43	(*6*)
	3.03	(*39*)
Entropy of fusion, cal/deg. mole	10.4	(*39*)
	11.7	(*7*)
Specific heat of solid at 18.45°C, cal/g	0.5	(*1*)
Heat of formation (graphite and $S_{rhomb.}$), kcal/mole	−47.7	(*2*)
Heat of combustion, cal/g	6050	(*2*)
Flash point, open vessel, °C	95	(*2*)
Molal f.p. depression, deg/mole/1000 g	4.4, 4.36	(*7*)
Refractive index (n_D^{20})	1.4783	(*1*)
Refractive index (n_D^{25})	1.47674	(*253*)
Molar refractivity, R_m	20.12	(*1*)
Polarizability, cm^3	7.97×10^{-24}	(*1*)
Dielectric constant at 20°C	48.9	(*1*)
Dielectric constant at 25°C	46.4	(*40*)
Dipole moment, 25°C, D	4.11	(*40*)
Viscosity, 20°C, cP	2.473	(*1*)
Viscosity, 25°C, cP	1.99	(*5*)
Surface tension at 20°C, dyne/cm	46.2	(*1*)
Surface tension at 25°C, dyne/cm	42.86	(*5*)
Specific conductance at 20°C, $ohm^{-1} cm^{-1}$	3×10^{-8}	(*1*)

It can be seen that in the case of DMSO the properties that characterize molecular association in some instances outdistance the equivalent properties of water—the prototype of a highly associated substance. This is particularly evident when the molar enthalpies and entropies of vaporization are considered.

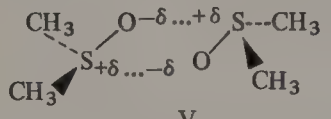
A recent x-ray study of a single crystal of DMSO *(23)* reveals two

interesting features which may be indicative of the nature of the strong molecular forces in the solid. First of all, it is found that along the *b* axis of the crystal, the molecules of DMSO alternate the orientation of the S—O bonds, and the distance separating the sulfur and oxygen atoms of neighboring molecules is 2.51Å. This leaves little doubt that the principal attractive force in an aggregate of DMSO molecules is the result of dipole-dipole attractions. Second, the x-ray study reveals a surprising disparity in the intramolecular S—C bond distances. It turns out that for the same DMSO molecule the C—S bond distance in one methyl group is 1.771, while that of the other is 1.805Å. This goes hand in hand with the observation that each oxygen atom is located relatively close to two of the three hydrogen atoms of one of the methyl groups of a neighboring molecule (2.40 and 2.51Å), but only to one hydrogen atom in the methyl group of another neighboring molecule (2.49Å). The "corrugated layer structure of the crystal, with S–O dipoles and methyl groups alternating in planes parallel to *ab*" *(23)* suggests the presence of a weak hydrogen bond that contributes to the sum total of the molecular forces in DMSO. Actually, a hydrogen-bond-like association was mentioned in the first extensive review of DMSO *(1)*. The recent x-ray study *(23)* provides experimental evidence that some intermolecular H···O bond distances in the DMSO crystals fall short of the sum of the accepted van der Waals' radii for hydrogen and oxygen (1.2 and 1.4Å, respectively). The ability of C—H bonds to function as hydrogen bond donors has been reviewed recently *(24)*, and there exists spectroscopic evidence *(41)* for hydrogen bonding between acetylenic hydrogen groups and DMSO. However, neutron inelastic scattering does not support *(42)* the participation of the methyl groups of DMSO in intermolecular hydrogen bonding.

In the liquid state DMSO is believed *(17,39)* to assume a chainlike structure held together by the alignment of S—O dipoles. This structure is believed *(1)* to suffer a partial breakdown between 40° and 60°C since certain properties of liquid DMSO, such as the refractive index, density, and viscosity, exhibit distinct changes in their temperature coefficients in this temperature range.

Some insight into the structure of DMSO aggregates can be attained by an examination of the concentration dependence of certain properties of DMSO solutions. Thus, for example, the analysis of dielectric constant data for benzene solutions of DMSO at 25°C *(40)* reveals interesting variations in the apparent dipole moment as a function of DMSO concentration. The infinitely dilute solution gives a dipole

moment of 3.88 D, but the moment decreases to the minimum value of 3.45 D when the DMSO concentration becomes 1.49 *M*, then as the DMSO concentration increases, it rises to remain constant at 3.89 D over the concentration range of 4.9–9.1 *M*, and finally it rises again reaching the value of 4.11 D for neat DMSO. These results are indicative of variable associations of the DMSO molecules. The minimum dipole moment observed for the 1.49 *M* solution corresponds to a maximum concentration of a relatively nonpolar aggregate of DMSO, and suggests the formation of a cyclic dimer (V).



V

The formation of a cyclic dimer was suggested *(43)* on the basis of an infrared examination of DMSO solutions in carbon tetrachloride at 27°C. Under these conditions the dimer seems to predominate in the concentration range 0.02–0.3 *M*, and the value of its formation constant is estimated as 0.9 M^{-1}. The discrepancy in the concentration ranges of DMSO required to produce a dimeric aggregate can be attributed to the difference in the association between DMSO and carbon tetrachloride or benzene, respectively, and the likelihood that the attractive interaction between DMSO and the π electrons of benzene *(44)* outweighs the association of DMSO with the polarizable nonbonding chlorine electrons in carbon tetrachloride (see Section III,A,1).

Cryoscopic results reported *(39)* for the DMSO-benzene system can be interpreted to mean that (between +6° and −6°C and up to a concentration of approximately 0.006 mole fraction DMSO) the formation of the DMSO dimer (with a formation constant of ca. 20 M^{-1}) competes favorably with the formation of chainlike aggregates for which the formation constant is ca. 9.5 M^{-1}. At DMSO concentrations greater than 0.10 mole fraction, the formation of chainlike aggregates begins to exceed the dimer formation. Also, the analysis of refractometric data for benzene solutions of DMSO *(40)* reveals a sharp break in the refractive index-concentration plot at the DMSO concentration of 0.155 mole fractions or 1.91 *M*. This too can be taken as evidence favoring the formation of cyclic dimer at low DMSO concentrations because the refractivity contribution of the sulfur atom (and particularly of its nonbonding electron pair) may be expected to increase in proportion to the tightness of the intermolecular association, and the

latter should be greater in the cyclic dimer than in the chainlike aggregate. Indirect evidence favoring the cyclic structure of the DMSO dimer is the NMR proof of a similar association in the dimer of 2-thiaindane-2-oxide *(45)*. The dimerization of alkyl sulfoxides is expected to be strongly solvent dependent, and thus it is not surprising that methyl *n*-butyl sulfoxide is reported *(46)* to be essentially completely dimeric in the poorly solvating solvent cyclohexane (0.018-0.055 *M* at 31°C).

It has been suggested *(40)* that at higher concentrations of DMSO there exists an equilibrium between extended chainlike and folded-chain or ring aggregates. Apparently the last-mentioned structures predominate, in view of the modest increase in the dipole moment observed at high concentrations of DMSO in benzene.

The view that DMSO dipoles tend to form a definite alignment in the liquid was challenged recently *(47)* on the basis of a nearly unity value of the Kirkwood correlation factor. Instead, the view of strong but nonspecific dipole-dipole forces is preferred.

The self-association of DMSO must be viewed in context with the association of DMSO and different solvent molecules. In this connection the infrared spectral characteristics of DMSO that relate changes in the stretching frequency of the S—O oscillator with perturbances induces at either terminal of the bond are of special interest. Table 2 summarizes some of the available data. We can see that the frequency that corresponds to the least perturbed DMSO molecule (1102 cm^{-1} in the gas phase) is displaced to lower values by interaction with solvent molecules through self-association, or through association with protic or Lewis acids. The frequency shifts caused by solvents increase according to the nature of the molecular interactions in the order: dipole-induced dipole, dipole-dipole, and finally, strong hydrogen bonding. These shifts in the stretching frequencies imply changes in the bond order of the S—O system, but these are relatively small and can be estimated *(58)* to range from the bond order 1.59 (attributed to the 1120 cm^{-1} frequency observed for the palladium complex) to 1.41 (in the cyclic dimer) and 1.28 in metal complexes in which the metal is associated with the oxygen terminal and which exhibit the lowest reported S—O stretching frequency at 904 cm^{-1}. It is of interest to note that metal complexes in which the metal is associated with the sulfur terminal of the S—O bond shift the stretching frequency to higher values.

TABLE 2

Effect of Perturbances on the S—O Stretching Frequency of DMSO[a]

Perturbance	cm^{-1}	Ref.
Complexing with Pd(II) (at S)	1116-1157	(*48,50,51*)
None (gaseous DMSO)	1102-1103	(*48,49*)
None [gaseous $(D_3C)_2SO$]	1096	(*48*)
Dilute solution (0.025 *M*) in hexane	1085	(*52*)
Dilute solution in cyclohexane	1083	(*53*)
Dilute solution in tetrachloroethylene	1075	(*52*)
Dilute solution in carbon tetrachloride	1072	(*52,53*)
Dilute solution in carbon disulfide	1071	(*53*)
Dilute solution in toluene	1070	(*49*)
Dilute solution in *trans*-1,2-dichloroethylene	1066.5	(*53*)
Dilute solution in acetone	1064	(*52,53*)
Dilute solution in acetonitrile	1061	(*52*)
Dilute solution in *cis*-1,2-dichlorethylene	1060	(*53*)
Dilute solution in pyridine	1060	(*52*)
Dilute solution in dichloromethane or nitromethane	1057	(*52,53*)
Dilute solution in chloroform	1054.5	(*53*)
Dilute solution in diiodomethane	1051	(*49*)
Dilute solution in acetic acid	1050	(*55*)
Neat DMSO	1046	(*48*)
Iodine in carbon tetrachloride	1020	(*56,57*)
p-Cresol in carbon tetrachloride	1016	(*52*)
Solution in water	1013	(*54*)
Solution in 2 *M* hydrochloric acid	1003	(*54*)
Solution >0.3 *M* in carbon tetrachloride	1000	(*43*)
Complex with UO_2X_2 (X = Cl, Br)	995	(*50*)
Metal complexes (at O)	904-955	(*48,51*)

[a]For the effect of additives for the purpose of hydrogen bond formation see Tables 8-10. The molar absorptivity is also affected by the perturbance of the S—O oscillator and ranges from 210 in hexane to 480 in carbon disulfide (*51,52*).

Additional structural implications of the infrared data are considered in Section III.

The absorption bands at 721 and 689 cm^{-1} are assigned *(48,50,59)* to the asymmetric and symmetric C—S—C stretching motions, and similarly those at 348-352 and 318-320 cm^{-1} are attributed to the vibrations of the C—S—O system, but little is known about shifts in these bands as a function of DMSO association.

III. THE ASSOCIATIVE PROPERTIES OF DMSO

One of the most fascinating properties of DMSO is its ability to associate with polarizable and polar neutral molecules as well as ionic species. Our limited understanding of the quantitative aspects of these associations has been derived from several experimental approaches. The most useful so far have been calorimetric determinations of the thermodynamic parameters of mixing; determinations of densities (i.e., molar volumes), viscosities and dielectric constants for binary systems at different temperatures; determinations of solubilities; conductometric determinations of dissociation and ion-pairing equilibria; spectroscopic determinations of specific associations attributed to the formation of charge-transfer complexes, intermolecular hydrogen bonds, or complex ions; and the titrimetric determinations of acid-base equilibria in which DMSO functions as the base. Rather than elaborate upon the results of each of the different experimental approaches, an attempt is made here to point out the more significant and relevant conclusions with regard to the associative properties of DMSO. The discussion is classified according to the chemical nature of the partner in the association with DMSO. The association of DMSO with nonionic derivatives of metals is discussed together with the solvation of metallic ions.

Minimal use is made here of deductions concerning molecular associations based on viscosity data. These often lead to "obscure discrepancies" *(60)* even in relatively clear-cut situations in which, for example, associations are motivated by strong intermolecular hydrogen bond formation. In less obvious situations the results are even more obscure *(61)*. The pitfall in the use of viscosity-composition data as a diagnostic approach to the nature of association between the components of a mixture can be traced to the nature of the relationship between the viscosity of a liquid and the free energy of flow. This is given by the Eyring equation *(62)*,

$$\eta = \frac{Nh}{V_M} \exp(-\Delta S/R) \exp(\Delta H/RT) = \frac{Nh}{V_M} \exp(\Delta G/RT)$$

We note that the free energy of viscous flow is affected by two factors, both of which reflect changes in molecular associations within the liquid structure, namely η and V_M. However, these two factors tend to compensate each other to a variable extent. On the one hand, it is to be

expected that strong intermolecular association in a binary mixture will increase its viscosity. However, on the other hand, the observed molar volume will be decreased. An excess viscosity-composition diagram thus reveals mainly which one of the two factors predominates in determining the free energy of flow. In some situations in which the association between two components of the mixture is very pronounced, the viscosity-composition plots point to the same ratio of components in the molecular complex as other methods mentioned here. Such a situation is present in the case of DMSO-water *(63,64)*. The use of the rheochor *(65)*, or excess rheochor, for the deduction of association phenomena has the same shortcomings because this quantity is simply the product of molar volume and viscosity with the arbitrary convention of reducing the effect of viscosity by taking its one-eighths power.

A. The Association of DMSO with Nonionic Substances

1. Nonpolar Substances

The association of a nonpolar substance with DMSO depends, most likely, on dipole-induced dipole interactions, and these interactions increase in proportion to the polarizability of the substance. Solubility in DMSO (see Table 3) is promoted not only by this type of molecular interaction but is also favored by the entropy of mixing. The latter factor, coupled with better packing of spherical molecules in the "free volume" of liquid DMSO, explains the greater solubility of cyclic, unsaturated hydrocarbons as compared to that of noncyclic saturated hydrocarbons. Since the interaction energy resulting from induced dipoles is a direct function of polarizability, but the latter is an inverse function of the binding energy of the electrons *(66)*, the solubility of DMSO increases with the introduction of π and nonbonding electrons, and with a decrease in the electronegativity of the atoms that constitute the substance in question.

The cryoscopic results obtained with dilute benzene solutions (0.04–0.16 mole fraction) of DMSO *(39)* can be interpreted to mean that two or more benzene molecules are associated. The possibility that this association is brought about through the intermediacy of solvent (DMSO) molecules is not excluded.

The association between DMSO and benzene molecules is supported by the notable solvent effects produced by benzene in the NMR spectra

TABLE 3

Solubility of Some Nonpolar Substances in DMSO (g/100 ml DMSO)[a]

Substance	Solubility	Substance	Solubility
Helium	Insol	Carbon dioxide	0.5
Hydrogen	0.00	Carbon disulfide	90
Nitrogen	0.00	Sulfur dioxide	57.4
Oxygen	0.01[b]	Dichlorodifluoromethane	1.8
Methane	0.00[b]	Carbon tetrachloride	Misc.
n-Pentane	0.36	Dichloromethane	Misc.
n-Hexane	2.9	1,2-Dichloroethane	Misc.
n-Decane	0.7	Ethylene	0.32
n-Dodecane	0.38	Butenes	2.1
Cyclohexane	3.67	1,3-Butadiene	4.35
Decalin	4.5	1- or 2-Pentene	7.1
Benzene	Misc.	Isoprene	Misc.
Naphthalene	40	Acetylene	2.99
Dioxane	Misc.	–	–

[a]Solubility data from Ref. (*4*). Data refer to the temperature range of 20-30°C, and in the case of gases to 1 atm at 20°C.

[b]Solubility of oxygen and methane, at 1 atm pressure of gas at 25°C, is reported (*67*) to be 1.57×10^{-4} and 3.86×10^{-4} moles/mole DMSO, respectively.

of complex sulfoxides. These solvent effects have become an important diagnostic tool of molecular geometry *(68,69,70)*.

The thermodynamic parameters for the formation of DMSO benzene solutions *(71)* support only partially the existence of a strong association between the two components. The enthalpy of mixing reaches a maximum *positive* value of 144 cal/mole at the equimolar composition (see Table 13, Section III,A,2). Apparently the association between DMSO and benzene takes place with some sacrifice in the total attractive interactions within the pure components. However, the negative entropy of mixing (at 0.4 mole fraction of DMSO) supports orderly structuring in the binary mixture and so does also the negative excess molar volume *(60)* which shows maximum deviation at 0.4 mole fraction DMSO.

Even though toluene is not, strictly speaking, a nonpolar substance, the negative excess molar volume (maximum deviation at $X_{DMSO} = 0.4$) *(60)* of this binary mixture can be cited in connection with the preceding discussion. As a matter of fact, the total picture presented by Lindberg and collaborators *(60)* of the interactions between DMSO and a series of benzene derivatives leaves little doubt that the attractive

forces are proportional to the electron-donating capacity of the aromatic compounds. This is shown by the linear relationship between the excess molar volumes and the Hammett σ_p values of the substituents. The formation of a π complex implies electrophilic behavior of the sulfur atom in DMSO.

The thermodynamic information concerning the formation of binary solutions of DMSO and carbon tetrachloride is fragmentary, but what little is known suggests *(44)* "a marked interaction between these species." The suspected association must depend by necessity on a dipole-induced dipole interaction.

The formation of a complex between DMSO and iodine occurs in carbon tetrachloride *(72)* at 25°C with an equilibrium constant of 11.6 M^{-1} *(73)* and an enthalpy of −4.4 kcal/mole *(74)*. Viewed in the context of analogous behavior of substances that are structurally related to DMSO, it is clear that DMSO now acts as an electron donor, that iodine is an electron acceptor, and that we are dealing with the formation of a charge-transfer complex. The characteristic absorption maximum is exhibited at 2720Å *(57)*.

An even stronger charge-transfer complex is formed *(75)* between DMSO and tetracyanoethylene in carbon tetrachloride solution. The equilibrium constant ranges from 95.4 M^{-1} at 25°C to 31.8 M^{-1} at 45°C, and the enthalpy of complex formation is −11.3 kcal/mole. Furthermore, it is noteworthy that the formation of the charge-transfer complex is accompanied by the production of an ESR signal. This signal is found to decay with second-order kinetics, and its production is attributed to the thermal excitation of the complex to the low-lying energy level of the triplet state by virtue of the relatively large heat of formation (above 11 kcal/mole).

Similar charge-transfer complexes are formed between DMSO and iodine cyanide *(56)*, and iodine monochloride *(57)*. They are mentioned here because the formation of the complexes is attributed to the electron acceptor qualities of the iodine compounds rather than to the polarity of the halogen compounds.

2. Polar Substances

a. Absence of Hydrogen Bonding. Molecular interactions between polar molecules of two kinds, in which one of them may play the role of solvent, are of great interest, but unfortunately quantitative information with regard to the magnitude of these interactions is still scarce. We can appreciate the magnitude of these interactions by examining

the case *(76)* of the polar solute nitromethane and a series of solvents, both polar and nonpolar, for which the formation constant of the equimolar "complex" and the thermodynamic parameters are given in Table 4. While the "complex" is short-lived and its life time may be not much greater than the duration of a molecular collision in a liquid, i.e., 10^{-11} sec, it nevertheless determines the structure of the liquid.

We can see that the associations listed in Table 4 are characterized by an equilibrium constant of approximately 0.1 M^{-1} and that the enthalpy and entropy of dimer formation are approximately −1 kcal/mole and −8 eu, respectively, regardless of whether the association is attributed to dipole-induced dipole or dipole–dipole interactions. An increase in the equilibrium constant is noted when a hydrogen bond is involved (last entry in Table 4). This trend can also be illustrated with the case of the classic hydrogen-bonded association between chloroform and acetone reported by the same authors *(76)* to have an equilibrium constant of 0.52 M^{-1} and an enthalpy and entropy of −3.4 kcal/mole and −12.2 eu, respectively.

Similar NMR studies of DMSO are not available, but its interactions with other polar, nonhydrogen donor molecules can be expected to be somewhat larger than those reported for nitromethane.

TABLE 4

Thermodynamic Parameters for the Formation of Nitromethane-Solvent Complexes[a] *(76)*

Solvent	K, M^{-1}	ΔH, kcal/mole	ΔS (eu)
Nonpolar solvents			
Carbon disulfide	0.03	−0.7	−9.1
Carbon tetrachloride	0.03	−0.3	−8.1
1,4-Dioxane	0.22	−0.9	−5.8
Benzene	0.17	−1.5	−8.5
Polar solvents			
Toluene	0.19		
Methyl iodide	0.09	−0.1	−5.4
n-Alkyl bromides (C_1-C_{15})	0.11	−0.9	−7.2
n-Butyl chloride	0.09	−1.0	−8.0
Bromoform	0.11	−1.5	−9.3
Chloroform	0.10		
n-Butyl alcohol	0.25	−1.1	−6.4

[a]From NMR solvent shifts at 43°C.

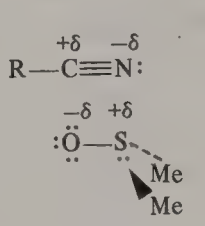
Information concerning associations of DMSO can be deduced from infrared and other spectral data.

The perturbation of the S—O oscillator by solvents has been noted in Table 2. Bellamy and co-workers *(49)* investigated extensively the effect of solvents on the characteristic S—O stretching frequency of DMSO (and related compounds containing the S—O moiety). Frequency shifts (relative to the least perturbed frequency and expressed in terms of $\Delta\nu/\nu \times 10^3$) were observed for a series of solvents ranging from hexane to methanol and were compared with those exhibited by acetophenone. The ratios of the frequency shifts in acetophenone to the frequency shifts in DMSO, diphenyl sulfoxide, and thionyl chloride are 0.32, 0.59, and 0.98, respectively. In other words, thionyl chloride exhibits frequency shifts nearly equal to those of acetophenone, while the frequency shifts in DMSO are three times larger than those of acetophenone. The frequency shifts appear to originate from associations between the solvent molecule and the oxygen terminal of the S—O dipole, and a gradual transition in the magnitude of these associations is noted as one proceeds from slightly polarizable to highly polar, hydrogen bonding solvents. The general nature of the phenomenon of solvent dependence of stretching frequency shifts has been discussed by Allerhand and Schleyer *(77)*.

A variety of solvent shifts of the S—O stretching frequency of DMSO is collected in Table 2. Thus, the frequency observed in hexane at 1085 cm^{-1} *(52)* or in cyclohexane at 1083 cm^{-1} *(53)* is found to shift to 1071.5 cm^{-1} in CCl_4, to 1071 cm^{-1} in CS_2 *(53)*, to 1054.5 cm^{-1} in $CHCl_3$ *(53)*, and to 1016 cm^{-1} upon hydrogen bonding with *p*-cresol *(52)*. The dipole–dipole interaction between DMSO and the solvent molecules is clearly revealed by the different frequencies observed in *trans*-1,2-dichloroethylene and *cis*-1,2-dichloroethylene. The former lacks a dipole moment, has a low dielectric constant ($D = 2.14$), and displaces the S—O stretching frequency to 1066.5 cm^{-1} *(53)*, presumably by way of a dipole-induced dipole interaction. The latter, however, is polar, is characterized by a higher dielectric constant ($D = 9.20$), and displaces the S—O stretching frequency by an additional 6.5 cm^{-1} *(53)*. Association of DMSO through hydrogen bonding is discussed in greater detail in the following section.

Association phenomena involving DMSO have also been investigated by observing changes in the spectrum of the partner substance. Thus, the increase in the intensity of the stretching frequency characteristic of

the cyano group (at ca. 2230 cm^{-1}) upon addition of DMSO to the carbon tetrachloride solution of nitrile was interpreted *(78)* to result from the dipole–dipole alignment (VI). The dissociation constants (at room temperature) for benzonitrile, as well as its *p*-chloro-, *p*-nitro- and *m*-nitro- derivatives, are given as 1.1 *M*, and for



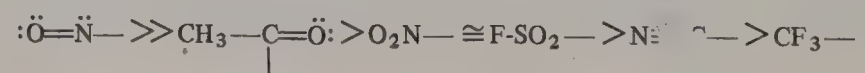
VI

p-anisonitrile and acetonitrile as 0.85 *M (79)*. Analogous complexing between DMSO and acetone or cyclohexanone is assigned dissociation constants of 2.7 *M (78)* and 1.5 *M (79)*, respectively. DMSO is thought to have a superior complexing capacity with benzonitrile than *N,N*-dimethylformamide or pyridine, in view of the relatively large dissociation constants of 1.6 and 6.2 *M*, respectively, for the latter substances. (The values of all dissociation constants reported in this study are probably not accurate to better than ±50%). This technique did not provide any evidence for the association between DMSO and ethyl benzoate.

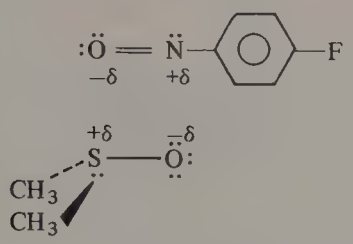
Among the nonhydrogen bond donors DMSO was found *(80)* to be the solvent that causes the greatest displacement of the rotamer equilibrium in haloacetaldehydes in favor of the most polar conformation.

The phase diagram of the binary mixture of DMSO and *p*-chlorobenzonitrile revealed the formation of an equimolar complex *(81)*. Adiabatic compressibility measurements and a negative excess volume indicate *(82)* an association between DMSO and acetonitrile.

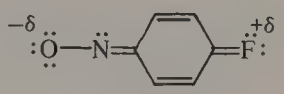
The intramolecular ^{19}F NMR shielding in substituted fluorobenzenes is affected by solvent molecules to the degree that they interact with the substituent *(83)*. The *para* substituents that appear to be most readily solvated by DMSO are



In this series solvation increases the electron-withdrawing character of the substituents. The strongest solvent effect was observed in the case of *p*-nitrosofluorobenzene and here the phenomenon can be explained either in terms of the formation of a complex (VII) with DMSO or by the enhanced contribution of the dipolar resonance structure (VIII) in a solvent of high dielectric



VII



VIII

constant [since the latter stabilizes the dipolar structure through electrostatic interaction *(84)*]. Generally speaking, the solvent effect of DMSO was greater than that produced by DMF, and it was even more pronounced than that observed with acetone. However, in the case of electron-withdrawing substituents, the solvent effect of 75% aqueous methanol exceeded that of DMSO.

Association between DMSO and polar groups, such as the cyano and the carbonyl, is believed to affect the chemical properties of these functional groups. Thus, the decrease in the acid-strengthening effect of the cyano group in 4-substituted bicyclo-[2.2.2]octane-1-carboxylic acids *(85)* in the presence of DMSO was explained in terms of a decreased cyano dipole attributable to the oppositely oriented sulfoxide dipole of a nearby solvent molecule.

The rates of lithium borohydride reduction of cyclohexanone and 3,3,5-trimethylcyclohexanone were found *(79)* to be strongly solvent dependent. The reduction of the two ketones in pyridine occurred with rate constants of 4.2×10^{-1} and 0.15×10^{-1} $M^{-1}\,min^{-1}$, respectively, but in DMSO the rates were decreased to 9.2×10^{-4} and 0.50×10^{-4} M^{-1} min^{-1}, respectively. The 1000-fold decrease in the reactivity of the carbonyl group in the presence of DMSO was attributed to dipole–dipole complex formation. In the same reaction it was observed that the reduction of 3,3,5-trimethylcyclohexanone gave a 91:9 ratio of the *cis:trans* alcohols in pyridine, while the ratio in DMSO was 36:64. The change in the stereochemical course of the reaction can be attributed to selective solvation of the carbonyl group by DMSO.

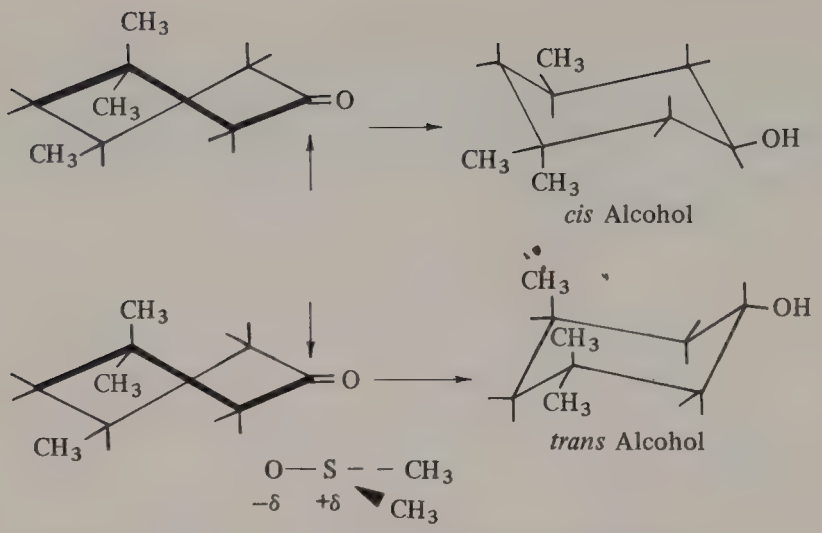
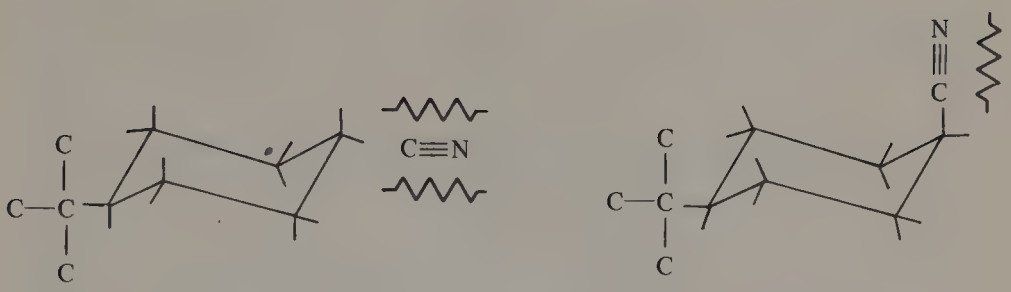


Fig. 1. Stereoselective reduction of 3,3,5-trimethylcyclohexanone.

In the graphic representation of the preferred stereochemistry (Fig. 1), the arrow near the carbonyl group symbolizes the axial attack of the borohydride ion from the less-hindered direction. The hindrance, however, can be produced either directly and exclusively by the 3-methyl substituent, or indirectly by the solvating DMSO molecule which orients itself preferentially on the side of the carbonyl group that is less hindered by the 3-methyl group.

Another stereochemical differentiation attributed to association of DMSO with a functional group comes from the base-catalyzed equilibration of *trans*- and *cis*-4-*t*-butylcyclohexanecarbonitrile *(79)*. While equilibration in *t*-butyl and methyl alcohols or tetrahydrofuran gives *trans*:*cis* ratios of 1.24–1.26 and 1.44, respectively, DMSO raises the ratio to 2.01. The greater stability of the *trans* isomer in DMSO is attributed to the greater solvation probability in the *trans* isomer in which the cyano group is forced into the equatorial conformation and can, therefore, be solvated from either direction (Fig. 2), while the axial conformation of the cyano group in the *cis* isomer allows solvation only from one side.

Since the solubility of nonpolar substances in DMSO was related (see Table 3, Section III,A,1) to the magnitude of the induced dipoles, it is to be expected that intrinsically polar substances exhibit large solubilities in DMSO by virtue of the strong dipole-dipole interactions. A listing of solubilities of polar substances in DMSO is, therefore, rather monotonous because most of the entries would indicate *(4)* either



trans Isomer *cis* Isomer

Fig. 2. Solvation possibilities in the 4-*t*-butylcyclohexanecarbonitriles. (Wavy lines symbolize possible alignment of DMSO molecules.)

miscibility at room or somewhat elevated temperatures. Of special interest here is the solubility, or total miscibility, of lipids or hydrophobic materials listed in Table 5.

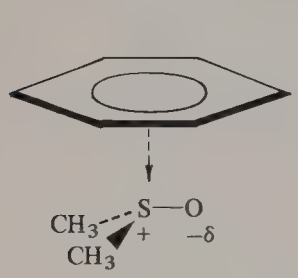
Some insight into the nature of association between DMSO and polar, but nonhydrogen bonding, substances is obtained from the study of excess molar volume relationships *(60)*. For a series of binary systems of DMSO and *para*-substituted benzenes, it is found that maximum deviations from ideal behavior are encountered with nearly equimolar mixtures and, most significantly, that the positive excess

TABLE 5

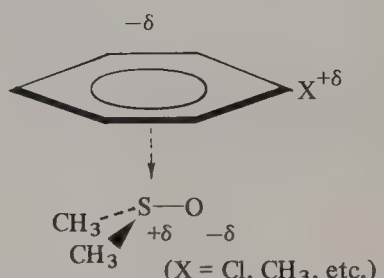
Solubility of Lipids or Hydrophobic Substances in DMSO (*4*)

Substance	Solubility (20-30°C)	Solubility, g/100 ml DMSO (90-100°C)	Misc. at, °C
Castor oil	Misc.	–	–
Coconut oil	0.3	1.3	160
o-Dichlorobenzene	Misc.	–	–
DDT	4	100	–
Diethyl ether	Misc.	–	–
Ethyl benzoate	Misc.	–	–
Ethylene chloride	Misc.	–	–
Methyl laurate	7	Misc.	–
Methyl palmitate	–	–	130-180
Nitrobenzene	Misc.	–	–
Polyacrylonitrile	–	ca. 20	–
Soybean oil	0.6	–	–
Tallow	–	1.9	–

volumes given by nitrobenzene and benzaldehyde and the negative excess volumes given by benzene and toluene (and also phenol, guaiacol, and aniline) are linearly related to the dipole moment of the aromatic component or to the Hammett σ_p values of the substituents. This implies that the electron-poor benzene rings (carrying nitro and carboxaldehyde substituents) interact with DMSO with an energy *smaller* than the average of the molecular forces in the pure components, i.e., these systems behave like "regular solutions." However, even the chloro-substituted benzene ring, and more so benzene itself, toluene, and the derivatives carrying distinctly electron-releasing substituents, associate with DMSO with an attractive energy *greater* than that given Berthelot's relationship, $E_{AB} = (E_{AA}\ E_{BB})^{1/2}$, for "regular solutions" *(86)*. These findings support the suggestion that the association between a relatively electron-rich aromatic ring and DMSO involves an interaction of the aromatic π electrons with the electrophilic sulfur center (IX) in addition to, or in conjunction with, an alignment of dipoles (X).



IX



X

However, the association between an electron-poor aromatic ring and DMSO may involve only the dipole–dipole interaction pictured in VI and VII.

b. Presence of Hydrogen Bonding. The hydrogen bond-accepting qualities of DMSO are evident from its hygroscopicity and its excellent solvent properties for materials that contain hydroxyl and other hydrogen bond donor groups even when the molecular weights of these solutes are relatively high. Solubility characteristics in DMSO of materials of special interest are listed in Table 6. DMSO is superior to water as a solvent for the materials listed in Table 6. An exception is the case of simple amino acids. For example, the solubilities of glycine and methionine in water at 25°C are 250 and 33.81 g/liter, respectively. If we consider the dipolar structure of a simple amino acid, and the fact that water is a hydrogen bond donor as well as an acceptor

(while DMSO is limited to the latter role), the superior solubility in water is not surprising:

Numerous studies of infrared spectral shifts attributable to hydrogen bonding have been published in order to elucidate either the relative hydrogen-bond donor qualities of various protic substances, or inversely, to compare the hydrogen-bond acceptor quality of DMSO with other acceptors (often referred to as "bases"). The majority of the quantitative studies follow the shift in the stretching frequency of the O—H group at ca. 3600 cm^{-1} because it is more readily resolved and more sensitive to perturbations than the S—O bond (see Table 2). The stretching frequency of the latter, reported recently at 1050 cm^{-1} for neat DMSO, was also shown *(42)* to be displaced to 1020 cm^{-1} in dilute aqueous solution (X_{DMSO} = 0.20), while the more concentrated solution containing 0.80 mole fraction of DMSO exhibited overlapping absorptions at 25°C that are clearly resolved at 1°C into peaks at 1050 and 1020 cm^{-1}.

TABLE 6

Solubility of Hydrogen-Bond Donors in DMSO (*4*)

Substance	Solubility, g/100 ml DMSO		
	20-30°C	90-100°C	150°C
Cellulose triacetate	10	20	–
Glycerine	Misc.	–	–
Glycine	<0.05	0.1	–
Lauryl amide	10	>20	–
Methionine	0.1	0.3	–
Nylon-6, 6	–	–	25
Nylon-6	–	–	40
Oleic acid	Misc.	–	–
Palmitic acid	100	–	–
Polyvinyl alcohol	>15	–	–
Sorbitol	60	7180	–
Starch ("soluble")	>2	–	–
Stearic acid	2	Misc.	–
Sucrose	30	100	–
Thiourea	40	85	–
Urea	40	100	–
Viscose rayon	–	<1	–

TABLE 7

Formation of Hydrogen-Bonded Complexes by DMSO

Hydrogen bond donor	Medium[a]	Temp., °C	ν_{OH}, cm^{-1}	K, M^{-1}	Ref.
Phenol	CT	20	350	250.2	(*87*)
Phenol	CT	25	366	216	(*88*)
Phenol	CT	25	*b*	183.0	(*74*)
Phenol	CT	27.3	*b*	169.3	(*74*)
Phenol	CT	30.7	*b*	149.5	(*74*)
Phenol	CT	36.8	*b*	121.5	(*74*)
Phenol	T	27	340	55	(*89*)
2,6-Di-*t*-butylphenol	T	27	–	2	(*89*)
p-Fluorophenol	CT	25	367	−6.9[c]	(*90*)
n-Butyl alcohol	T	27	–	15	(*89*)
Cyclohexanol	CT	27	206	6.4	(*91*)
2,2,2-Trifluorethanol	CT	25	306	161.8	
				−5.63[c,d]	(*92*)
1,1,1,3,3,3-Hexafluoro-2-propanol	CT	25	437	1432	
				−6.97[c,e]	(*92*)
Chloroform	CL	RT	*f*	0.30	
				−3.3[c]	(*97,98*)

[a] CT, Carbon tetrachloride; T, toluene; CL, chloroform.

[b] Based on changes of absorbance of phenol band at 2840 Å.

[c] Enthalpy of association in kcal/mole.

[d] The free energy and entropy of association are given as − 3.01 kcal/mole and −8.79 eu, respectively.

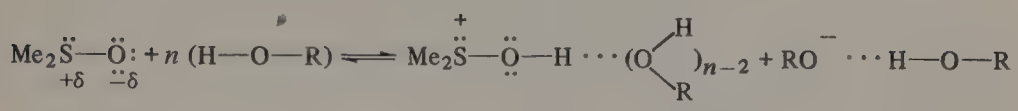
[e] The free energy and entropy of association are given as − 4.30 and − 8.96 eu, respectively.

[f] Based on proton chemical shifts.

The results of some of the quantitative studies of hydrogen bonding involving DMSO, and utilizing the hydroxyl stretching frequency shifts, are summarized in Table 7.

It is to be noted that the results of acid-base studies in which the proton transfer is complete are dealt with elsewhere (Section IV). Hydrogen-bond acceptor capacity and proton acceptor capacity go hand in hand with each other, but they are not identical and do not have to be linearly proportional. The first primarily depends on the polarity of the acceptor site, while the latter is determined by the relative energies of bond formation and bond breaking and on the solvation energy of both ions. The difference between these two processes can be represented by means of the following equations:

(n = 1-2)



($n \cong 4$)

The information in Table 7 can be supplemented with data summarized by Drago and co-workers *(74)*, who compare the hydrogen-bonding accepting capacity of DMSO with that of other compounds of interest. These data are reproduced in Table 8.

It is of interest to note that under equivalent conditions the enthalpies for the association of DMSO with phenol and iodine are –6.5 and –4.4 kcal/mole *(74)*, i.e., association through hydrogen bonding is only ca. 50% more favorable than association through charge-transfer complexing. However, the association of DMSO with the Lewis acids antimony pentachloride and boron trifluoride gives enthalpies of –29.8 *(93)* and –42.3 kcal/mole *(94)*, respectively.

The rather unexpected feature of the association of DMSO with phenols is the formation of a complex containing two molecules of phenol to each DMSO molecule, in addition to the equimolar complex.

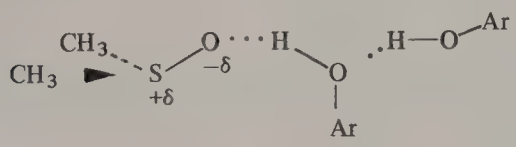
TABLE 8

Thermodynamic Parameters of Hydrogen-Bonding between Phenol and DMSO and Some Other Compounds[a] (*74*)

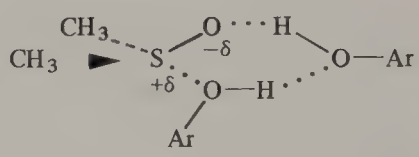
Hydrogen-bond acceptor	K, M^{-1}	$-\Delta H$, kcal/mole
Benzene	–	1.5
Acetonitrile	5.0 ± 0.2	3.3 ± 0.5
Acetone	13.5 ± 1.0	3.3 ± 0.5
Diethyl sulfide	–	4.6
Diethyl sulfone	17.1 ± 0.7	4.9 ± 0.3
Diethyl ether	–	5.0
N,N-Dimethylformamide	64 ± 1	6.1 ± 0.2
N,N-Dimethylacetamide	134 ± 1	6.4 ± 0.2
DMSO	182 ± 1[b]	6.5 ± 0.2
Tetramethylene sulfoxide	–	7.0

[a] In carbon tetrachloride at 25°C.
[b] Note that the values of K reported in Table 7 are higher.

This was first noted by Cairns and collaborators *(52)* using *p*-cresol at two levels of concentration, and was further substantiated by Szmant and co-workers *(89)* using phenol. While the former investigators suggested an open structure, (XI) for the 2:1 complex, the latter preferred the cyclic structure (XII) in which the outstanding feature is assumed to be an attractive force between the oppositely charged dipole terminals in the phenol and DMSO.



XI



XII

In favor of the cyclic structure is the fact that 2,6-di-*t*-butylphenol fails to give the 2:1 complex and the observation that while the formation of the 1:1 complex follows the Hammett relationship in a series of diaryl sulfoxides, this is not true in the case of the formation of the 2:1 complex. It is reasoned that the latter behavior is attributable to the conflicting effects of the substituents in the phenol on the hydrogen bonding, on one hand, and the interaction between the phenolic oxygen electrons and the electrophilic sulfur center, on the other. This argument is supported by observations *(95)* of the formation of 2:1 complexes in the case of the cresols, while only equimolar complexes are given by *m*- and *p*-nitrophenols and picric acid and none by *o*-nitrophenol. Picric acid also forms a 1:2 complex with DMSO but its structure probably is that of an ion pair, $(Me_2SO{\cdot}H{\cdot}OSMe_2)^+$ picrate. It should be mentioned that the capacity to form 1:1 and 2:1 complexes with *p*-cresol is also exhibited by acetone and cyclohexanone *(96)*.

The formation of an analogous 2:1 complex between water and DMSO is mentioned later in this discussion. Curiously enough, a 2:1 complex between chloroform and DMSO is suggested on the basis of spectroscopic data *(97,98)*. If true, the second molecule of chloroform may be held in part by the interaction between the polarizable, negative end of the chloroform dipole and the electrophilic sulfur center of DMSO.

TABLE 9

Hydrogen-Bond Acceptor Capacity Based on Hydroxyl Stretching Frequency Shifts

Hydrogen-bond acceptor	Hydrogen bond donor ($\Delta\nu_{OH}$, cm^{-1})		
	Water (*101*)	Phenol in CCl_4	Phenol in toluene (*89*)
1,2-Dichloroethane	–	30 (*102*)	–
Carbon tetrachloride	40	–	–
Benzene	75	–	–
Nitromethane	78	–	–
Phenol	–	136 (*102*)	–
Acetonitrile	115	–	–
Dimethyl sulfone	–	145 (*103*)	–
Ethyl acetate	–	182 (*104*)	–
Acetone	127	200 (*105*)	–
N-Nitrosodimethylamine	–	240 (*104*)	–
1,4-Dioxane	153	–	–
Tetrahydrofuran	196	294 (*104*)	–
Diphenyl sulfoxide	–	305 (*87*), 299 (*106*)	256
Water	276	–	–
Di-*p*-tolyl sulfoxide	–	320 (*104*)	–
Phenyl methyl sulfoxide	–	330 (*87*)	–
Triethyl phosphate	–	345 (*104*)	–
DMSO	334	350 (*99,104*), 366 (*87*)	340
Diethyl sulfoxide	–	385 (*87*)	–
Pyridine *N*-oxide	–	441 (*104*)	–
Pyridine	292	770 (*102*)	–
Trimethylamine	358	–	–

In addition to the hydrogen bond results listed in Tables 7 and 8, more extensive comparisons of acceptor capacity based on the frequency shifts of different hydrogen bond donors could be cited. From the results summarized in Table 9, it must be deduced that frequency shifts depend to some extent on the nature of the hydrogen bond donor and of the medium. However, regardless of the yardstick employed, it is of interest to note that DMSO occupies a high-ranking position among the acceptors. The shifts of the O—H stretching frequency of phenol in a carbon tetrachloride solution can be transformed *(73)* into the enthalpy of complex formation by means of the relationship

$$\Delta H = (0.0105\ \Delta\gamma_{OH} + 2.99)\ \text{kcal/mole}$$

As a matter of fact, most of the enthalpy values reported in Table 8 were calculated in this fashion. An analogous relationship between

enthalpy of hydrogen bonding and the chemical shift of the phenolic proton is derived *(99)* for dilute solutions of the components in methylene chloride. The relationship

$$\delta_{obs.} = 0.748\ (\Delta H) - 4.68$$

is believed to hold within ± 0.5 kcal/mole or ± 0.38 ppm.

Association constants between chloroform and a series of sulfoxides and analogous ketones were determined in solutions of carbon tetrachloride and cyclohexane by the proton chemical shift of chloroform *(100)*. It is of interest to note that while the equilibrium constants retain the value of ca. 12 M^{-1} in the case of the dimethyl, diethyl and diisopropyl ketones, they decrease from the value 31 M^{-1} in the case of DMSO to ca. 22 M^{-1} for the higher sulfoxides. The larger dependence on structure suggests that the association between chloroform and alkyl sulfoxides is more complicated than what would be expected in the formation of a simple equimolar complex derived from unassociated precursors.

The strength of hydrogen bonding depends, among other things, on the polar nature of the proton donor. This point is brought out when the hydrogen bonding data for DMSO presented in Tables 7, 8 and 9 is compared with more recent results *(107)* dealing with 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) (summarized in Table 10). The last-mentioned work also demonstrates the high acceptor capacity of DMSO approached only by that of substituted amides. The work with TFE and HFP also revealed the existence of complexes of two hydroxylic molecules to one of DMSO, and it is noteworthy that the ratio of K_{11}/K_{12} in the case of HFP at 25°C was found to be 43, i.e., of the same magnitude as the value 25 reported for phenol in toluene *(89)*. The ratio of K_{11}/K_{12} for tetramethylurea was found to have the values of 28 and 19 at 25° and 50°C, respectively *(107)*.

The hydrogen-bond acceptor function cannot be divorced from the less polar interactions resulting from dipole-induced dipole interactions and dispersion forces. Changes in the relative contributions of the electrostatic and nonpolar forces account for shifts in the relative positions of the different acceptors listed in Table 9. A quantitative treatment of such interactions is given by Drago and Wayland *(108)*,

who propose that the enthalpy of the formation of a molecular complex between A and B be expressed in terms of the equation

$$-\Delta H = E_A E_B + C_A C_B$$

where E and C represent parameters characteristic of the electrostatic and covalent interactions for each substance. For carbon tetrachloride solutions at 25°C, and relative to the arbitrarily assigned E_A and C_A values of 1.00 for iodine, the parameters for DMSO and some other substances of interest here are listed in Table 11. An inspection of Table 11 reveals that, except in the case of acetone, the covalent interaction parameters predominate among the electron donors shown. This implies that the covalent interactions are chiefly responsible for the enthalpy of association, and more so in the case of the acceptors that have low electrostatic interaction parameters. The following sample calculations of the enthalpies of association are revealing.

DMSO and chloroform:

$$-\Delta H = 0.10 \times 0.969 + 5.11 \times 3.42 = 17.6 \text{ kcal/mole}$$

Acetone and chloroform:

$$-\Delta H = 0.10 \times 0.706 + 5.11 \times 0.66 = 3.45 \text{ kcal/mole}$$

Pyridine and chloroform:

$$-\Delta H = 0.10 \times 0.88 + 5.11 \times 6.92 = 35.5 \text{ kcal/mole}$$

We notice that DMSO associates with chloroform five times more readily than acetone mainly because of the relatively low polarizability of the latter. However, pyridine is approximately twice as effective as DMSO, again on account of its relatively high polarizability. Similar calculations utilizing the hydrogen donor phenol give enthalpy values of -3.5, -16.6, and -35.5 kcal/mole for acetone, DMSO, and pyridine, respectively. While these results parallel those shown above for chloroform, the main difference between the two sets of results is the relatively high (11%) contribution of electrostatic interaction in the case of the acetone-phenol system.

TABLE 10

Hydrogen Bonding by 2,2,2-Trifluoroethanol (TFE) and 1,1,1,3,3,3-Hexafluoro-2-propanol (HFP) (*107*)

Hydrogen-bond acceptor	TFE					HFP				
	K,[a] M^{-1}	$\Delta\nu$, cm^{-1}	$-\Delta G$, kcal/mole	$-\Delta H$, kcal/mole	$-\Delta S$, eu	K,[a] M^{-1}	$\Delta\nu$, cm^{-1}	$-\Delta G$, kcal/mole	$-\Delta H$, kcal/mole	$-\Delta S$, eu
Sulfolane	12.66	123	1.50	4.02	8.46	67.6	195	2.50	4.91	8.09
Acetone[b]	8.91	172	1.30	3.72	8.12	49.0	297	2.30	5.94	12.21
Diisopropyl ether	6.40	251	1.10	5.07	13.32	36.4	382	2.13	6.92	16.07
Tetrahydrofuran	10.04	253	1.37	5.11	12.55	77.7	389	2.58	6.26	12.35
Tetramethylurea[c]	100.3	277	2.73	5.84	10.44	1301	390	4.25	8.05	12.75
N,*N*-Dimethylacetamide	99.7	284	2.73	5.75	10.13	1522	405	4.34	7.68	11.21
DMSO	161.8	306	3.01	5.63	8.79	1432	437	4.30	6.97	8.96

[a]Values for the formation of the 1:1 complex in carbon tetrachloride at 25°C.

[b]The corresponding values for complexing with ethanol are 1.143, 113, 0.08, 2.90, and 9.46.

[c]The corresponding values for complexing with ethanol are 5.39, 177, 1.00, 4.18 and 10.67.

TABLE 11

Electrostatic and Covalent Interaction Parameters (*108*)

Substance	E_A	C_A	E_B	C_B
Iodine	1.00	1.00	–	–
Phenol	0.574	4.70	–	–
Methanol	0.14	3.41	0.78	1.12
Chloroform	0.10	5.11	–	–
DMSO	–	–	0.969	3.42
Acetone	–	–	0.706	0.66
Pyridine	–	–	0.88	6.92
Tetrahydrofuran	–	–	0.61	4.69
Benzene	–	–	0.143	1.36
Acetonitrile	–	–	0.533	1.77

It is of interest to compare the above thermochemical results with those obtained *(94)* in the formation of complexes with the Lewis acid boron trifluoride. The heats of formation of the condensed phase products, DMSO·BF_3 and the corresponding pyridine and ethyl acetate complexes, from the gaseous components are –42.3, –46, and –33 kcal/mole, respectively.

Implicit in the values listed in Table 10 is the use of carbon tetrachloride solutions (at 25°C) for the experimental foundation on which the whole structure of parameters is erected. This tends to weigh rather heavily the covalent interactions attributable to favorable solvation of the nonpolar complexes by carbon tetrachloride. A shift to polar solvents can be expected to require corrections because the solvation of polar complexes will then contribute significantly to the enthalpy of association.

Enthalpies of DMSO mixing are found in Table 12. It is difficult to draw quantitative conclusions concerning the attractive forces in binary mixtures from the enthalpies of mixing because the latter result from relatively small differences between the sum of molecular forces that are overcome in the individual components and the attractive forces in the mixed molecular aggregate. In other words, the enthalpies listed in Table 12 are affected by the magnitudes of three quantities, of which only one, namely, the attractive energy in liquid DMSO, remains constant throughout all comparisons. For example, we note that the formation of the DMSO–water and the DMSO–chloroform systems is nearly equally favorable, but this is probably true for two different

TABLE 12

Enthalpies of DMSO Mixing

Second component	x_{DMSO}[a]	ΔH, cal/mole[a]	$\overline{\Delta H}_s$(DMSO), cal/mole[b]
Acetic acid	0.45	−760	–
Water	0.36[c]	−715[c]	−1280
Chloroform	0.50[d]	−657	−1320
Methanol	0.50	−85	−340
Carbon tetrachloride	0.50[d]	+46	–
Ethanol	0.35	+122	+280
Acetone	–	–	+370
Benzene	0.50	+143.9	+650
n-Propanol	0.40	+188	+610
Toluene	–	–	+890
n-Butanol	0.45	+225	+990
t-Butanol	0.45[e]	+255	+1210
Isopropylbenzene	–	–	+1300

[a] Extreme values at the indicated concentrations and at 22-25° C (*44*).

[b] Partial molal heats of solution (*109*).

[c] A value of −620 cal/mole at $x_{DMSO} = 0.37$ is also reported (*64*).

[d] Determined only at the concentration shown.

[e] At 33° C.

reasons. The formation of the DMSO–water mixture is exothermic because the attractive forces in the mixed aggregate (corresponding, by the way, to a ratio of two moles of water to one of DMSO) are considerably larger than the strong attractive forces present in the pure components. However, the formation of the DMSO–chloroform mixture is exothermic because the attractive forces in the mixture exceed by a relatively small margin those in pure DMSO and the rather insignificant attractive forces in pure chloroform.

It is of interest to compare the partial molal heats of solution of DMSO listed in Table 12 with the analogous data for water *(109)*. Positive heats are listed only for the aromatic compounds: 720, 770, and 1060 cal/mole for benzene, toluene and isopropylbenzene, respectively. The remaining solutions give exothermic heats: −1740, −2450, −2480, −2160, and −4100 cal/mole with methyl, ethyl, *n*-propyl, *n*-butyl, and *t*-butyl alcohols, respectively, and −2370 cal/mole in the case of acetone. The comparison reveals that the main driving force in the formation of aqueous solutions is the hydrogen-bond donor

capacity of water, while DMSO functions in hydrogen bonding as a hydrogen bond acceptor and otherwise by participating in dipole-dipole interactions. The similarity of the heats in the formation of solutions with aromatic hydrocarbons seems fortuitous since DMSO functions by way of dipole-induced dipole forces, while water probably associates through weak hydrogen bonding with the aromatic π systems.

Table 12 also contains entries for carbon tetrachloride, benzene, and so forth, and we note that the behavior of these nonpolar but polarizable components blends into the behavior of the polar components.

TABLE 13

Comparison of Extreme Thermodynamic Parameters of Mixing of DMSO with Water and Benzene

	Water (*105,110*)			Benzene (25°)(*71*)	
	25°C	10°C			
ΔG,cal/mole	−315	−254	(0.40)[a]	+190	(0.47)[a]
ΔH,cal/mole	−715[b]	−727	(0.36)[a]	+144	(0.50)[a]
$T\Delta S$,cal/mole	−408	−482	(0.32-0.35)[a]	− 48	(0.40)[a]

[a] Mole fraction of DMSO.
[b] The value −620 cal/mole is reported (*64*) at $x_{DMSO} = 0.37$.

Possibly more revealing with regard to the association in the mixtures is the comparison of the organizational energies obtained from the entropies of mixing. These are shown for the DMSO–water and DMSO–benzene systems in Table 13. We note that the organizational energy in the benzene mixture is about one-ninth that of the water mixture, and that the organizational energy of the DMSO–water system actually increases with rising temperature. The latter observation implies that the structure of the mixed aggregate survives thermal agitation better than the structures of the individual components. The same conclusion can be drawn on the basis of the shifts of the O—H stretching frequency of monomeric, gaseous water (3700 cm^{-1}) when the latter is introduced into a variety of substances *(101)*. These results are included in Table 9 and show that the shift is greater for the DMSO–water system than for water–water system itself. The same authors collected values for the heats of mixing per mole of water at infinite dilution (ΔH_{mix}^{∞}), assumed that the effective hydrogen bond energy between two molecules of water is ca. 4 kcal/mole, further assumed that at infinite dilution each water molecule is associated with two

TABLE 14

Energies of Interaction in the Formation of Water-Hydrogen Acceptor (1:1) Complexes (*101*)

Donor	ΔH^{∞}_{mix}, kcal/mole	E_H, kcal/mole
Trimethylamine	−1.6	4.8
DMSO	−1.3	4.65
Pyridine	−0.65	4.32
Acetone	1.05	3.48
Acetonitrile	1.89	3.05
Nitromethane	2.4	2.8

hydrogen acceptor molecules, and thus estimated the energy (E_H) of a single water–hydrogen acceptor interaction by means of the relationship

$$E_H = 4.0 - \Delta H^{\infty}_{mix}/2$$

The resulting values are given in Table 14.

Recent investigation of the effect of DMSO on the structure of liquid water by neutron inelastic scattering, radial distribution function of x-ray diffraction, as well as infrared spectroscopy, leads to the conclusion *(42)* that at low DMSO concentrations the effect of DMSO is to stabilize ("rigidify") the normally present water aggregates without major perturbations of the characteristic intermolecular distances. At higher concentration of DMSO the formation of hydrogen-bonded DMSO–water complexes causes a breakdown of the structure of liquid water and inhibits the formation of hexagonal ice. The last-mentioned effect might explain the cryoprotective properties of DMSO in living cells.

Turning now to NMR, it is not easy to relate the strength of the water–hydrogen donor associations with the observed chemical shifts when water is present at high dilution in a variety of substances, because the NMR results are affected by bulk diamagnetic effects and by diamagnetic anisotropies. Nevertheless, the order of relative basicities is nearly the same (Table 15) as that shown in Table 9.

In another NMR study *(111)* the thermodynamic parameters were determined for the equilibrium

$$H—O—H\cdots S + S \rightleftharpoons S\cdots H—O—H\cdots S$$

by extrapolating the proton chemical shift of water to infinite dilution.

TABLE 15

Proton Chemical Shifts of Water at High Dilution (*101*)

Solvent	$\Delta\nu^0_{obs.}$, cps	$\Delta\nu^0_{corr.}$, cps
Pyridine	28.1	18.7
DMSO	60.5	50.4
Triethylamine	71.5	58.4
Acetone	105.2	83.3
Tetrahydrofuran	88.0	86.5
Dioxane	97.6	88.0
Nitromethane	139.9	106.5
Acetonitrile	119.3	118.0

The chemical shifts were determined for water concentrations X_{H_2O} = 0.01-0.03 in dilute solutions of DMSO or DMF in carbon tetrachloride or cyclohexane, respectively. The values for the equilibrium constant (at 19°C), enthalpy, and entropy for the two systems are reported as 3.7, −2.6 kcal/mole, −7.4 eu and 9.4, −4.4 kcal/mole, −12.4 eu, respectively. The relative magnitudes of the results for the two systems are surprising because it seems that DMF is a better hydrogen bond acceptor than DMSO, contrary to the conclusions based on data shown in Table 9. A possible reason for the apparent discrepancy is the greater self-association of DMSO and the greater solvation of DMSO in carbon tetrachloride than the corresponding solvation of DMF in cyclohexane. The validity of this explanation is supported by the larger negative entropy in the case of the DMF equilibrium.

The hydrogen-bond acceptor capacity of DMSO was compared with that of a series of other acceptors by measuring the proton chemical shift of chloroform *(112)*. The chemical shifts (δ values) are reported as follows:

$(Me_3N)_3PO$	DMSO	DMF	Sulfolane	Ethylene carbonate	MeCN
1.89	1.05	0.98	0.48	0.41	0.31

Similarly, the proton chemical shift of a large number of halogenated methanes and ethanes was employed in the study of self-association of the halogen compounds and the formation of hydrogen-bonded complexes with DMSO *(97,98)*. A representative result obtained for the DMSO–chloroform system is included in Table 7.

A comparison of the proton chemical shift of the N—H bond gave the following order of apparent hydrogen-bond acceptor strength *(113)*:

$$\text{DMSO} > \text{pyridine} > \text{acetone} > \text{R—NO}_2 > \text{CHCl}_3$$

Hydrogen bond formation also affects the magnetic resonance spectrum of DMSO. Thus, the proton chemical shift of DMSO (relative to tetramethylsilane) in a carbon tetrachloride solution $\delta = 2.50$ is displaced to $\delta = 2.62$ for a solution of DMSO in deuteriochloroform *(7)*.

The examination of proton chemical shifts of DMSO–water solutions, over the concentration range $X_{H_2O} = 0.189$–0.942 and the temperature range 238–280°K, failed to reveal the formation of long-lived complexes *(114)*. The chemical shift of hydroxyl proton increased linearly with an increase in the concentration of water and as the temperature was lowered. These observations suggest that the net electronic shielding of the hydroxylic protons results from a dynamic hydrogen bond equilibrium between water–water and water–DMSO complexes, that the deshielding effect produced by another water molecule is greater than that caused by a DMSO molecule, and that at lower temperatures the equilibrium shifts in favor of the DMSO–water complexes. The latter is predicted by Le Chatelier's principle, in view of the exothermic heat of mixing (Tables 12 and 13) and the relatively strong interaction between DMSO and water (Table 14). Work with the DMSO–water system at still lower temperatures may hopefully reveal the existence of longer-lived complexes such as those suggested by the examination of excess molar volumes for DMSO–water mixtures.

Excess-volume as well as viscosity results from several laboratories *(61–64,82,110,115)* argue in favor of the formation of aggregates containing a ratio of two molecules of water for each one of DMSO. This conclusion is supported by the enthalpy of mixing data (Table 13). A structure of the DMSO–water complex similar to that suggested for the complexes of DMSO and phenols (XI or XII) was proposed by Tommila *(116)* but this need not be so because of the greater structural complexity of the water aggregates. Chemical reactivity changes in aqueous DMSO support the conclusion *(117)* that hydrogen bonding between water and DMSO molecules is stronger than that between water molecules themselves.

In view of the extraordinary interest in the structure of the DMSO–water system, it is appropriate to summarize at this point the present

views with regard to this question. The addition of small amounts of DMSO to water seems to increase the organization of the water aggregates *(42)*. This effect reaches a maximum when X_{DMSO} reaches the value 0.33 and the composition of the liquid corresponds to DMSO·2 H_2O. The latter is also suggested on the basis of the observed maximum deviation from ideality of the molar volume *(64,110)*, maximum viscosity *(63,64,110,115)*, maximum heat of mixing *(110)*, minimum excess $T\Delta S$ *(110)*, and so forth. However, a phase diagram of DMSO–water does not show the formation of a compound *(105)* but rather exhibits a eutectic at $X_{DMSO} = 0.33$. Thus, it seems that "three zones of different solvent structure" *(118)* exist. As the concentration of DMSO approaches 0.33 mole fraction, the structural change can be symbolized by

$$(H_2O)_X \cdot HOH \cdot DMSO \xrightarrow{DMSO} DMSO \cdot 2\,H_2O$$

In the concentration range X_{DMSO} 0.3–0.5, the changes in the composition can be represented by

$$DMSO \cdot 2\,H_2O \xrightarrow{DMSO} DMSO \cdot H_2O$$

and, finally, as X_{DMSO} exceeds the value 0.5, the composition changes as follows

$$DMSO \cdot H_2O \xrightarrow{DMSO} DMSO \cdot HOH \cdot DMSO$$

Naturally, in addition to the above-mentioned mixed complexes of DMSO and water, there are also present aggregates of molecules of each component. Mixtures of DMSO and methanol, ethanol, *n*-propanol or acetic acid exhibit negative excess volumes *(63,65,82)* similar to those of the DMSO–water system.

The strong hydrogen bonding of DMSO with hydroxyl groups has been utilized for the structural differentiation of hydroxyl functions in rather complex substances. Ordinarily it is difficult to interpret the NMR spectra of solutions of polyfunctional alcohols, or alcohols in conformational equilibrium, because of rapid proton exchange. The latter phenomenon is suppressed in the presence of DMSO, and then the hydrogen-bonded hydroxyl groups exhibit resolved chemical shifts of $\delta > 4.0$ and splitting characteristic of structural surroundings. The usefulness of this technique was demonstrated independently by Chapman *(119)* and Casu *(120)* in structural studies of carbohydrates

(119,120) and osazones *(119),* as well as simple molecules, and it was also applied to conformational studies of cyclohexanols *(91)* and sterols *(92).* The analogous examination of the protons in pyrimidines and nucleosides requires the use of hexadeuterio-DMSO *(121).*

Recent work *(122)* on the hydroxyl proton shift variation produced by DMSO leads to the conclusion that the relative strength of association between alcohol and DMSO depends on steric crowding about the hydroxyl group: primary and secondary alcohols with little steric crowding autoassociate in preference to hydrogen bond formation with DMSO, while the reverse is true in the case of tertiary and otherwise sterically hindered alcohols. The same author used DMSO solutions of alcohols to determine the preferred conformations on the basis of the coupling in the H—C—O—H system.

The association of DMSO with other proton donors has also been investigated, e.g., polyfunctional alcohols *(123),* carbohydrates *(124, 125),* aliphatic carboxylic acids *(43,126,127),* alkaryl carboxylic acids, anilines *(128,129),* and aminosilanes *(130).*

The selectivity of the periodic acid degradation of carbohydrates is greater in DMSO than in aqueous solutions because of the strong preferential solvation of certain hydroxyl groups by DMSO *(131).* Also, the hydrogen bonding between DMSO and *n*-butyl alcohol may be responsible in part for the observed resistance of the latter to oxidation by lead tetraacetate in DMSO solution *(132).*

There exist *(7,10)* many technological applications of DMSO based on its excellent solvent properties for polar materials such as dyes, starch, cellulose and its derivatives, lignin *(133),* proteins, vinyl polymers and copolymers such as polyacrylonitrile, polymethacrylates, polyvinyl alcohol, acetate, halides, and so forth, as well as polyurethans, polyamides, polyesters, and epoxy and phenol–formaldehyde resins. Numerous industrial extraction and purification processes using DMSO have been described *(7,10,17)* and DMSO has also found interesting uses *(7)* in chromatographic separations.

Of great interest here is the effect of DMSO on proteins and nucleic acids. In the natural state proteins are associated with water molecules in several different ways *(134–137).* Water molecules act as hydrogen bond donors and as hydrogen bond acceptors, and as highly ordered aggregates. Water forms "icelike" structures around the hydrophobic parts of proteins and promotes the formation of "hydrophobic bonds." From the preceding discussion of the associative properties, it follows that DMSO can replace water molecules in the role of hydrogen bond

acceptors, and thus it is to be expected that DMSO can associate with the N—H and O—H moieties commonly present in the protein structure. DMSO is, for all practical purposes, unable to compete with water as a hydrogen bond donor, but it can be expected to be superior to water in associations based on the induction of dipoles in aromatic rings, methylmercapto and disulfide bonds, and so forth. The quantitative aspects of these interactions of DMSO with the different components of proteins are difficult to evaluate, but it is reasonable to expect that the ability of DMSO to exchange sites with "bound" water molecules in relatively immobile protein structures might account for the transfer of DMSO across the dermal barrier without attendant tissue damage *(138)*. The association of DMSO with the polar portion of lipids cannot be ignored *(139)* in this connection. It has been suggested *(140)* that the rather indiscriminate passage across the epidermis of materials dissolved in DMSO is possible because of the loosened protein structure that results from the replacement of some "bound" water by DMSO. The interaction of DMSO with relatively mobile proteins has been investigated by following conformational changes, and in the case of enzymes, by following changes in enzymic activity. In bovine serum albumin DMSO was found to be very effective in changing the chemical *(141)* environment of the tryptophan units as evidenced by solvent-induced shifts in the absorption bands attributed to this moiety. The effect of DMSO was greater than that caused by the presence of polyfunctional alcohols. The optical rotary dispersion of lysozyme was found *(142)* to vary drastically in an aqueous solution containing approximately 70 vol.% DMSO (i.e., corresponding to the 1:2 molar ratio of DMSO and water). This and other observations *(143,144)* suggest reversible changes in the conformation of the protein upon adjusting the composition of the water–DMSO medium. The effect of DMSO on enzymic activity cannot be generalized because of the multiplicity of enzymic systems and mechanisms. In the case of the hydrolytic activity of trypsin toward casein, it is found *(144)* that the activity reaches zero when the concentration of DMSO approaches 70% by weight, but it is partially restored between 75 and 90% DMSO. The solubility of trypsin is unaffected by DMSO up to a 30% concentration, then decreases, and again partially recovers above 70%. Light-scattering experiments suggest *(144)* that DMSO causes a gradual dissociation of the cluster of protein molecules in trypsin and that the molecular shape changes from a compact coil in the large aggregate of molecular weight 1.32×10^6 to an elongated coil of molecular weight 75.5×10^3 in 56%

DMSO. A new compact coil of molecular weight 25×10^3 is present in 80% DMSO, and finally a spheric unit of molecular weight 18×10^3 in essentially neat DMSO. The absorbancy at 2800Å reaches a maximum at approximately 70 wt.% DMSO, presumably because of the exposure of the hydrophobic tryptophan residues from the interior of the coiled structure. Decreased catalytic activity in the presence of DMSO was observed *(145)* in the case of one phosphomonoesterase, while another one isolated from a different organism was affected in an opposite manner. Unlike the above-mentioned trypsin result with casein, the presence of DMSO up to 20 vol.% was found *(145)* to be beneficial in the hydrolysis of a simple substrate like the methyl ester of the tosyl derivative of L-arginine, and this suggests the possibility that in the trypsin-casein case DMSO affects adversely the structure of the substrate casein. The activity of trypsin toward lipids is retained in DMSO solutions *(146)*.

DMSO can be used as a solvent in the synthesis of polypeptides *(147)* and as solvent for the reactions of proteins *(148)*.

DMSO induces conformational changes in nucleic acids *(149,150)* and is an effective solvent for the denaturation of DNA. The helix of calf thymus DNA is believed to change to a random coil configuration as evidenced by the abrupt change in optical rotation when the DMSO concentration reaches 0.27 mole fraction. Rather analogous results were obtained *(151)* with salmon testes DNA and attributed to the DMSO-induced breakdown of hydrophobic bonds in the double-stranded DNA helix. However, in view of the similarity of the effects of DMSO and DMF and the milder effect caused by formamide, it seems that the monofunctional hydrogen-bonding solvents (hydrogen bond acceptors) deplete the helix of water molecules and disrupt the hydrogen bond cross-linking between purine and pyrimidine bases, while the bifunctional solvents formamide and water (which are both hydrogen bond acceptors and donors) are partially interchangeable. The vertical stacking association of purine and β-methylpurine was compared *(150)* in water and in solutions of DMSO, DMF, and 50% aqueous DMSO, and the hydrogen bond acceptor capacity of DMSO is believed to be responsible for the dissociation of the purine aggregates.

B. The Association of DMSO with Ionic Substances

Much has been written *(14,17,18)* about the differences in the *modus operandi* of polar, protic solvents of which the foremost representative

is water, and the dipolar, aprotic solvents that include DMSO. When we deal with ionic substances, the differences in solvation can be analyzed by considering separately the behavior toward cations and anions. The dipolar, aprotic solvents excel in the solvation of metallic cations. This is true because the negative terminal of the dipolar solvent molecules is exposed, and the cluster formed around the cation produces a spherical, protective layer of relatively large hydrophobic alkyl groups on the periphery of the solvated ion (XIII).

$$[M\,(\overset{-\delta}{O}\!-\!\overset{+\delta}{S}Me_2)_n]^{+m}$$

XIII

Thus, the cation is for all practical purposes "buried" inside the resulting structure, and the coulombic forces that normally function between ions of opposite charge tend to be greatly reduced. This situation is apparent, among other things, from the examination of solubilities, ion-pairing equilibria, ionic conductivities, and also from the increased activity of the anions. Generally speaking, the solvation of the anions is more effective with protic solvents because of the formation of hydrogen-bonded complexes. However, when hydrogen bonding becomes negligible in the absence of a localized negative charge (either because an electronegative atom is absent or because the negative charge is delocalized over a large area of the resonance-stabilized system), then the dipolar, aprotic solvents exhibit solvating capacity superior to that of the protic solvents by virtue of dipole-induced dipole interactions. The latter situation exists in the case of iodide or picrate ions, for example.

The quantitative aspects of the difference in solvation by water and DMSO are given by the enthalpy contributions of individual ions to the total enthalpy of solvation in both solvents *(109)*. The results can be expressed in terms of the enthalpy of transfer of a given ion from water to DMSO. Unfavorable enthalpies are obtained in the case of hydrogen-bonded and relatively unpolarizable ions such as chloride, bromide, and tetramethylammonium (4.89, 1.34, and 0.55 kcal/mole, respectively). However, increasingly favorable heats of transfer from water to DMSO are obtained as the ions become more polarizable and capable of coordinating DMSO molecules:

Ion	$(C_6H_5)_4B^-$	$(C_6H_5)_4As^+$	I^-	Na^+	K^+	Cs^+
kcal/mole	−2.31	−2.32	−2.52	−7.15	−7.78	−8.84

More extensive comparisons of the behavior of ions in the protic solvents methanol, water, and formamide, and dipolar solvents DMSO, DMF, HMPT, and AN are found in the literature *(152,153),* and an attempt has been made to establish a quantitative basis for the energy of transfer of a given ion from the reference solvent (methanol, 25°C).

The interaction between a cation and the dipolar, aprotic solvent can range from a simple ion–dipole attraction to the overlap of nonbonding electrons of the solvent molecules with vacant orbitals of the metal.

The free energy of solvation in the case of an ion–dipole attraction is believed to be given *(154)* by the relationship

$$-\Delta G_{\text{solv.}} = Zen_s M_s/(r + r_s)^2$$

where, Ze = charge of ion; n_s = number of solvating molecules; M_s = dipole moment of solvent molecule; r_s = radius of solvent molecule; and r = radius of ion.

In DMSO, nonbonding electrons are available at both the oxygen and sulfur terminals of the dipole and thus coordination with a metal can occur at either site. Thus, DMSO is an "ambident nucleophile" *(155)* and can act both as a "hard" and "soft" base *(19).* Actually DMSO attaches itself to metals through the oxygen atom, except in complex ions derived from extremely "soft acids" such as platinum *(48, 156, 157),* palladium *(158),* iridium *(159),* and rhodium *(160).* The usual coordination number in case of the oxygen-bound complex ions is six, while the sulfur-bound square planar complexes contain, of course, four DMSO molecules. Recently it was shown *(51)* that the $Pd(DMSO)_4^{2+}$ complex contains both sulfur- and oxygen-bonded DMSO molecules.

DMSO is an excellent ligand and in the case of antimony pentachloride, for example, it is excelled only by pyridine *(93):*

$$\text{Pyridine} > \text{DMSO} > \text{MeCONMe}_2 > \text{DMF} > \text{Et}_2\text{O} > \text{Me}_2\text{CO} > \text{MeCN}$$

Salts of the transition and heavier metals readily form DMSO solvates *(1,161)* when the anhydrous or hydrated substances are crystallized from this solvent. The ease of solvation by DMSO accounts for the favorable solubility of many salts *(4).* Noteworthy is the relatively high solubility (as compared to that in water) of salts containing highly polarizable anions such as iodide, thiocyanate, picrate, and so forth.

Among the numerous studies of DMSO-solvated salts one can mention investigations of structure by means of x-rays *(158–160,162)*, infrared *(48,156,157,163,164)*, and electronic spectra *(165)*, investigations of solvent exchange by means of NMR *(114,166–169)* or electronic spectroscopy *(169)*, investigations of ion-pairing equilibria by means of conductivity determinations *(170–172)*, investigations of viscosity–composition relationships *(171)*, interconversion of geometrical isomers in complex ions *(173)*, ESR spectra *(174)*, and polarographic behavior *(175–177)*.

IV. PROTON-TRANSFER REACTIONS IN DMSO

DMSO is a strong proton acceptor but it can also function as a proton donor under certain conditions. The basic properties of DMSO were recognized at the time of its discovery in 1866 by Saytzeff *(178)* who isolated a number of DMSO salts of mineral acids. The structures of the crystalline DMSO adducts with nitric, hydrochloric, hydrobromic, and trichloroacetic acids were studied more recently by means of infrared spectroscopy *(179)*. Electrical conductance measurements of hydrogen chloride solutions in DMSO reveal *(172)* a low dissociation of the electrolyte ($K_{diss.}$ and $K_{ass.}$ at 25°C are 8.64×10^{-3} M and 115.7 M^{-1}, respectively) in contrast to the behavior of other electrolytes *(170)*. The limiting conductance of hydrogen chloride in DMSO is 38.7 ohm^{-1} cm^{-1} at 25°C *(172)*, which compares favorably with the values of 32.1 and 93 ohm^{-1} cm^{-1} for solutions in acetonitrile and pyridine, respectively, in spite of the high viscosity of DMSO (2.002 cP) as compared to that of acetonitrile and pyridine (0.345 and 0.974 cP, respectively). Also, the limiting conductance of hydrogen chloride in DMSO is of the same magnitude as that of lithium, sodium, potassium, rubidium, and cesium perchlorates, which ranges from 35.7 to 40.5 ohm^{-1} cm^{-1}.

DMSO is a relatively weak protic acid. The self-ionization constant is estimated *(180)* to have a value of 5×10^{-18} M^2 at 25°C. On the Hammett acidity scale, determined by means of an appropriate series of indicators, the pK_a of DMSO is reported *(181)* to be 31.3. (By personal communication, Steiner recently indicated this value to be 32.5.) Thus, DMSO is a weaker acid than diphenylmethane and dimethyl sulfone by 2.7 *(181)* and 2.8 *(182)* pK_a units, respectively. Nevertheless, DMSO is readily converted to its alkali metal salts and

the resulting dimsyl ion has been found to have numerous synthetic applications (Section VI,B).

The relative acidity of weak hydrocarbon acids is in good agreement when pK_a values determined in DMSO are compared *(181)* with those determined in aqueous or methanolic DMSO. This is no longer true when one deals with the stronger acids. Even though the basicity of DMSO is considerable *(vide infra),* it is the solvation of the acid anion by the protic solvent that exerts decisive control of the dissociation equilibrium as shown in Table 16. We note that for all the acids shown in Table 16 the acidity in water is greater than that in DMSO by 2.5–6.8 pK units in spite of the belief *(184)* that the basicity of DMSO (toward a proton) is greater than that of water by 1.8 pK units. A more recent estimate places the value at 1.5 ± 0.5 pK units *(185).*

TABLE 16

Comparison of pK_a Values in DMSO and Water *(183)*

Acid	pK_a	
	DMSO	Water
Hydrazoic	7.9	4.74
Benzoic	10.0	4.2
Acetic	11.6	4.76
Benzoylacetone	12.1	9.6
Hydrocyanic	12.9	8.68
Acetylacetone	13.4	9.0
Nitromethane	15.9	10.2

Surprisingly enough, the pK_a of malononitrile is found *(183)* to be essentially the same in water and DMSO, namely, 11.1. This is most likely attributable to the dipole–dipole interactions of the nitrile function with DMSO (see Section III,A,2,a). The resulting solvation of the malononitrile anion by DMSO appears to be competitive with the hydrogen bonding between the anion and water.

The effect of delocalization in the anion on the relative acidity in DMSO and water is illustrated by the work of Kolthoff and collaborators *(185)* who showed that, while the acidity of *o*- or *p*-nitrophenol in water exceeds that in DMSO by 3.8 pK units, the difference is decreased to 1.3 and 0.5 pK units in 2,6-dinitrophenol and its 4-chloro derivative, respectively, and picric acid turns out to be more acidic in DMSO by approximately 1.3 pK units.

In aqueous DMSO of *low* water content the value of the equilibrium constant of the proton transfer reaction at 25°C

$$(DMSO \cdot H)^+ + H_2O \rightleftharpoons DMSO + H_3O^+$$

is given *(180)* as 0.45 M^{-1}. This result agrees with the above-mentioned greater basicity of DMSO as compared to that of water. However, the true picture of the equilibrium should take into account the probable solvation of all species,

$$(Me_2SOH \cdots OSMe_2)^+ + H_2O(DMSO)_2 + (DMSO)_x \rightleftharpoons (DMSO)_{x+1} + H_3O^+(DMSO)_3$$

and now it appears that as the equilibrium is displaced to the right one of four tightly bound DMSO molecules is required to join the liquid DMSO aggregate. Evidence exists that in DMSO a proton is associated with two solvent molecules *(186)*. On the basis of the molarity of DMSO and water in the respective liquids, the calculated pK_a of $(DMSOH)^+$ is –2.48 *(187)*.

In aqueous DMSO of *high* water content, the pK_a of $(DMSOH)^+$ is estimated *(187)* to have the value –2.01 (at 21°C). Considering again the probable solvation of all species,

$$(Me_2SOH \cdot 2H_2O)^+ + (H_2O)_x \rightleftharpoons Me_2SO(H_2O)_2 + [H(H_2O)_4]^+ + (H_2O)_{x-4}$$

we note that as the equilibrium is displaced to the right four molecules of water from the liquid water aggregate become more tightly bound in the solvated proton cluster. This may be expected to favor loss of the proton from DMSO, and explains the observed increase in basicity of DMSO in solutions of high water content.

Recent determinations of the basicity of DMSO by potentiometric titration with perchloric acid in acetic anydride give a pK_a value of 0.91–1.00 *(188,189)* for the ionization of $(DMSOH)^+$. This value is probably valid for a comparison of the relative basicities of DMSO and related substances shown in Table 17. We note a rather strong structural dependence of the relative basicity, and the apparent operation of both electronic and steric effects. Basicity values more compatible with those cited above for DMSO in more-or-less protic surroundings are obtained *(190)* by following proton chemical shifts of DMSO in the presence of sulfuric acid. This technique gives pK_a values of –2.78 and –3.38 for protonated DMSO and methyl phenyl sulfoxide, respectively.

TABLE 17

Relative Basicities of Sulfoxides (*189*)

Sulfoxide	pK_a $(R_2SOH)^+$
DMSO	0.91
Methyl *p*-anisyl	+0.550
Methyl *p*-tolyl	0.014
Methyl phenyl	−0.488
Diethyl	−1.19
Methyl *p*-chlorophenyl	−1.57
Methyl *p*-nitrophenyl	−3.51
Diphenyl	−3.58
Di-*p*-chlorophenyl	−6.07
Di-*p*-nitrophenyl	−6.898

An indirect estimate of the relative basicities of sulfoxides is obtained *(191)* by following the rate of oxidation of benzyl mercaptan by molecular oxygen in the presence of different sulfoxides which are assumed to act as basic solvents. By this token, the relative basicities are proportional to the relative rates of oxidation, namely, diphenyl sulfoxide 1.0, phenyl methyl sulfoxide 6.2, DMSO 33.3, and tetramethylene sulfoxide 159.

A different approach to the evaluation of the basicity of DMSO and related aprotic solvents is the determination of the ionization of water present in extremely low concentrations *(101)*. The equilibrium constants for protolysis of water by solvent S

$$S + H_2O \rightleftharpoons (SH)^+ + HO^-$$

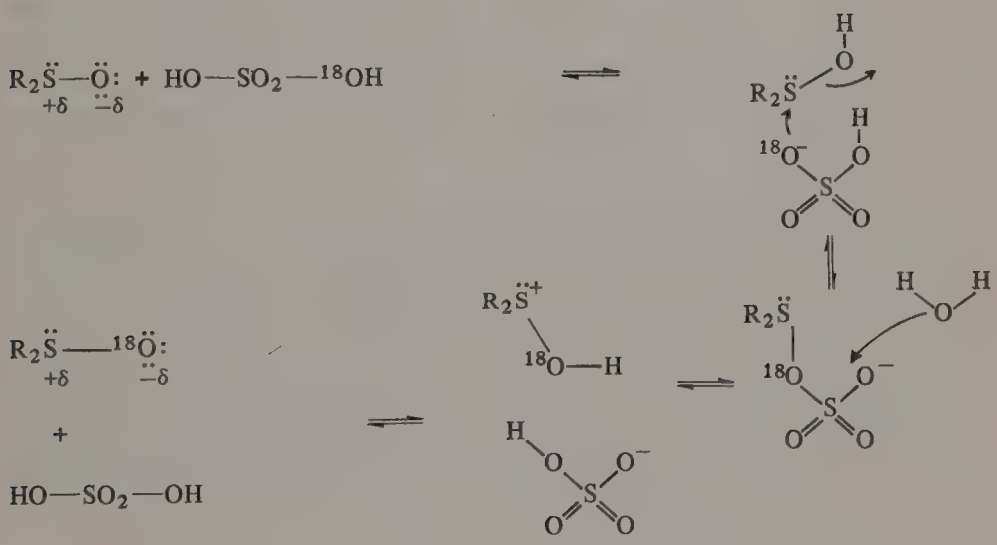
are given as follows:

S =	DMSO	Pyridine	Acetone	AN	CH_3NO_2
K_B 25° =	5×10^{-9}	3×10^{-9}	2×10^{-22}	2×10^{-23}	2×10^{-25}

We note that by this measure DMSO is even more basic than pyridine, probably because of the considerably more favorable solvation of the protonated species.

The linear relationship between the proton chemical shifts of DMSO solutions of phenols and the σ^- values of their substituents, as well as the ρ value of 1.29, suggest an association of high ionic character *(192)*.

The fact *(193)* that DMSO is almost fully protonated in anhydrous sulfuric acid and produces a colorless solution of van't Hoff *i* factor 1.90 is not surprising. Rather unexpected, however, is the reported *(194,195)* oxygen exchange when sulfoxides are dissolved in ^{18}O-labeled sulfuric acid. At 0°C and after 5 min. DMSO is found to incorporate 0.45 atom % of ^{18}O. While the authors prefer a mechanism of oxygen exchange that involves a nucleophilic attack of the nonbonding sulfur electrons in the protonated sulfoxide on the oxygen atom of sulfuric acid *(194)*, or the short-lived formation of a radical cation by the loss of water from the double-protonated sulfoxide *(195)*, a more reasonable mechanism seems to be a concerted nucleophilic displacement of the original sulfoxide oxygen by a bisulfate ion (probably, the bisulfate ion present in the solvent cage from the initial protonation of the sulfoxide), followed by the immediate collapse of the water and the dipolar sulfoxide SO_3 addition product to a new protonated sulfoxide and bisulfate ion pair:



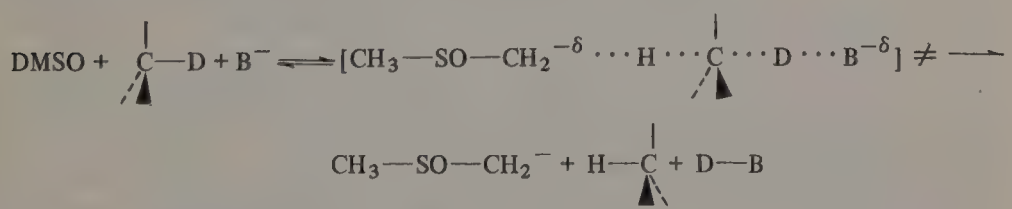
This mechanism is compatible with the now generally recognized isomerization or stereomutation *(38)* of sulfoxides by means of a nucleophilic attack on sulfur by oxygen-containing reagents such as trifluoroacetic acid *(196)*, chlorinated acetic acids *(197)*, phosphoric acid *(198,199)*, acetic anhydride *(200,201)*, hydroxide, and *t*-butoxide ions *(202)*. The ESR signal reported in a more recent paper *(195)* dealing with sulfuric acid solutions of sulfoxides, previously unobservable *(194)*, probably results from minor oxidative side reactions.

Independent of the thermodynamics of proton-transfer reactions in DMSO is the kinetic aspect of the process. The proton transfer from DMSO to dimsyllithium at 25°C was found *(203)* to occur with a rate constant of $7M^{-1}$ sec^{-1}, and in the case of dimsylsodium the rate constant increased to 12 M^{-1} sec^{-1}. The proton transfer between fluorene and fluorenyllithium in DMSO solution is estimated to have a rate constant of 0.5 M^{-1} sec^{-1} at 38°C *(204)*, contrary to the 1000-fold greater rate reported by other workers *(205)*. The discrepancy between these results may be traced to the finding *(206)* that the addition of relatively small amounts of an alcohol (*t*-butyl) causes significant increases in the rate of proton transfer from DMSO. The maximum rise in rate occurs at a 2:1 molar ratio of the alcohol and base. The rate of proton transfer from triphenylmethane to potassium *t*-butoxide in a mixture of DMSO-*t*-BuOH (80–20% by volume) is of the same magnitude ($k = 0.134$ M^{-1} sec^{-1}) *(207)*. Also, the proton transfer from benzophenone hydrazone to potassium *t*-butoxide in dry DMSO was found *(208)* to occur at 25°C with a rate constant of $7\,M^{-1}$ sec^{-1}. It is apparent that the rate of proton-transfer reactions in DMSO is rather rapid when compared to the slow proton exchange ($k = 1.9 \times 10^5$ M^{-1} sec^{-1}) between fluorene and fluorenyllithium in diethyl ether at 25°C *(204)*, or between triphenylmethane and the methoxide ion in methanol (10^9 M^{-1} sec^{-1} at 45°C) *(209)*. However, the proton-transfer reactions in DMSO are more than a billion times slower than those given by the diffusion-controlled rates of hydrogen-bonded acids in hydrogen-bonded solvents such as water *(210)*. Relatively slow proton-transfer reactions are expected when the activity of the base is decreased because of ion pairing or because of the formation of strong, hydrogen-bonded complexes such as $RO^-\cdots HOR$. These reasons may be invoked to explain the relatively slow exchange reactions cited above. Furthermore, the absence of significant association between the potential acid and the proton acceptor does not aid the cause of proton transfer. At the other extreme of the rate spectrum, we can have the potential acid hydrogen-bonded to, say, water, and the nearly indistinguishable existence of hydroxide ion and water molecules in the hydrated hydroxide ion complex. The rate of proton exchange in DMSO falls almost halfway between these extremes. It is reasonable to assume that DMSO promotes the dissociation of ion pairs and that the base is quite free and active. The base, regardless of whether it is dimsyl, alkoxide, or a carbanion, is very likely associated with DMSO via a weak hydrogen bond, in view of the suspected existence of a hydrogen bond in neat DMSO (see Section II)

and the expectation that carbanions *(211)* or alkoxide ions are better hydrogen bond acceptors than DMSO itself. The ready proton exchange between DMSO and alkoxide ions and, in the presence of alcohol, between DMSO and dimsyl ion, has even led some investigators to question *(205)* the wisdom of designating DMSO an "aprotic solvent." The practical aspect of this situation is the feasibility of using DMSO solutions of potassium *t*-butoxide for the purpose of bringing about reactions of the dimsyl ion. The kinetic protic behavior of DMSO may call for a reinterpretation of the results in the study of competitive exchange and racemization processes of optically active deuterio–carbon systems carried out in DMSO *(213)*, which show that the rates of deuterio–hydrogen exchange and of racemization are equal and that the kinetic isotope effect is very small. These observations were interpreted to mean that an asymmetrically solvated carbanion (XIV) is formed in equilibrium (described by K_1) with the starting material by a relatively slow process (k_1), and that the process of hydrogen exchange can proceed by way of a symmetrically solvated anion,

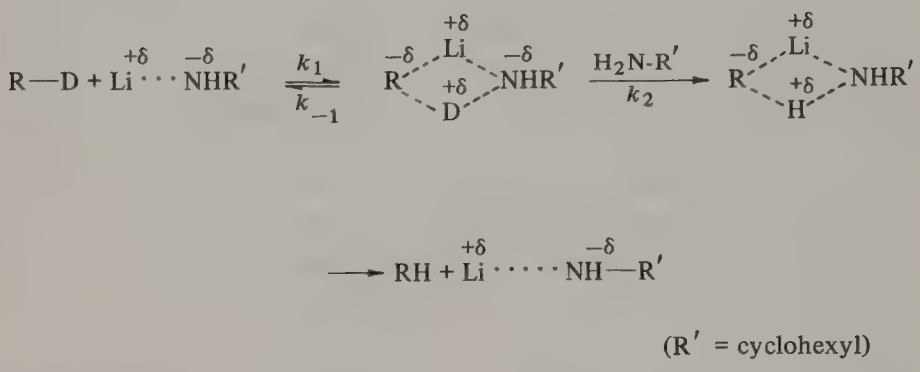
XIV

Application of the steady-state approximation and the assumption that $k_1 >> k_2$ predicts an observed rate that approaches the value of the product of K_1 and k_2 and also predicts an insignificant isotope effect. However, the assumption that DMSO is capable of rapid hydrogen exchange with the carbanion and that the transition state for this process is best described by hydrogen bonding between DMSO and the nascent carbanion also fits the experimental results.



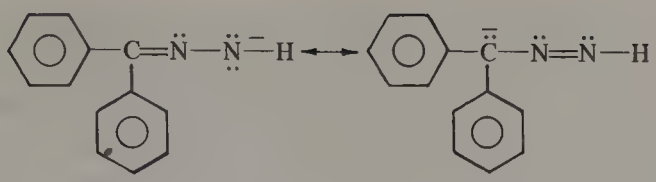
A similar mechanism may explain the absence of a primary kinetic isotope effect observed *(214)* in the base-catalyzed proton exchange of toluene in DMSO. It is of interest to note that in the related exchange of benzylic hydrogen atoms carried out in cyclohexylamine in the

presence of the corresponding lithium salt, the reaction occurs *(215)* with a significant kinetic isotope effect and with predominant retention of the initial configuration. Since the solvent cyclohexylamine should certainly be superior to DMSO in hydrogen bonding of a nascent carbanion, the fundamental difference between the processes must be traced to the nondissociated nature of the base lithium cyclohexylamide in cyclohexylamine and the formation of tight ion pairs in the course of the exchange reaction,



The observed isotope effect agrees with the assumption that $k_2 >> k_{-1}$ and with $k_{obs.} = k_1$.

The ionization of triphenylmethane in the solvent mixture DMSO:*t*-butyl alcohol (80:20 by volume) and in the presence of potassium *t*-butoxide was found *(207)* to exhibit a significant kinetic isotope effect unlike the previously mentioned proton-transfer reaction of benzylic hydrogen atoms carried out in DMSO *(213)*. However, the different behavior of the triphenylmethyl system may be simply a result of steric inhibition of solvation by DMSO of the nascent trityl carbanion from the rear of the ionizing C—H bond. Under this circumstance the energy of activation would depend solely on the breaking of the C—H bond with the consequent presence of a significant kinetic isotope effect. In this connection it is of interest to note that DMSO is apparently incapable of proton transfer in the course of the base-catalyzed Wolff–Kishner reaction of benzophenone hydrazone *(216)*. The failure of DMSO to act as a protic solvent can also be attributed to the poorly developed carbanion character in the benzophenone hydrazone anion (XV) and steric inhibition of solvation at the potential diphenylmethyl carbanion terminal.



XV

It is difficult to relate the results obtained *(217,218)* in a study of isotope exchange and racemization of diarylmethane derivatives with the preceding discussion because the experiments were carried out in methanol:DMSO (75:25 by volume). This solvent mixture represents an approximate 4.4:1 molar excess of methanol, and thus relatively "free" methanol is available for association with the potassium methoxide catalyst and for solvation of the nascent carbanion. The predominant protic character of the solvent mixture is also suggested by the relatively slow reaction rates ($k = 10^{-5}\ M^{-1}\ \text{sec}^{-1}$ at ca. 100°C). The increase in the apparent molecularity in base with increasing concentrations of potassium methoxide is explained *(217)* in terms of the presence of two solvated methoxide species differing in the degree of association and, hence, activity. The ratio of exchange and racemization rates is slightly greater than unity (1.06–1.13) and the kinetic isotope effect is significant. Both observations are indicative of a situation in which back-door solvation by the protic solvent is partially competitive with an exchange process similar to that pictured above for the cyclohexylamine–cyclohexylamide lithium system.

It is obvious that further work is needed to define more clearly the conditions under which DMSO is capable of functioning kinetically as a protic solvent.

V. SOLVENT EFFECTS OF DMSO IN CHEMICAL REACTIONS

This section is devoted to a brief discussion of the effect of DMSO on chemical reactions when it functions as a chemically inactive solvent. The preceding sections dealing with the associative properties of DMSO and with its participation in relatively simple acid–base reactions form the essential background for the present topic.

Widespread interest in the effects caused by DMSO and other dipolar, aprotic solvents was awakened by the accumulation of results pointing to a phenomenal rate acceleration of certain reactions when these solvents were utilized in addition to, or in place of, the traditional protic solvents such as water, the alcohols, formic or acetic acid, and aqueous acetone, dioxane, and so forth. Soon the nature of the dipolar, aprotic solvents became the subject of extensive reviews *(3–8, 13–17, 219–222),* and because of the often sensational acceleration of reaction rates the name "super solvents" was applied occasionally *(18)* to describe DMSO, DMF, and certain other solvents. Out of the analysis of the effects produced by the dipolar, aprotic-solvents there came a better understanding of the whole picture of solvent participation in chemical reactions. The solvent effects under discussion can be attributed to one or more of the following factors.

A. Change in the Dielectric Constant of the Medium

This effect is pronounced when the polarity of the transition state differs significantly from that of the initial state of the reacting species. For example, if we deal with a bimolecular nucleophilic substitution reaction symbolized by

$$Q^- + R{-}S \longrightarrow Q{-}R + X^-$$

in which the transition state is $[Q^{-\delta} \ldots R \ldots X^{-\delta}]^{\neq}$, we note that charge dispersal in the transition state is equivalent to an effective decrease in charge density. In this case a polar solvent stabilizes the initial state (by virtue of a large interaction with the anion) more than the transition state, and therefore a less-polar solvent allows the reactions to proceed with a smaller sacrifice of solvation energy. This mechanism of solvent intervention in chemical processes was recognized some time ago by Hughes and Ingold *(223)* and is diagnosed by the linearity of the relationship between log k and the reciprocal of the dielectric constant of the medium *(224,225)*. Generally speaking, the effect of changing dielectric constant of the reaction medium is not very large (because of partial cancellation of the entropy effects), and the rates may change by 1 to 2 powers of 10.

B. Change in the Effective Concentration and the Chemical Potential of Ionic Reactants

We can recognize the operation of two separate but related solvent effects when we deal with ionic reactants, namely, the change in the

ion-pairing equilibrium and the change in the intrinsic activity, or chemical potential, of the reacting ion.

It is now well recognized *(226)* that the reactivity of an anion increases the less its negative charge is neutralized by the coulombic attraction exerted by the partner cation. One speaks of contact ions (Q^-, M^+), solvent separated ions ($Q^-//M^+$), and finally fully dissociated ions (Q^-) and (M^+), and the equilibrium between these species depends on the ability of the solvent molecules to overcome the coulombic forces. Naturally, the mere fact that a polar solvent can decrease the coulombic forces by virtue of its elevated dielectric constant already contributes to the dissociation of contact ion pairs, but this rather small effect can be included under the preceding solvent mechanism (Section V,A).

The polar, aprotic solvents are highly effective *(14,227)* in bringing about the dissociation of ion pairs, primarily because they "bury" metallic cations in the center of a solvent cluster. For this reason the nature of the cation causes profound changes in the rates of reactions carried out in polar, aprotic solvents. In DMSO, for example, a change from a sodium to potassium salt can cause a 100-fold rate acceleration, with an additional 3-fold acceleration when the cesium salt is used in place of potassium *(228,229)*. Similarly, the equilibrium between a metal hydroxide and an alcohol is affected by the nature of the cation. In DMSO, potassium or cesium hydroxides generate the corresponding alkoxides, while lithium or sodium hydroxides fail to do so *(230)*. The large steric requirement of each DMSO-solvated molecule explains the fact that steric considerations, rather than charge density, control the solvation of metallic cations. A DMSO-solvated alkali metal ion is thought *(133)* to occupy a volume equivalent to that of the tetra-*n*-propylammonium ion.

In a polar, aprotic solvent the anion Q^- finds itself, solvation-wise, rather neglected, and thus is endowed with a high free energy or chemical potential. This situation contrasts with that prevalent in protic media in which solvation via hydrogen bonding stabilizes, and thus deactivates, the anions *(16,231,232)*. The difference between the solvent effects exhibited by polar, aprotic and polar, protic solvents is larger the more readily the anion is hydrogen bonded, and the rate constants can differ by 6 or more powers of 10. The largest rate differences (up to 10^{12}) are observed in relatively simple proton-transfer reactions in which the transition state can be represented by

$$[Q^{-\delta} \cdots H \cdots R^{-\delta}]^{\neq}$$

Somewhat smaller rate differences are found in bimolecular nucleophilic substitution reactions in which the above-mentioned deactivation

of the nucleophile by the protic solvent is partially compensated by the electrophilic catalysis of the departure of the leaving group X by the same protic solvent SH. The transition state for this situation can be represented by

$$[SH \cdots Q^{-\delta} \cdots R \cdots X^{-\delta} \cdots HS]^{\neq}$$

It follows, then, that the difference between aprotic and protic solvents under these circumstances will also depend on the hydrogen-bonding capacity of the leaving group X. In a case in which the nucleophile Q is subject to a relatively weak hydrogen bonding while the opposite is true for the leaving group X, the rate difference for polar, protic solvents and polar, aprotic solvents can be rather small. When the nucleophile is affected by hydrogen bonding significantly more so than the leaving group, then the polar, aprotic solvents can easily accelerate the reaction by 6 powers of 10, but this effect is also subject to small changes as the cation is varied. The latter is expected on the basis of stronger solvation of the cation with an increase of its charge density *(233)*.

C. Specific Solvation of Reactive Species

The solvent effects classified under this heading are more subtle than the solvation of cations considered in Section V,B, but admittedly it can be argued that the solvent effects differ in degree rather than kind.

A strong association between a solvent molecule and a particular site of the reacting molecule can produce several consequences.

(1) It can affect the conformational equilibrium of the reacting molecule and change the effective concentration of the more reactive conformer.

(2) It can affect the steric surroundings near the site of chemical change in the reacting molecule, and this may be expected to decrease the probability of successful collisions as well as to increase the energy of activation of the process.

Examples of situations (1) and (2) have already been mentioned in connection with the association between DMSO and the nitrile function (see Section III,A,2,a). It is noteworthy that specific solvation of this kind influences the steric course of the reaction and causes moderate (10^2–10^4) changes in reaction rates. An additional example of situation (2) presents itself in the case of reactions of neutral nucleophiles such as amines, sulfides, and so forth, in which hydrogen bonding by protic

solvents is detrimental in proportion to the strength of the hydrogen bonding, and by contrast, dipolar, aprotic solvents are beneficial.

(3) It can affect the dissociation of a certain bond in the reacting molecule, and hence the activation energy of the overall reaction if the particular bond breaking is part of the rate-determining step.

One can illustrate situation (3) with cases in which either protic or aprotic solvents are beneficial. Thus, for example, the heterolysis of R—X is aided by hydrogen bonding with a protic solvent S—H and leads to a transition state represented by

$$[R^{+\delta} \cdots X^{-\delta} \cdots HS] \neq$$

However, a polar, aprotic solvent such as DMSO is beneficial in the removal of a potential proton from a hydrogen-bond donor group because of its superior "basicity."

An example of this situation is the oxidation of thiols with molecular oxygen *(191),* for which the rate increases as a function of the "basicity" of the solvent (see Section IV for relative rates in different sulfoxide solvents). The evidence in favor of the superior basicity of aprotic solvents, as compared to that of the traditional protic solvents such as water, alcohol, and so forth, is discussed in Sections III,A,2,b and IV.

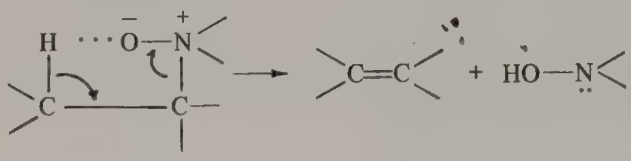
Numerous examples of the effect of DMSO on the rates of chemical reactions are reviewed by Parker *(14,17)* and are also discussed elsewhere *(220–222).* The intention of the presentation that follows is to bring out certain highlights in the context of the preceding discussion.

The shift from potassium methoxide–methanol to potassium *t*-butoxide–DMSO conditions can produce sensational billionfold increases in rates *(228)* of reactions which depend on the dissociation of a C—H bond. The subsequent reaction of the carbanion may consist of isomerization, elimination, oxidation, condensation, and so forth, hence, the importance of the potassium *t*-butoxide–DMSO system in chemical synthesis. The activity of the hydroxide ion is enhanced even more than that of the alkoxide ion, the acidity function of which increases by 14 powers of 10 when compared in water and 99% DMSO *(234).* Similar changes in the acidity function are observed in the case of the methoxide ion.

The fluoride ion, a notoriously poor nucleophile or base in traditional protic solvents, reveals its hidden capabilities in dipolar, aprotic

solvents and is an effective nucleophile in substitution reactions upon carbon *(235,236),* or an effective base in the removal of potential protons in elimination reactions *(237,238).*

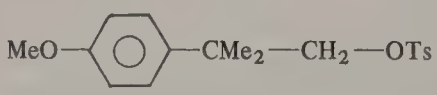
The Cope elimination proceeds with intramolecular proton abstraction by the *N*-oxide group,



and it stands to reason that hydrogen bonding of the oxide terminal by protic solvents will obstruct the reaction. Thus, it is not surprising that DMSO allows the reaction to occur at room temperature *(239)* while protic solvents require temperatures of ca. 135°C, and that the rates in DMSO are accelerated by 4 to 5 powers of 10.

The site of chemical change is shifted in ambident nucleophiles when one changes from protic to aprotic media *(219).* Thus, for example, sodium 2-naphthoxide is benzylated *(240)* in DMSO predominantly at the oxygen terminal to give 2-naphthyl benzyl ether, while in a strongly protic medium such as 2,2,2-trifluoroethanol the benzylation occurs mainly at the carbon site to give 1-benzyl-2-naphthol. A similar shift from carbon to nitrogen alkylation is observed *(241)* in pyrrole when one changes from a protic reaction medium to DMSO.

Differences in specific solvation and nucleophilic character explain the opposite effects of DMSO, ethanol, and acetic acid on the solvolytic rates *(242)* of *p*-methoxyneophyl tosylate,



and ethyl tosylate. In the three solvents under discussion, the relative solvolytic rates of the two tosylates are 1:3.42:9.23 and 1:0.101:0.0016, respectively. Neophyl tosylates are known to react with "neighboring-group participation" and the deactivating effect of DMSO must be attributed to strong solvation of the anisyl group. Such a phenomenon can be expected because of the electron-donating nature of this benzene derivative, but at the same time the solvation must interfere with the anchimeric effects of the anisyl group. A consideration of the dielectric constants of the three solvents would predict the greatest reactivity in DMSO. The solvolysis of ethyl tosylate seems to proceed mainly by a S_N2 process and the relative rates reflect the nucleophilicity of the solvents.

TABLE 18

Rates and Thermodynamic Parameters
for the Reaction of Sodium Azide and *n*-Butyl Bromide (*112*)

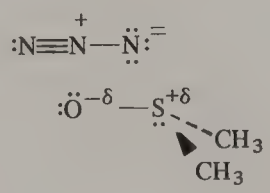
Solvent	D^a	$k^{25°}, M^{-1}\,sec^{-1}$	$E^{\neq}$, kcal/mole	$\Delta S^{\neq}$, eu
N-Methylacetamide	183	7.35×10^{-5}	20.10	+2.19
Formamide	109	9.62×10^{-5}	20.08	+2.11
Water	78.7	5.80×10^{-5}	21.41	+5.93
Methanol	32.6	8.61×10^{-6}	21.18	+1.44
Ethylene glycol carbonate	89.6[b]	1.48×10^{-4}	16.40	−9.20
DMSO	48.9[c]	1.14×10^{-2}	16.91	+1.62
Sulfolane	44[d]	3.24×10^{-2}	15.36	−2.49
DMF	36.7	2.37×10^{-2}	16.45	+0.29
Acetonitrile	36.7	4.31×10^{-2}	17.12	+4.19
Hexamethylphosphorotriamide	30[c]	1.75	13.40	−0.73

[a] At 25° C unless otherwise noted.
[b] At 40° C.
[c] At 20° C.
[d] At 30° C.

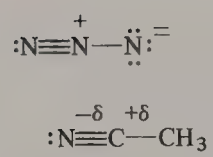
The complexity of the factors that contribute to the net solvent effect is brought to light by recent work *(112)* in which the kinetics of the reaction of sodium azide with *n*-butyl bromide is compared in four protic and six aprotic solvents. The results are summarized in Table 18.

The behavior of the protic solvents is rather consistent; they give slow reactions in spite of the positive entropies of activation, and primarily so because the strong solvation of the azide ion raises the energy of activation of the reaction. Solvent molecules are released in the transition state and this accounts for the positive entropies. As may well be expected, water solvates the azide more strongly than the remaining protic solvents. The rates in the protic solvents appear to be essentially independent of the dielectric constant and, indeed, the plot of log k versus $1/D$ exhibits an approximate zero slope. The behavior of the aprotic solvents is more complex. First of all, the plot of log k versus $1/D$ suggests that the rates are partially dependent on the dielectric constant of the medium, and in line with the principle of Hughes and Ingold, the reaction is favored by a decreased dielectric constant. However, scatter of the points from the best linear log k versus $1/D$ plot indicates that other factors enter into the picture, and the determination of energies and entropies of activation gives one an idea concerning the relative significance of these factors. The exceptionally low energy of activation, in conjunction with an insignificant entropy change, suggests that HMPT is most effective in dissociating

sodium azide ion pairs (in spite of its relatively low dielectric constant), while at the same time its solvation of the initial azide and the nascent bromide ions is of the same magnitude (probably nearly zero). DMSO, and especially acetonitrile, give positive entropies of activation, suggesting that solvent molecules are released as the transition state is approached, and this implies that the azide ion is subject to relatively strong solvation in DMSO and acetonitrile. This conclusion is supported by the trend in the direction of higher energies of activation exhibited in these two solvents. One is tempted to visualize the association in terms of structures



and



The exceptional negative entropy of activation in the case of ethylene glycol carbonate is responsible for the relatively slow reaction rate. It seems that this solvent associates relatively poorly with the azide but much better with the nascent bromide ion. In this respect the behavior of sulfolane resembles that of ethylene glycol carbonate except that the dissociation of the ion pair is somewhat more effective.

Very interesting solvent effects on reaction rates are observed in processes in which solvation by both protic and aprotic solvents affects the activation energy of the rate-determining step. Since the dual solvation of the reacting species depends on the availability of both types of solvents, and the latter can associate with each other, the interplay of these equilibria causes the rates to vary in a highly nonlinear fashion as the composition of the solvent mixture is varied. Possibly the most irregular behavior described so far is that of the Wolff–Kishner reaction of ketone hydrazones in a mixture of DMSO and hydroxylic solvent *(243)*. The rate of this reaction is found to increase as the hydroxylic solvent (butyl carbitol) is partially replaced

by DMSO. At first the rate increases probably because of the favorable increase in the dielectric constant of the medium. However, as additional DMSO replaces the hydroxylic solvent, the beneficial effect of DMSO decreases and finally disappears. Thus, the plot of the rate constants as a function of DMSO concentrations exhibits a maximum. Of further interest is the observation that the rate maxima appear at higher DMSO concentrations as the temperature is lowered. Since it is known *(208)* that the anion of benzophenone hydrazone does not undergo a Wolff–Kishner reaction in the absence of a protic solvent, it is believed that the rate-determining step of this reaction involves a more-or-less concerted proton transfer from the hydroxylic solvent to the hydrazone anion and a proton abstraction from the hydrazone anion by a relatively "basic" solvent. Such a transition state can be represented by

$$[S \cdots H \cdots \overset{(-)}{R_2CN_2} \cdots H \cdots S'] \neq$$

where SH and S′ symbolize the protic and "basic" solvent molecules, respectively. The presence of both protic and "basic" solvent molecules generates the solvated hydrazone anion that reacts with a lowered energy of activation, but the picture is complicated, first, by the equilibrium

$$S—H + S' \rightleftharpoons S—H \cdots S'$$

and second, by the ion-pairing equilibrium of the sodium salt of the hydrazone. The temperature-induced shift of the rate maxima to compositions containing a higher concentration of the protic solvent is believed to be attributable, to a large extent, to the relatively weak solvation of the carbon terminal in the hydrazone anion by the hydroxylic solvent. In other words, since the "hydrogen bond" between the hydroxylic solvent and the carbanion terminal of the hydrazone anion is probably a weaker association than that involved in alcohol–alcohol or alcohol–DMSO complexes, and since, as the temperature is raised, the weakest association is most readily broken down, the disruptive effect of higher temperatures on the concentration of the solvated hydrazone anion can be partially compensated by an increased concentration of the hydroxylic solvent. The effect of DMSO on the Wolff–Kishner reaction is, then, attributed to its relatively high dielectric constant, its capacity to dissociate ion pairs, but foremost, to

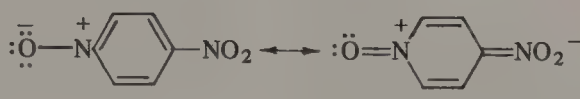
the fact that its significantly high basicity lowers the contribution of the N—H bond dissociation energy to the total energy of activation.

Less pronounced, but otherwise of similar nature, is the rate response of the neutral hydrolysis of 2,4-dinitrofluorobenzene to changes in the composition of the DMSO–water medium *(244)*. The rate increases about 50-fold, reaches a maximum value when the concentration of DMSO is 88 wt.%, and then decreases. The presence of water is an obvious requirement in this reaction. It is needed as the reacting nucleophile and probably also as the electrophilic catalyst whose function is to solvate the fluorine substituent. The analogous hydroxide ion-catalyzed hydrolysis does not exhibit such a rate maximum over the solvent composition range that was studied. This is probably so because the greater nucleophilicity of the hydroxide ion does not require the above-mentioned electrophilic catalysis of water.

The active participation of both water and DMSO molecules is invoked *(245)* to explain the nonlinear rate dependence of the acid-catalyzed hydrolysis of ethyl acetate on the water content in the reaction mixture. The rate is seen to increase up to 70 vol. % in water, but then it decreases, revealing the involvement of DMSO in the mechanism. The role of DMSO is believed to be that of a basic solvent that aids in the removal of a proton from the tetrahedral intermediate in the hydrolysis. The inverse effect of DMSO is observed *(118)* in the acid-catalyzed hydrolysis of acetal, since in this case addition of DMSO produces a minimum of approximately 0.7 mole fraction of DMSO. Considering the effect of DMSO on the rate of this reaction with that of other nonaqueous solvents *(118)* one is led to conclude that, in the lower concentration range of DMSO, the rate decreases mostly because of a decreased solvation of the protonated acetal intermediate, while in the higher concentration range the reaction tends to be favored by a decrease in the dielectric constant.

The nucleophilic displacement of the nitro group in 4-nitropyridine N-oxide by hydroxide ion was studied over a large range of DMSO–water compositions *(246)*. The rates increase by 3 to 4 powers of 10 in the presence of DMSO, but no maxima are exhibited. Significantly enough, however, the plot of log k against concentration of DMSO reveals that the acceleration induced by the presence of DMSO (attributable to the increased activity of hydroxide ion) diminishes as its concentration is increased. This implies that the presence of water is also helpful in this particular reaction. However, this does not appear to be the case for hydroxide ion reactions with methyl iodide, *p*-fluoroni-

trobenzene, and the already mentioned case of 2,4-dinitrofluorobenzene. The beneficial presence of water in the nucleophilic reaction of 4-nitropyridine N-oxide is not difficult to understand if the electron distribution in the molecule is considered.



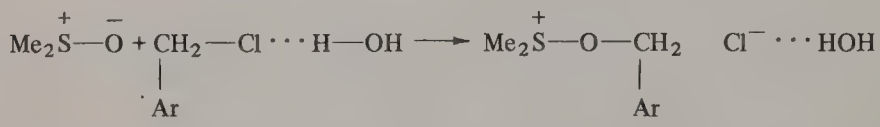
It is obvious that hydrogen bonding at the *N*-oxide terminal will decrease the delocalization of the negative charge into the nitro group and thus render the molecule more susceptible to nucleophilic attack by hydroxide ion at the 4-position.

Nucleophilic substitutions of nitrophenyl halides have been studied in methanol and DMSO. The latter was found *(236)* to cause a 100-fold greater acceleration of the reaction of azide ion as compared to thiocyanate. The view that this difference relates to the degree of solvation of the nucleophile in protic and aprotic solvents has been questioned in more recent work *(247)*.

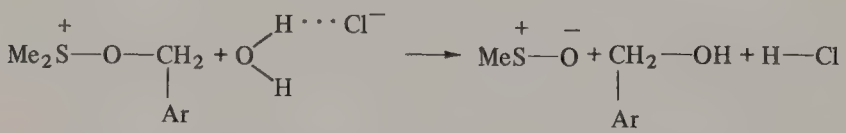
The complex interplay of the known, and still unknown, solvent-produced factors that affect the rates of reaction makes it risky to draw mechanistic conclusions from a limited set of experimental results. Often, the more data that are accumulated, the more difficult it is to arrive at an understanding of the whole picture. This situation is well exemplified by the series of papers by Tommila and co-workers *(248)* dealing with the neutral or base-catalyzed solvolysis of benzyl chlorides in DMSO–water, DMSO–methanol, aqueous acetone, and aqueous dioxane. The results reveal variation in energies and entropies of activation as a function of substituents and solvent composition and also show temperature coefficients of the solvent effects, dependence of the rates on the dielectric constant and substituents (Hammett relationship), and so forth. Some of the observations of interest follow.

The noncatalyzed solvolysis of benzyl chlorides is retarded by increasing the concentration of DMSO in the DMSO–water medium, while the base-catalyzed reactions are accelerated by DMSO more so in methanol than in water. The noncatalyzed hydrolysis in aqueous acetone or dioxane is about equally dependent on the concentration of water regardless of the nature of substituent, but in aqueous DMSO the dependence on water decreases rapidly as one shifts from electron-donating to electron-withdrawing substituents. This suggests the possibility that DMSO enters actively into the solvolytic process. In the case

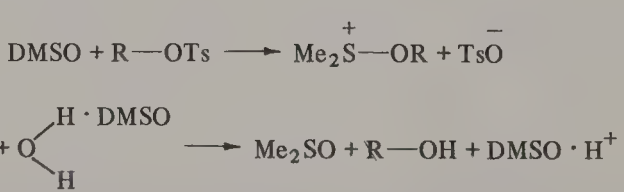
of *p*-nitrobenzyl chloride, basic solvolytic conditions are known to produce *p,p'*-dinitrostilbene and the mechanism of this reaction is believed to start with the formation of the *p*-nitrochlorobenzyl carbanion. Electron-donating substituents accelerate the reaction in the noncatalyzed solvolysis, but the substituent effect decreases as the concentration of DMSO or the temperature of the reaction are increased. These observations again suggest that the reaction mechanism shifts from an essential clean S_N1 process (in the case of the electron-rich benzyl chlorides) to a bimolecular process with an active participation of DMSO. This conclusion is supported by the linear enthalpy–entropy plot in the case of *p*-nitrobenzyl chloride over the whole range of DMSO–water compositions, while a highly irregular plot is given by, for example, *p*-methylbenzyl chloride. A possibility not reported by the Finnish workers is that in the case of the benzyl chlorides containing electron-withdrawing substituents the slow reaction step may consist of a bimolecular attack of DMSO on the benzyl chloride solvated by the protic solvent.



and that the benzyloxysulfonium ion is rapidly decomposed, perhaps by a nearby water molecule,



The hydrolysis of tosylates of optically active alcohols with retention of configuration *(249)* can also be explained by an active participation of DMSO in the process



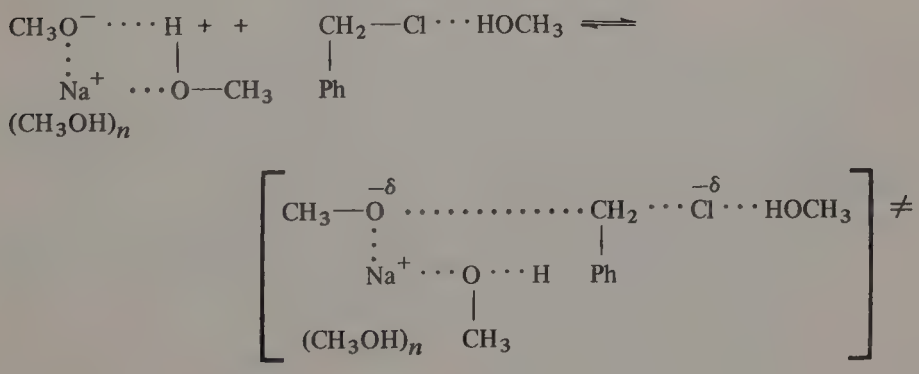
An analogous mechanism was invoked *(250)* to explain the beneficial effect of DMSO in the reaction of benzyl chloride with thiosulfate.

The multiplicity of possible mechanisms that contribute to the experimentally observed rates explains the inconsistent substituent

effects for the alkaline hydrolysis of benzyl chlorides *(248)* carried out in aqueous DMSO. As stated above, DMSO accelerates the base-catalyzed reaction, and especially so when its concentration is greater than 0.3 mole fraction. Since this reaction is an example of charge dispersal, it would be expected to benefit from a decrease in the dielectric constant of the medium. This is indeed the case in aqueous DMSO. Because higher concentrations of acetone in aqueous acetone retard the reaction, it follows that under these conditions the rates are more strongly affected by the removal of solvated water molecules from the hydroxide ion than from the decrease in the dielectric constant. DMSO is superior to acetone in associating with water molecules. The solvation of the methoxide ion by methanol probably involves only one solvent molecule, while the solvation of the hydroxide ion by water could easily involve three or more solvent molecules. This difference explains why DMSO is more effective in accelerating the reaction of benzyl chlorides in methoxide–methanol than in the hydroxide–water system. Highly irregular enthalpy–entropy plots in the methoxide-catalyzed DMSO–methanol system speak strongly in favor of the coexistence of different mechanisms that differ in the nature of solvation. Examination of the variation in enthalpies of activation in different DMSO–methanol mixtures reveals a nearly linear relationship. However, the entropies of activation form a minimum centered about an equimolar mixture of DMSO and methanol. This result suggests that the fixation of solvent molecules in the activated complex reaches a maximum near the equimolar solvent composition.

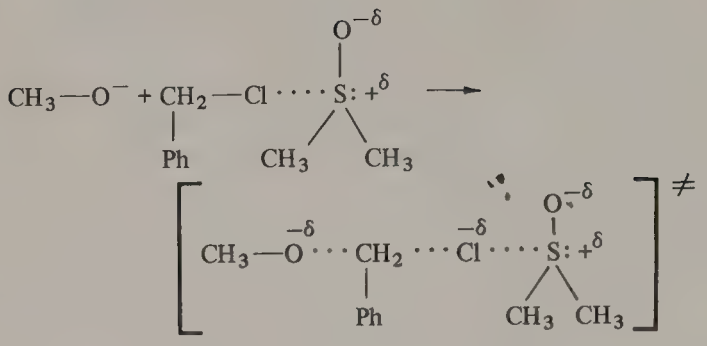
The subtleties of the solvation differences can be represented as follows:

a. In nearly pure methanol:



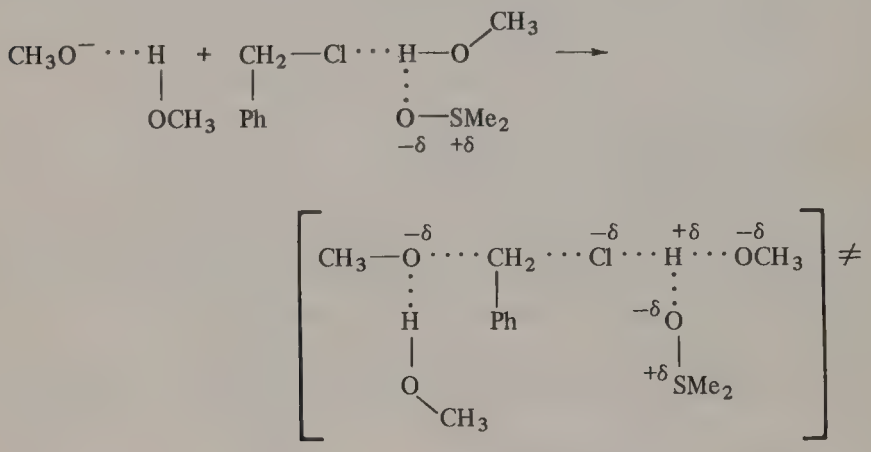
High $E_{act.}$, small negative $\Delta S^{\neq}$

b. In nearly pure DMSO:



Low $E_{act.}$, small negative $\Delta S^{\neq}$

c. In ca. equimolar DMSO–MeOH:



Medium $E_{act.}$, large negative $\Delta S^{\neq}$

Changes in composition of the DMSO–water reaction medium also cause complex variations in the thermodynamic parameters of the alkaline hydrolysis of esters *(251,252)*.

A detailed review of the literature of solvent effects is convincing in that while the generalizations stated at the beginning of this section represent useful guidelines for the prediction of gross effects expected of DMSO, additional work is needed in order to understand more fully the exact nature of the participation of solvent molecules in the drama of a chemical change.

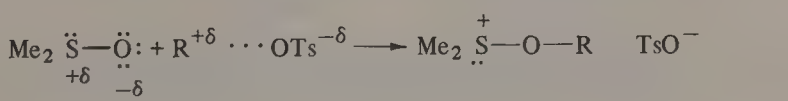
VI. CHEMICAL PROPERTIES OF DMSO

DMSO is an extremely versatile reagent and there are several extensive reviews *(3,7,8,11,12,220–222)* of its chemical properties. In the present limited account of this subject, the intention is to examine systematically the fundamental types of chemical behavior of DMSO, and we shall start with aspects already touched upon in the preceding sections.

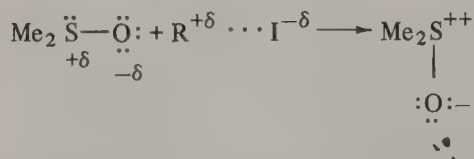
A. Electron-Donating Properties of DMSO

We recall that DMSO can function as a "base" with respect to a proton source (Section IV), as a hydrogen bond acceptor (Section III,A,2,b), as a ligand in the formation of loose solvates or more tightly bound complex ions with metals (Section III,B), and as an electron donor in charge-transfer complexes (Section III,A,1). To this already extensive list of electron-donating properties of DMSO, we must still add two other modes of action, namely, those of a nucleophilic reagent and of a reducing agent. The latter behavior is dealt with separately in Section VI,D. With regard to the ligand and nucleophilic behavior of DMSO, its versatility is compounded further by the fact that electron donation can materialize by way of nonbonding electrons present at either the oxygen or sulfur atoms. In other words, DMSO is an ambident electron donor, base, ligand, or nucleophile. The greater polarizability of the nonbonding electrons at the sulfur atom allows one to predict *(19)* that this "softer" terminal of the base will interact preferentially with a corresponding "softer" electron acceptor, or acid. This has already been pointed out in Section III,B in connection with the ligand behavior of DMSO.

The polarizability of a carbon group subject to a nucleophilic displacement reaction depends on the ionic character developed in the transition state, and this in turn is a function of the electronegativity of the leaving group. These concepts, together with the "hard and soft acid–base theory" allow us to understand why the rather ionic tosylates react with DMSO to give dimethylalkoxysulfonium ions *(254)*,

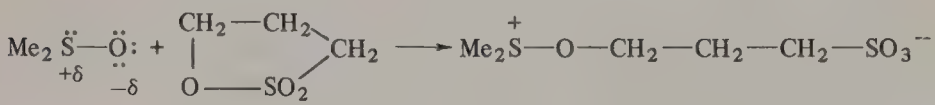


while the poorly ionized but rather polarizable alkyl iodides react with DMSO to give dimethylalkylsulfoxonium ions *(254–256),*



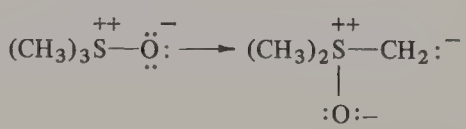
The reaction of DMSO with methyl-^{14}C iodide, followed by heating of the sulfoxonium salt with pyridine, produces DMSO with retention of two-thirds of the radioactivity originally present in the methyl iodide, as well as *N*-methylpyridinium iodide. This sequence was used *(256)* to demonstrate the occurrence of *S*-methylation, and as a practical synthesis of ^{14}C-labeled DMSO *(257).* It is noteworthy that the dimethylalkoxysulfonium ions tend to isomerize in solution to the dimethylalkylsulfoxonium ions *(254).* This isomerization is understandable in view of the higher nucleophilic character of the lone pair at the sulfur atom. The less-stable alkoxylsulfonium ions are also produced by means of a Meerwein reagent *(258)* such as triethyloxonium fluoborate *(259–261),*

or a sulfone *(260),*

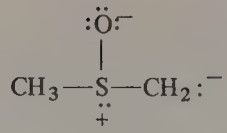


Both of these reagents are similar to the tosylates in the sense that they possess electronegative, oxygen-containing "leaving groups" and consequently develop a highly positive charge at the carbon atom in the transition state.

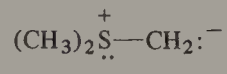
The trimethylsulfoxonium ion is readily converted to an ylid by means of sodium hydride *(262),*



and the latter is one of three sulfur-containing variations on the theme of the classic Wittig reagent. The other two are the dimsyl ion,

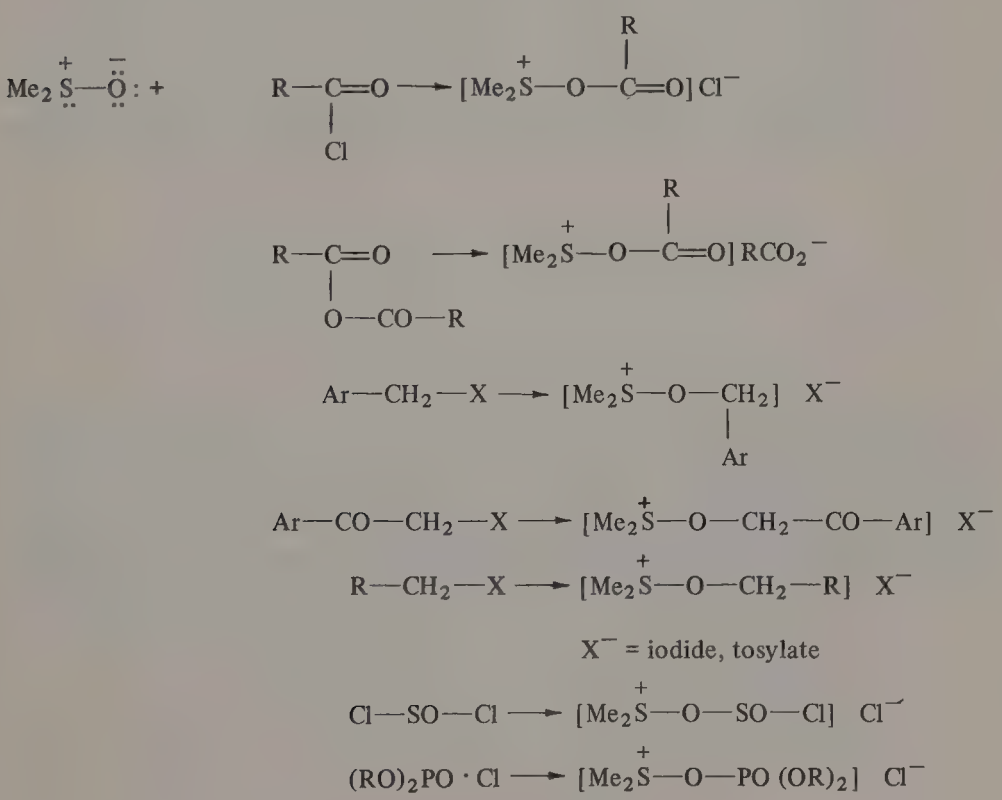


referred to more extensively in Section VI,B, and the anion derived from a trimethyl sulfonium salt,



The useful synthetic applications of these sulfur ylids *(257-267)* are beyond the scope of this discussion.

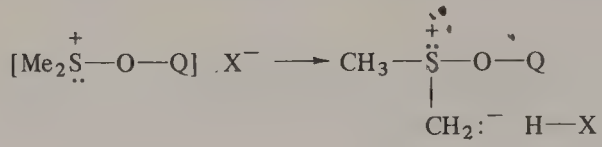
The electron-donating character of DMSO is responsible for numerous reactions initiated by a nucleophilic attack of the oxygen terminal upon carbon, phosphorus, sulfur, and other atoms that contain a good leaving group. These reactions are often highly exothermic and, unless properly controlled, can be explosive *(268,269)*. Let us first indicate the common pattern of the nucleophilic attack, although the postulated intermediates may not be stable or even known.



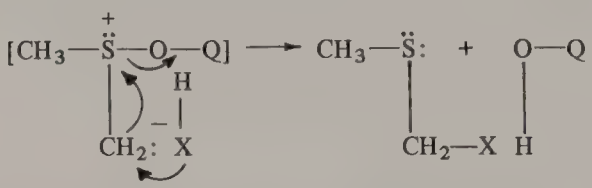
X^- = iodide, tosylate

The decomposition of these initial products of the nucleophilic substitution reaction can take two routes:

(1) If the most available "active hydrogen" is that of the original DMSO molecule, then the original leaving group (X) functions as a base,



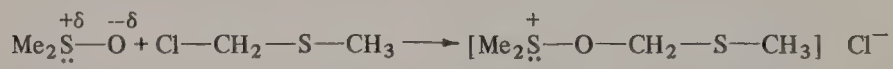
and further decomposition occurs according to the scheme:



In this fashion the reaction of DMSO with carboxylic acid chlorides *(270)*, sulfonyl chlorides *(271)*, thionyl chloride *(272)*, sulfinyl and sulfenyl chlorides *(273)*, chlorophosphates *(274)*, and acid anhydrides *(275,276)* leads to the formation of methylmercaptomethyl chloride or esters. The conversion of a methyl sulfoxide with an acid anhydride to the corresponding sulfide ester is just another example of the Pummerer reaction

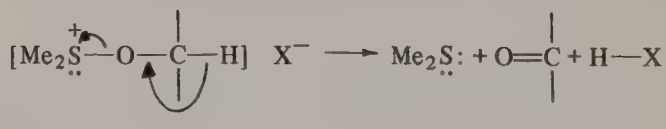
$$R{-}SO{-}CH_3 + HQ \longrightarrow R{-}S{-}CH_2{-}Q + H_2O$$

Since chloromethyl methyl sulfide can also be attacked by DMSO,



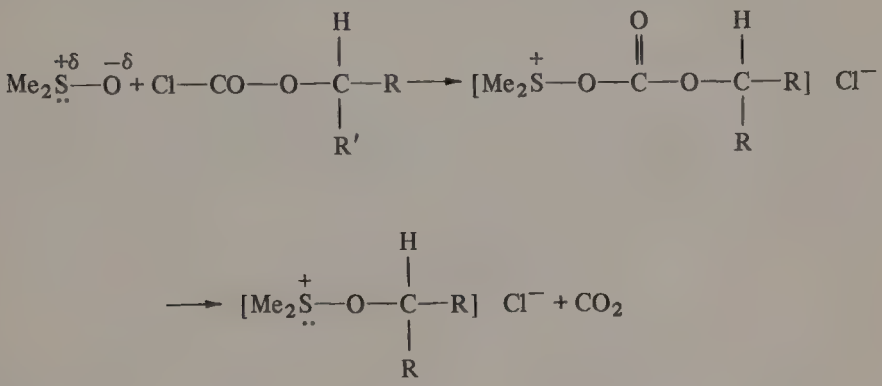
the reaction tends to become more complex *(274)* as explained in the paragraph that follows:

(2) If an "active hydrogen" is available on the moiety that underwent nucleophilic attack by DMSO, then the decomposition of the initial product occurs with dimethyl sulfide becoming the new leaving group

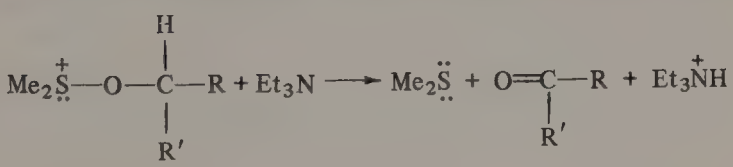


The net result of this change is the conversion of a primary halide or tosylate to the corresponding carbonyl compound, with the incidental formation of dimethyl sulfide. The oxidation of primary halides and arylsulfonates by DMSO is now identified as the Kornblum reaction *(277,278)*.

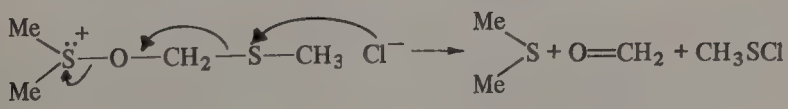
An oxidation scheme somewhat more complex than the Kornblum reaction has been devised *(279)* in which the initial intermediate is generated by nucleophilic attack of DMSO upon the chloroformate of the alcohol in question, whereupon the complex carbonate spontaneously rearranges with the extrusion of carbon dioxide,



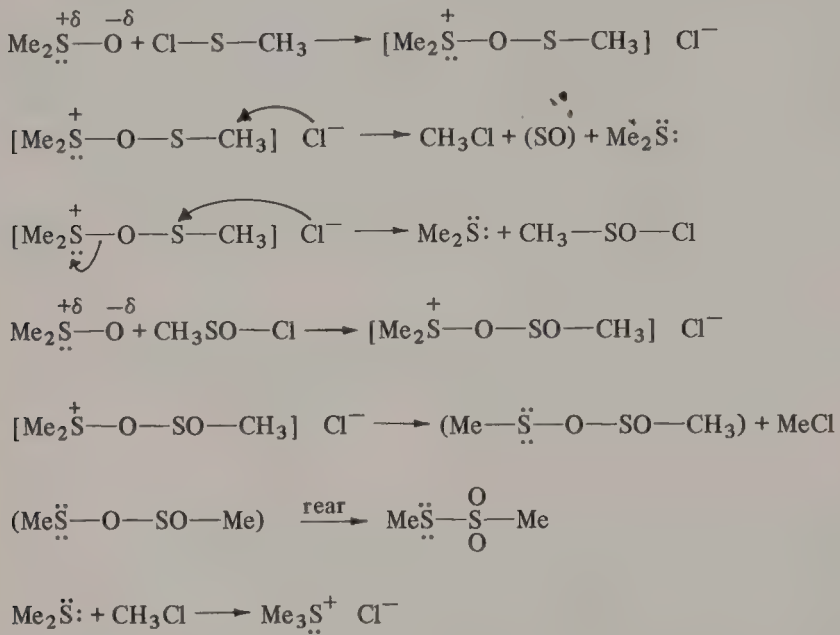
and the final decomposition to dimethyl sulfide and the carbonyl compounded is facilitated by the use of a tertiary amine,



The product of the nucleophilic attack of DMSO upon chloromethyl methyl sulfide decomposes in a way that resembles the general pattern described here. The original leaving group Cl^-, rather than returning to an attack upon an "active hydrogen," finds the sulfide group more favorable since this leads to a concerted fragmentation giving rise to such stable products as formaldehyde and dimethyl sulfide:

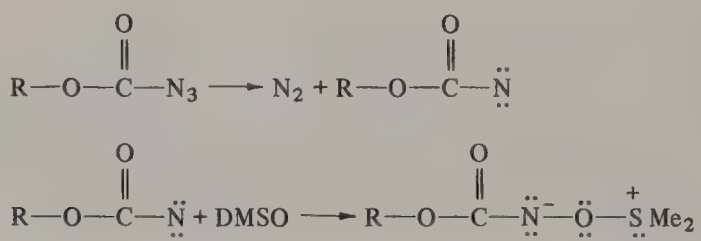


However, the chain of events does not stop here because methanesulfenyl chloride *(280)* is not a stable product and undergoes further reaction with DMSO.

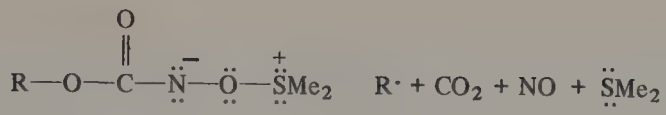


Thus, a series of transformations can be suggested that explain the fact that the reaction of chloromethyl methyl sulfide and DMSO produces *(274)*, in addition to dimethyl sulfide and formaldehyde, also trimethylsulfonium chloride, *S*-methyl methanethiosulfonate, as well as methanesulfenyl chloride *(280)*.

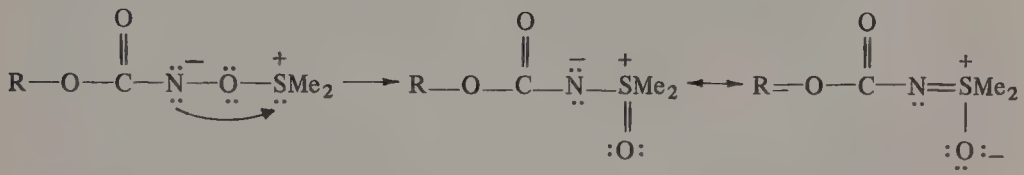
The electron-donating character of DMSO can be invoked in order to formulate the products of the reaction with an electron-deficient nitrogen species such as a nitrene *(281)*:



The hypothetical dipolar addition product can be visualized as decomposing by two reaction paths. First, it can cleave to give nitric oxide, carbon dioxide, dimethyl sulfide, and a hydrocarbon radical (which undergoes subsequent reactions),



and second, an intramolecular nucleophilic attack of the negatively charged nitrogen upon sulfur triggers a rearrangement to a sulfoximine,



It is possible that the nucleophilic attack of a sulfoxide upon a nitrene requires that the latter be negatively substituted as is true in the case mentioned above and also with the sulfonylnitrenes, $R—SO_2—\ddot{N}$ *(282).*

B. Electron-Accepting Properties of DMSO

The capacity of DMSO to accept electrons was noted in Section III,A in connection with the negative excess volumes observed in the case of binary mixtures containing benzene derivatives carrying electron-donating substituents. Furthermore, the electron-accepting character of DMSO was invoked in connection with the oxygen-exchange reaction (Section IV), and it manifests itself in its protic acidity, also discussed in Section IV, and in the formation of the dimsyl ion.

A full account of the chemistry of the dimsyl ion *(283)* is beyond the scope of this review. Suffice to say, that it is not only useful for the generation of anions from stronger protic acids *(284,285),* but also functions in its own right as a strong nucleophile, and as such undergoes addition and condensation reactions *(283,286–290)* characteristic of more conventional carbanions.

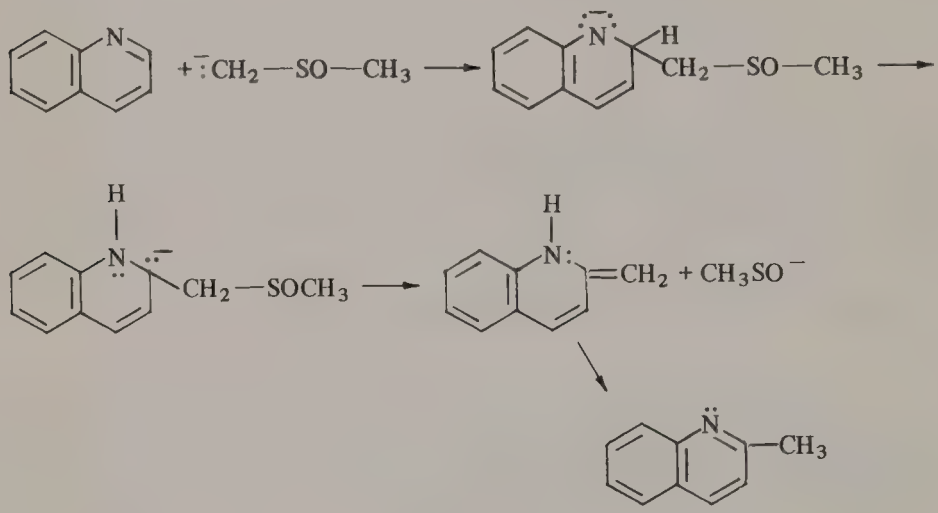
The condensation of the dimsyl ion with esters produces β -keto sulfoxides, $R—CO—CH_2—SO—CH_3$, which have been shown *(283, 291–293)* to be extremely versatile starting materials for the synthesis of a variety of families of organic compounds.

An interesting feature of the dimsyl addition reactions is the possibility of a base-catalyzed prototropic shift in the original addition product to give a carbanion which expells the methansulfenate leaving group,

$$Q{-}CH{=}CH_2 + :\overset{(-)}{C}H_2{-}SO{-}CH_3 \longrightarrow Q{-}\underset{\cdot\cdot}{CH}{-}CH_2{-}CH_2{-}SO{-}CH_3$$

$$\longrightarrow Q{-}CH_2{-}\underset{\cdot\cdot}{CH}{-}CH_2{-}SOCH_3 \longrightarrow Q{-}CH_2{-}CH{=}CH_2 + CH_3SO^-$$

The net synthetic result is a methylation reaction by means of the dimsyl ion, and this has been observed to occur in activated olefins *(286)*, conjugated dienes *(287)*, and in polynuclear aromatic carbo- and heterocycles susceptible to nucleophilic attack *(294)*. The last mentioned reaction is illustrated with the case of quinoline.



The electrophilic nature of the methanesulfinate moiety makes it possible to utilize the dimsyl ion in the manner of a Wittig reagent *(258,262)*, except that the situation is somewhat complicated by the susceptibility of the initial olefinic product to undergo further reactions with the dimsyl ion. *(262)*.

Another synthetic twist in the use of the dimsyl ion takes advantage of the lability of the C—S bond to reductive cleavage. Thus, the sodium amalgam reduction of the condensation reaction of the dimsyl ion with an ester converts the latter to the corresponding methyl ketone *(262)*:

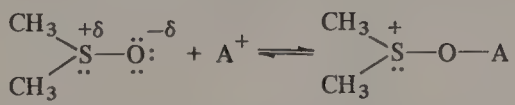
$$R{-}CO{-}OCH_3 \xrightarrow{CH_3SOCH_2^-} R{-}CO{-}CH_2{-}SO{-}CH_3 \xrightarrow[H_2O]{Na{-}Hg} R{-}CO{-}CH_3$$

Before we leave the subject of the dimsyl ion, it should be noted that for all practical purposes *(290,294)* it can be generated from DMSO and potassium *t*-butoxide in spite of the unfavorable equilibrium,

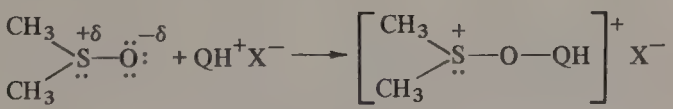
$$t\text{-}BuO^- + DMSO \rightleftharpoons t\text{-}BuOH + :\overline{C}H_2SOCH_3$$

for which the equilibrium constant is reported *(295)* to have the value of 1.5×10^{-7}. The explanation of this apparent anomaly lies in the high reactivity of the dimsyl ion so that it is efficiently consumed in the desired nucleophilic reaction. Furthermore, the kinetic situation under discussion may not be as unfavorable as one would be led to believe on the basis of the relatively high thermodynamic acidity of *t*-butyl alcohol when this is determined in protic media. Strong solvation of this alcohol by DMSO would be expected to decrease the kinetic acidity of *t*-butyl alcohol to the point at which the rate of the nucleophilic reactions of the dimsyl ion become competitive.

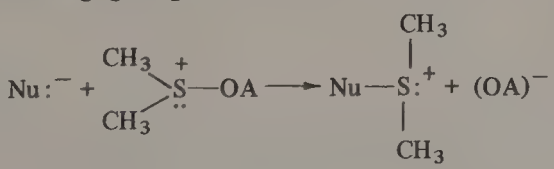
Next let us consider the electron-accepting character of DMSO manifested by nucleophilic attack on the sulfur atom. Naturally, the lone pair present at the sulfur of DMSO cannot be expected to favor the approach of a nucleophile in spite of the presence of a partial positive charge and vacant *d* orbitals. Thus, it is not surprising that most if not all reactions in which a nucleophilic attack on sulfur seems to be the initial, critical step are aided either by electrophilic catalysis at the oxygen atom,



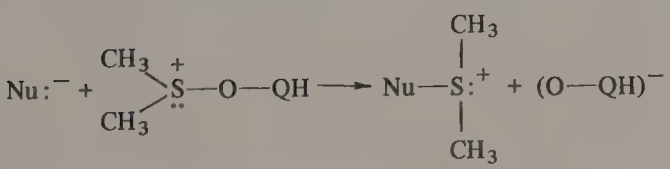
or occur in derivatives of DMSO in which (through a previous nucleophilic attack by DMSO upon an appropriate reagent) a good leaving group is built in,



Now, a nucleophile (Nu:) can bring about a displacement of either one of the two leaving groups,



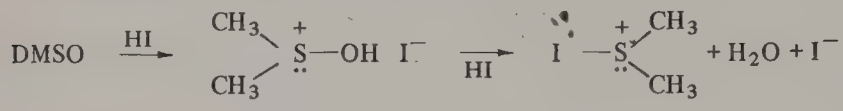
or

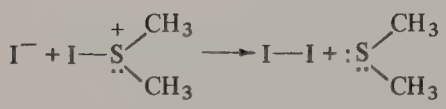


The formation of $Nu—\overset{+}{S}(CH_3)_2$ can, and usually is, followed by additional reactions.

The preceding general scheme can be illustrated with the following examples:

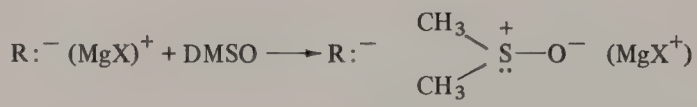
(1) The reduction of DMSO by HI *(296–298)*:

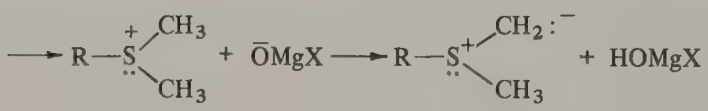


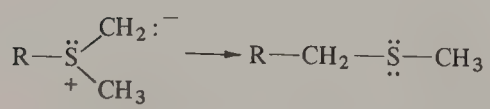


A similar reaction occurs with hydrobromic acid, and the electrophilic character of the dimethylbromosulfonium ion explains the unexpected aromatic brominations encountered during the reaction of 2-aminofluorenone with alkyl bromides in DMSO *(299)*.

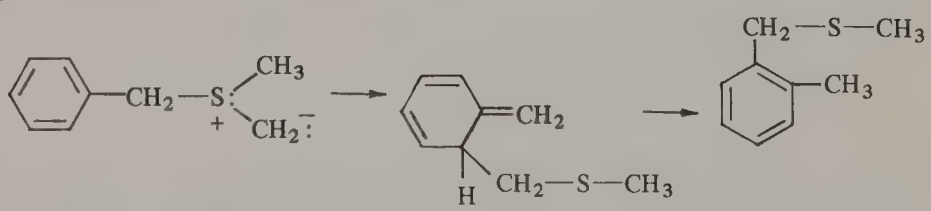
(2) The reaction of Grignard reagents with DMSO *(300)*:



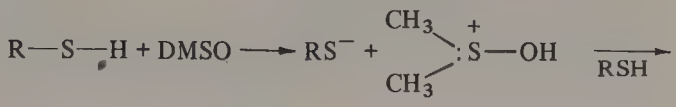


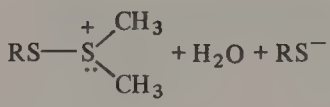


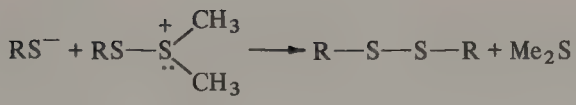
When R represents a benzyl group, the reaction takes the following course:



(3) The reaction of thiols with DMSO *(191,346,347)*:

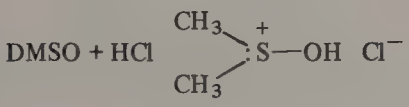


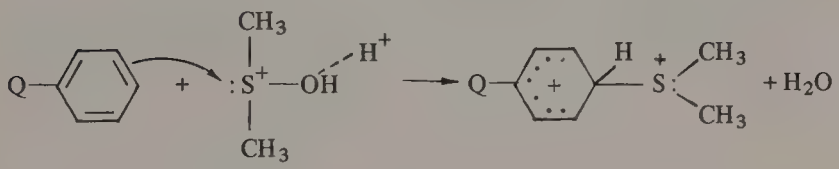


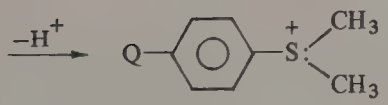


This mechanistic scheme is supported by observations that the reaction is favored by acidic thiols and is catalyzed by mineral acids.

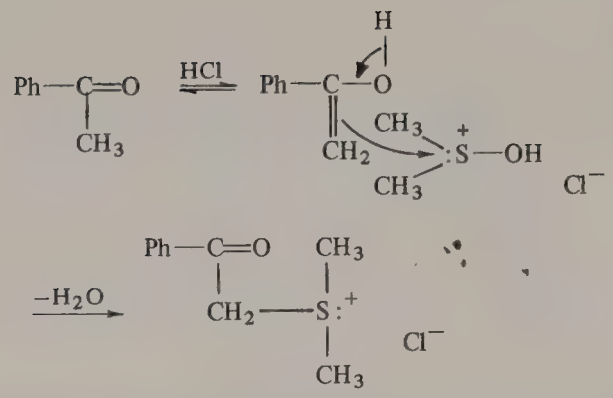
(4) Electrophilic substitution by DMSO of aromatic and enolic systems *(301)*. This reaction functions only with aromatic compounds most susceptible to electrophilic attack, and then only in the presence of acid catalysts.



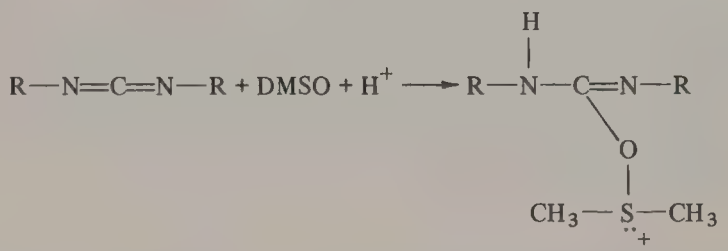




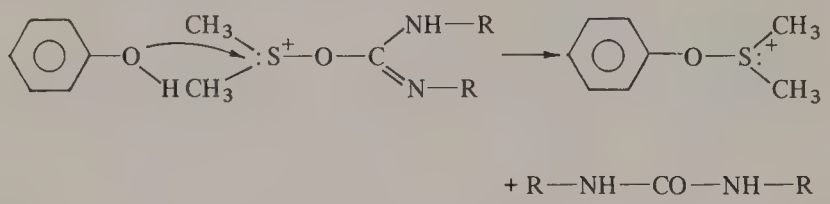
Hydrogen chloride catalyzes not only the formation of the electrophilic sulfonium ion center in DMSO, but also the formation of the nucleophilic tautomer of a ketone, and in the case of acetophenone, for example, dimethyl phenacyl sulfonium chloride is obtained,



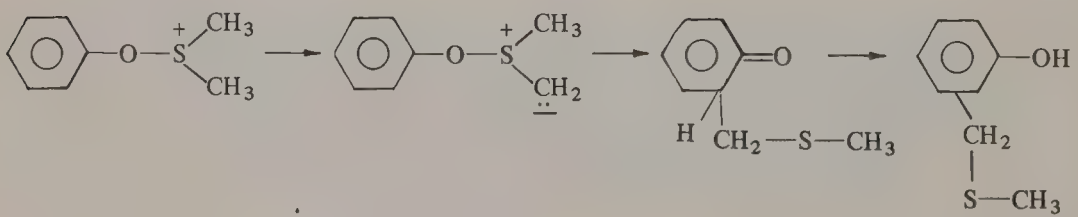
(5) Intensification of electrophilic character of DMSO via its isourea derivative *(302)*. A nucleophilic attack of DMSO upon dicyclohexylcarbodiimide (in the presence of an acid catalyst) leads to an isourea derivative,



which, generated *in situ,* is now subject to the attack by the weakly nucleophilic phenol because of the presence of a good leaving group in the form of the disubstituted urea.

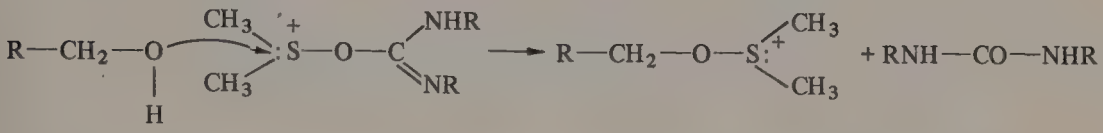


The phenoxydimethylsulfonium ion is unstable and undergoes a rearrangement analogous to that shown above for the benzyldimethylsulfonium ion.

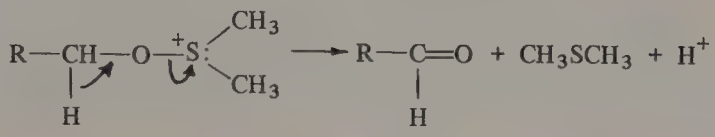


The net result is that DMSO functions as a methylmercapto-methylating agent *(303)*.

The reaction of an alcohol with the isourea derivative of DMSO follows the same pattern *(304)*

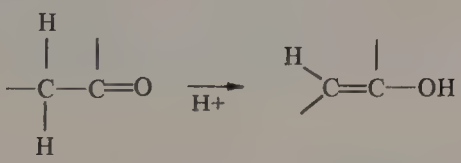


and the alkoxysulfonium ion decomposes as if it were generated from the Kornblum reaction shown in Section VI,A

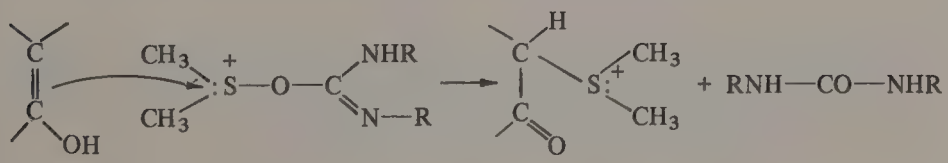


The net result of this and the Kornblum reaction is the conversion of the precursor R—CH_2—X to the identical oxidation product R—CHO, with the variant that in the Kornblum reaction DMSO initiates the process through a nucleophilic attack while in the carbodiimide derivative the DMSO moiety is the recipient of a nucleophilic attack by the alcohol.

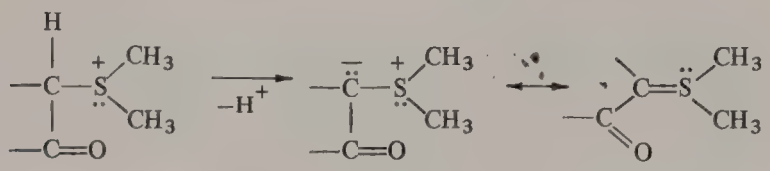
The electrophilic character of the isourea derivative of DMSO also accounts for the reaction of DMSO with a potential enol in the presence of dicyclohexylcarbodiimide and phosphoric acid *(300)*. The latter serves to catalyze a tautomerization,



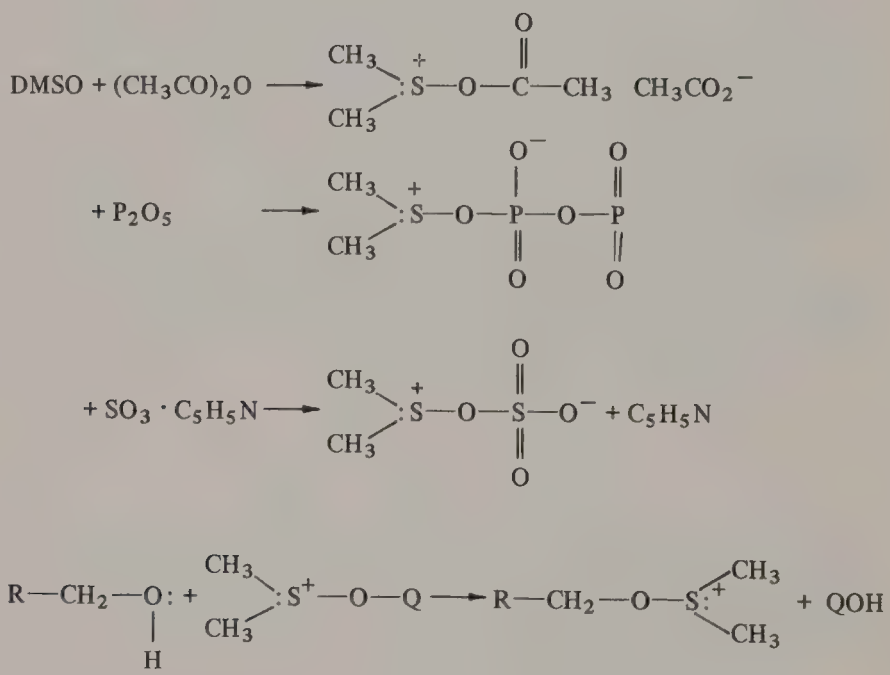
and the newly formed nucleophile is trapped by the reaction with the isourea derivative of DMSO,



The presence of two (or more in the case of a doubly activated methylene derivative) electron-withdrawing groups facilitates the proton loss from the intermediate sulfonium ion to give a stable sulfonium ylide,



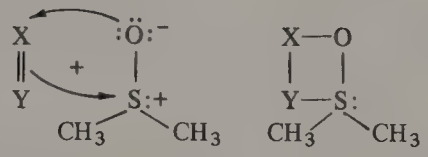
(6) Intensification of electrophilic character of DMSO via acid anhydride derivatives. Nucleophilic attack of DMSO on acetic anhydride *(305)*, phosphorus pentoxide *(306)*, or the sulfur trioxide–pyridine complex *(307)*, is expected to produce species in which the electrophilicity at the sulfur center is highly intensified. Now, the DMSO derivatives are susceptible to the attack of the weakly nucleophilic alcohol molecule,



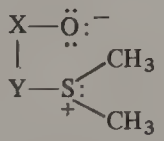
where QOH = CH_3CO_2H,HO—PO_2—O—PO_2,HSO_4^- and the oxidation of the alcohol results from the previously described fate of the alkoxydimethylsulfonium ion.

C. Dipolar Cycloaddition Reaction of DMSO

In view of the coexistance of electron-accepting and electron-donating properties in DMSO, it is not surprising that this reagent should also participate in polar, 1,2-cycloaddition reactions represented in a general manner as follows:

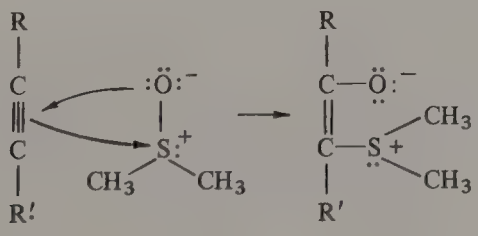


The only deterrent to such a process is the formation of the tetravalent sulfur product, a structure not commonly encountered in the realm of sulfur chemistry. However, cycloaddition concerted with heterolysis of the S—O bond yields the dipolar structure



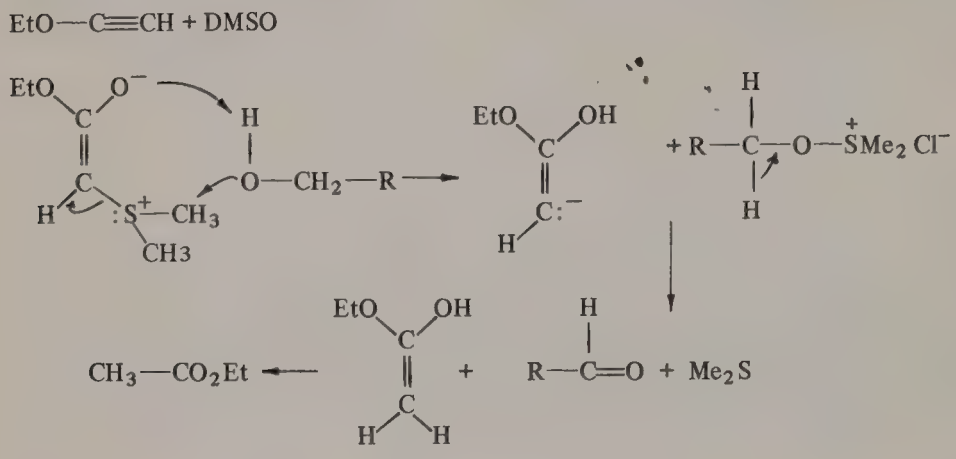
The dipolar ion derived from the addition of DMSO to benzyne was shown to be formed by its conversion to the picrate *(308)*, and other investigators *(309–311,365)* isolated the substance derived from the base-catalyzed degradation of the sulfonium ion, namely, the *o*-methylmercaptophenolic product.

Another example of the dipolar cycloaddition reaction of DMSO is the reaction with acetylenes *(312)*.



(R,R′ = CO_2CH_3; R′ = CO_2CH_3, R = H)

The dipolar addition product of DMSO and ethoxyacetylene is believed *(305)* to be responsible for the oxidation of alcohols to aldehydes brought about in the presence of a phosphoric acid–pyridine mixture.



D. Oxidation-Reduction Reactions of DMSO *(2,313)*

Oxidative behavior of DMSO was noted in connection with its electron-donating properties (Section VI,A) which initiate a sequence of events of the Kornblum reaction or its variation. Also, the electron-attracting properties of DMSO were shown (Section VI,B) to initiate reactions that again terminate in the formation of dimethylsulfide simultaneously with the formation of iodine or bromine from hydrogen halides, disulfides from mercaptans, or aldehydes from alcohols. DMSO can participate in the dethionylation reaction of sulfoxides capable of this extrusion process *(314)*.

More conventional procedures for the reduction of DMSO include the use of zinc in the presence of sulfuric acid *(178)*, stannous or titanous chloride in the presence of hydrochloric acid *(315)*, aluminum hydride *(316)*, and diborane *(317)*. However, DMSO is sufficiently resistant to reduction to be a useful solvent for polarographic reductions *(318,319)*, in electrolysis *(320)*, and in reductions employing sodium borohydride *(78)*. A mild and selective method for the reduction of sulfoxides to sulfides, and one that is also applicable to DMSO, employs trivalent phosphorus compounds *(321–323)*. It is of interest to note that depending on the nature of the phosphorus compound DMSO can act either as a nucleophile and attack the phosphorus center by means of its nonbonding oxygen electrons, or it can act as an elec-

trophile and be the object of nucleophilic attack by the nonbonding electrons of phosphorus. In the latter case the electrophilicity is augmented by acid catalysis *(323)*.

Generally speaking, DMSO functions more readily as an oxidizing agent, with the evolution of volatile dimethyl sulfide, than as a reducing agent. Nevertheless, conventional oxidizing agents convert it readily to dimethyl sulfone. Among the practical methods employed for this purpose are those that employ permanganate *(324)*, dichromate *(325)*, ozone *(326)*, chlorine in an acidic medium *(327)*, and peroxides *(328,329)*.

Oxidation of DMSO with chlorine or hypobromite under alkaline conditions produces hexachlorodimethyl sulfone *(330)* or hexabromodimethyl sulfone *(331)*, respectively. Substitution probably occurs subsequent to oxidation and is expected to be a rapid reaction in view of the acid-strengthening effect of the sulfone group on α-hydrogen atoms. This situation is similar to the classic base-catalyzed halogenation of acetone. Chlorination of DMSO to the mono and dichloro derivatives is possible using nitrosyl chloride and pyridine in a chloroform solution *(332)*.

Just as in the case of reductions, DMSO is sufficiently resistant to oxidation to be a useful solvent for base-catalyzed oxidations employing molecular oxygen *(333)*, or oxidation reactions employing nitrogen tetroxide *(334)*, the triiodide ion *(335)*, periodic acid *(131)*, lead tetraacetate *(132)*, and in electrolytic processes *(320,336)*. With regard to the last-mentioned reaction, the possibility exists that a product of the electrolytic reaction is capable of attacking DMSO. This is the case in the electrolytic oxidation of triethylamine in DMSO *(337)*.

$$Et_3N: \xrightarrow[\text{Pt electr.}]{-e^-} (Et_3N)^+$$

$$(Et_3N)^+ + DMSO \longrightarrow (Et_3N{:}H)^+ + CH_3{-}SO{-}CH_2{\bullet}$$

The fate of the DMSO-derived radical was not determined. Also, in the electrolytic reduction of oxygen *(338)* it is found that one mole of DMSO is oxidized for each mole of reduced oxygen according to the mechanism

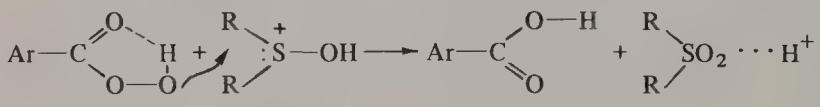
$$O_2 \xrightarrow{+e^-} (\cdot\ddot{O}{-}\ddot{O}{:})^- \xrightarrow{+e^-} O_2^=$$

$$O_2^= + Et_4N^+ \longrightarrow HO_2^- + CH_2{=}CH_2 + Et_3N$$

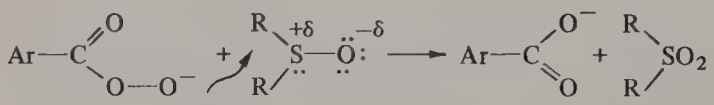
It is noteworthy that the oxidation of DMSO to dimethyl sulfone is brought about by the nucleophilic attack of the hydroperoxide anion rather than by an intermediate radical:

$$HO_2^- + DMSO \longrightarrow CH_3—SO_2—CH_3 + HO^-$$

Peracids are common reagents in the chemistry of organic sulfur compounds and therefore the mechanism of peracid reactions is of great interest. Numerous studies by Modena and collaborators *(339, 340)* attempted to answer the question as to whether, in the reaction between peracids and sulfoxides, the latter behave as electrophilic or nucleophilic reagents. In aqueous dioxane at 25°C, DMSO is oxidized by perbenzoic acid 13 times more rapidly at pH 12 than at pH 3.4. In working with other sulfoxides it is found that the substituent effects in the perbenzoic acids are rather small, and a positive value of 0.57–0.75 is obtained under either acidic or basic conditions. However, substituent effects in the sulfoxide moiety are large and of opposite character in acidic ($\rho = -2.63$) and alkaline ($\rho = +1.75$) media. Also, the oxidation reaction in alkaline media is strongly inhibited by steric crowding at the sulfoxide site, while under acidic conditions the change from DMSO at diisopropyl sulfoxide actually accelerates the rate, but further branching of the dialkyl groups causes the rates to fall off rapidly. All of these observations are consistent with a single mechanism proposed sometime ago *(341)* in which the rate-determining step is a nucleophilic attack of the oxidizing agent on the (electrophilic) sulfoxide. Naturally, the change in the pH of the medium does influence the structures of the acidic and basic participants of the reaction. Under acidic conditions,



while under basic conditions,



The nucleophilic character of the peracid anion is greater than the nucleophilic character of the undissociated peracid, and this accounts for the generally higher oxidation rates observed at high pH. The difference between oxidation rates in acidic and alkaline media would be larger than what is actually observed were it not for the fact that

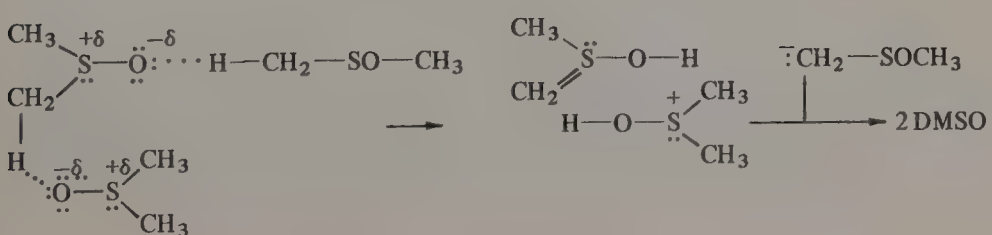
under acidic conditions the reaction involves the more electrophilic protonated sulfoxide species. While the basicity of the alkyl sulfoxides is expected to be increased by replacing the methyl by more strongly electron-releasing alkyl groups, this effect is soon overshadowed by steric inhibition to protonation and/or nucleophilic attack by the perbenzoic acid, and this explains the maximum in the relation between rate and the size of the alkyl groups for the oxidation under acidic conditions. The substituent effects in the peracid structure are small enough to be attributed to solvation phenomena. Finally, under neutral aqueous conditions it is to be expected that the acidity of the peracids outweighs the basicity of the sulfoxides and therefore the substituent effects should be small but on the side of the acidic pH situation. This expectation is in line with the ρ values of -0.64 and -0.54 observed *(342)* for the oxidation of methyl aryl and diaryl sulfoxides, respectively, using perbenzoic acid in 50% aqueous dioxane at 25°C.

E. Decomposition Reactions of DMSO

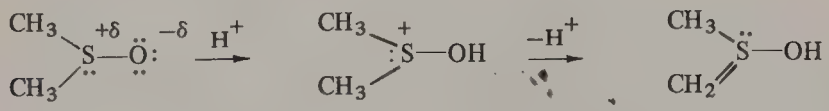
DMSO is a relatively stable substance and, in the absence of extraneous catalytic impurities, it can be refluxed at its elevated boiling point of 189°C with minimal decomposition; 3.7% of volatile products are produced over a 72-hr period *(343)*. As implied by the preceding statement, the resistance of DMSO to decomposition is affected by experimental conditions.

1. Thermal and Acid-Catalyzed Decomposition of DMSO

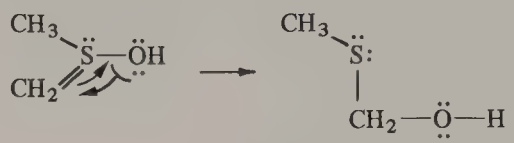
The decomposition of DMSO seems to follow a similar or identical pattern under both experimental conditions mentioned in the title, and thus one is tempted to suggest that, under purely thermal activation, the decomposition is autocatalytic in the sense that intermolecular hydrogen bonding aids in the formation of an enol analog of the sulfoxide *(344)*.



The same, short-lived species can be assumed to be generated by protic acids and hydrogen bond donors better than DMSO such as alcohols, amides, and so forth *(343)*.

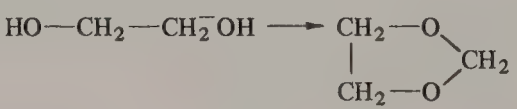


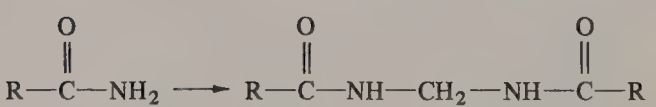
The rapid decomposition of the hypothetical tautomer of DMSO can be visualized to follow the well-established behavior pattern mentioned in Section VI,A in connection with the Pummerer-like conversion of DMSO to methylmercaptomethyl chloride and esters,



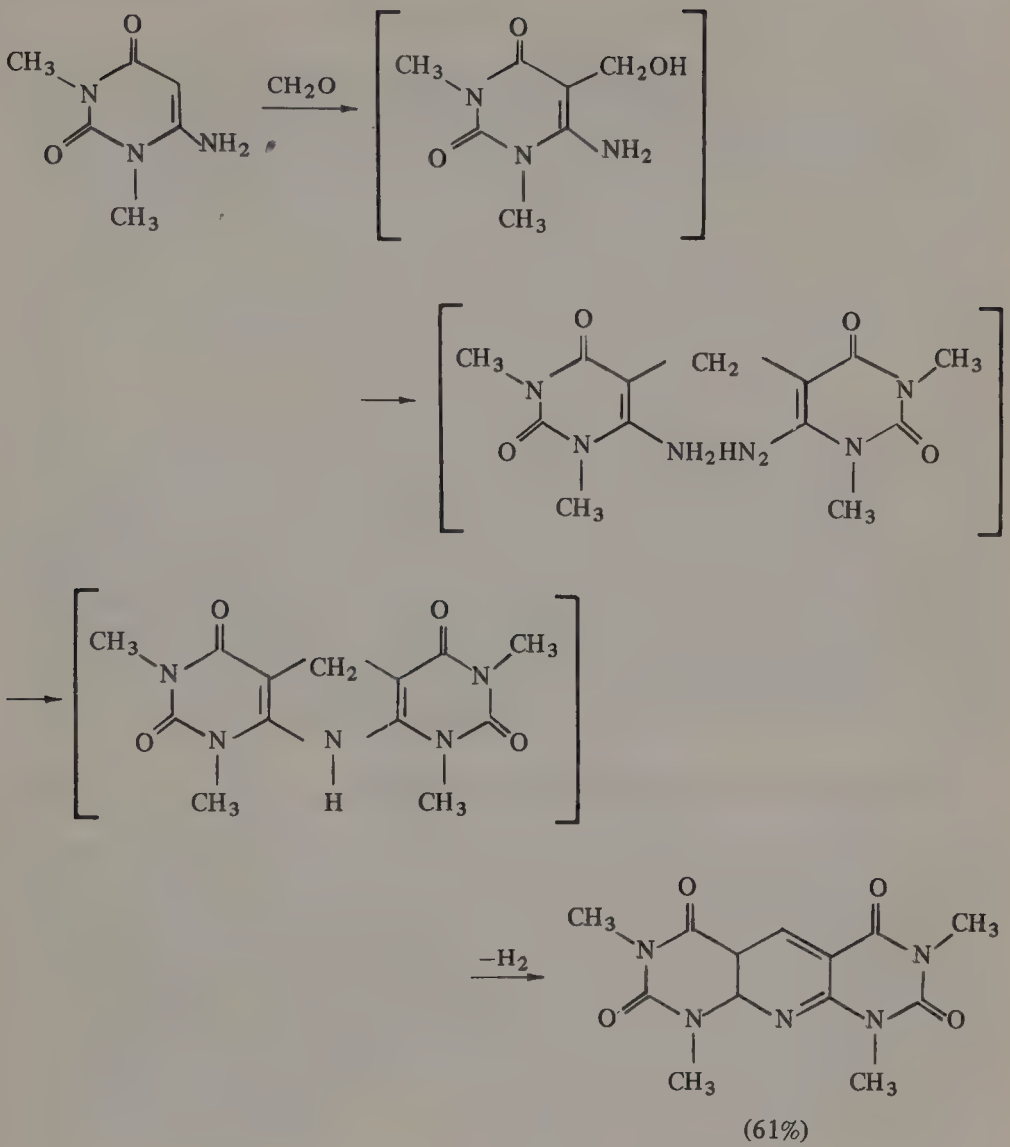
except that the product of this rearrangement is a labile hemithioformal and thus undergoes further decomposition,

The initial formation of formaldehyde and methyl mercaptan explains *(343)* the origin of paraformaldehyde, methyl thioformal, and water, and the formation of the formaldehyde derivatives of glycols or amides when DMSO is heated in the presence of the latter.





A formaldehyde condensation, followed by cyclization and aromatization, explains the interesting transformation of 6-amino-1,3-dimethyluracil *(345)* when this substance is heated for 48 hr in DMSO:

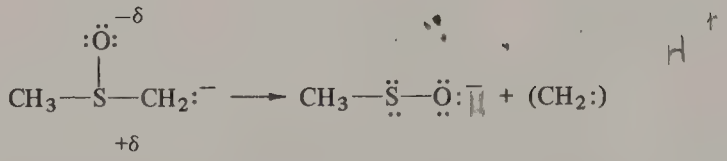


The formation of some methyl disulfide *(343)* during the thermal decomposition of DMSO can follow the mechanism suggested in Section VI,B for the general oxidation reaction of thiols to symmetrical disulfides by means of DMSO *(191,346,347)*.

Finally, the formation of some dimethyl sulfone can be explained by invoking a bimolecular disproportionation mechanism *(343)* for which there exist precedents *(314)* in the case of other sulfinyl group-containing compounds.

2. Base-Catalyzed Decomposition of DMSO

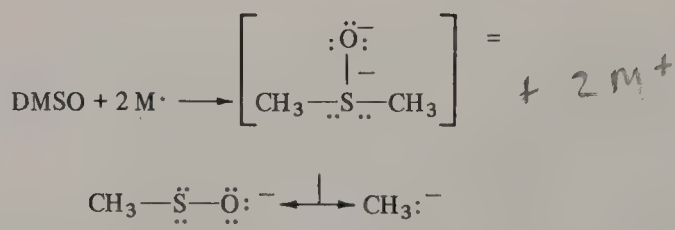
Base-catalyzed decomposition of DMSO centers around the known instability of the dimsyl ion in solution *(283)*. This behavior is understandable in view of the presence of the methanesulfenate leaving group,



The fate of the carbene is debatable, but the formation of sodium methanesulfenate is unquestionable because it tends to accumulate as an insoluble material in concentrated DMSO solutions of dimsylsodium. The heterogeneous mixture frequently undergoes violent decomposition reactions *(348,349)* and thus must be handled with proper precautions. The unstable nature of sulfenic acids and their derivatives is well known *(350)*. Additional insight into the complex decomposition of the dimsyl ion is provided by recent work *(367)*.

3. Radical-Initiated Decomposition of DMSO

Generally speaking, DMSO is remarkably resistant to the attack of neutral, unpaired electron-containing species in solution. This includes resistance to molecular oxygen, and explains the successful solvent use of DMSO in the molecular oxygen oxidations of ketones, anilines, nitrotoluenes, and so forth *(333)*. The inert behavior of DMSO toward lithium *(259)* can be included under this heading. More electropositive metals, such as sodium or potassium, do bring about a decomposition of DMSO *(351)* but the reaction apparently consists of a *two*-electron transfer *(352)*,



The decomposition of the intermediate dianion, followed by an immediate neutralization of the methane carbanion by another DMSO molecule,

$$CH_3:^- + DMSO \longrightarrow CH_4 + CH_3—SO—CH_2^-$$

gives an overall reaction between DMSO and active metals that produces methane and the methanesulfenate and dimsyl salts. Radical ions, such as the triethylamine cation or the peroxide anion mentioned at the beginning of this discussion, can provoke reactions of DMSO.

Vinyl polymerizations can be brought about in DMSO. This is particularly useful in the preparation of polyacrylonitrile which is insoluble in most other solvents *(353,354)*. It has been suggested *(355)* that the persulfate-catalyzed polymerization of acrylonitrile occurs by way of an electron transfer from DMSO to the persulfate ion,

$$2\,DMSO + S_2O_8^{=} \longrightarrow 2\,SO_4^{=} + 2\,(CH_3—SOCH_3)^+$$

and that the radical cation derived from DMSO functions as the catalyst. However, there seems to be little if any reaction between ammonium persulfate and DMSO *(356)*, and it is more likely that the favorable polymerization conditions for acrylonitrile in DMSO *(357)* result from the combination of its low reactivity toward radicals and good solvent properties for the polymer.

4. Photochemical Decomposition of DMSO (13)

DMSO seems to be stable under photochemical conditions employed for the oxidation of substituted benzyl alcohols to the corresponding benzaldehydes by molecular oxygen *(358)*. This is probably so because DMSO is transparent to ultraviolet light above 2300 . Also, DMSO does not appear to quench photoexcited solutes *(359)*.

The irradiation of DMSO at 2537 Å in the presence of catalytic amounts of iodine produces *(366)* dimethyl sulfide and trimethylsulfonium methanesulfonate

$$3DMSO \longrightarrow Me_2S + Me_3S^+\,MeSO_3^-$$

A mechanism that explains the stoichiometry of the reaction is also suggested:

$$DMSO + I_2 \xrightarrow{h\gamma} (Me_2\overset{O}{S}I)^+\,I^-$$

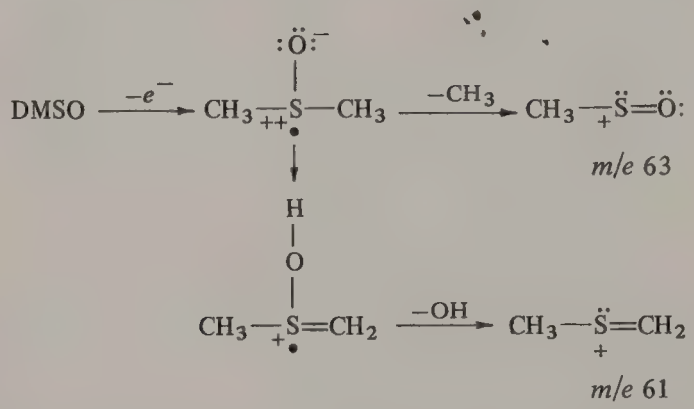
$$DMSO + (Me_2\overset{O}{S}I)^+ \longrightarrow (Me_3SO)^+ + MeSO - I$$

$$(Me_3SO)^+ + MeSO - I \longrightarrow (Me_3S)^+ + MeSO_2 - I$$

$$MeSO_2 - I + DMSO \xrightarrow{I^-} MeSO_3^- + Me_2S + I_2$$

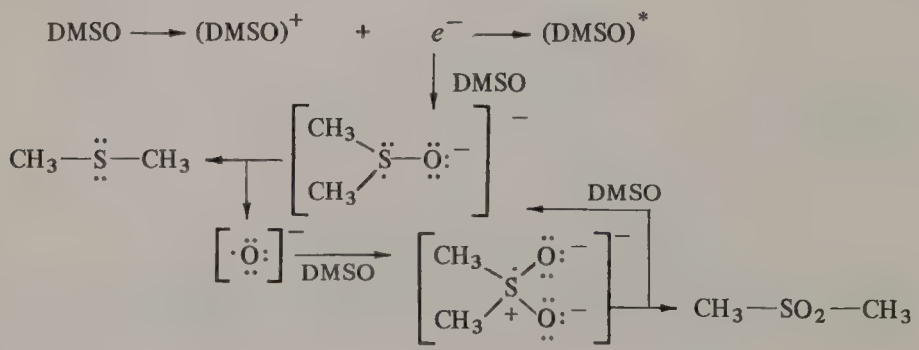
5. Decomposition of DMSO by Electron Impact and Ionizing Radiation

Electron impact upon DMSO produces *(360)* in addition to the parent ion, the fragments of *m/e* 63 and 61 which arise by the loss of methyl or hydroxyl:



The postulated fragmentation is in line with the mass spectral behavior of methyl alkaryl sulfoxides *(361)* in particular and sulfur compounds *(362)* in general. A sample of ^{18}O-labeled DMSO was analyzed *(363)* by mass spectrometry and, in addition to the above mentioned fragments, there were also observed peaks of *m/e* 48 and 50 that correspond to the formation of the $(SO)^+$ ions.

The principal sulfur-containing products of the gamma radiolysis of degassed DMSO *(364)* are dimethyl sulfide and dimethyl sulfone. The formation of these products is dose dependent and the initial *G* values are 2.2 and 0.4, respectively. A third, minor radiolytic product was tentatively identified as methyl methanethiosulfonate. The principal radiolytic products are believed to arise in the following fashion:



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Chapter 2

Pharmacology of DMSO

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I. INTRODUCTION

The pharmacological actions of dimethyl sulfoxide (DMSO) have stimulated much research, and the purpose of this chapter is to

summarize current concepts in this area. Among the primary pharmacological actions of DMSO which have been demonstrated in laboratory models are the following: (1) membrane penetration; (2) membrane transport; (3) effects on connective tissue; (4) antiinflammation; (5) nerve blockade (analgesia); (6) bacteriostasis; (7) diuresis; (8) enhancement or reduction of effectiveness of other drugs; (9) cholinesterase inhibition; (10) nonspecific enhancement of resistance to infection; (11) vasodilation; (12) muscle relaxation; (13) antagonism to platelet aggregation; (14) influence on serum cholesterol in experimental hypercholesteremia; (15) radioprotective action (see Chapter 5).

II. PRIMARY PHARMACOLOGICAL ACTIONS

A. Membrane Penetration

DMSO readily crosses most tissue membranes of lower animals and man. It does not penetrate nails or the enamel of the tooth.

Employing DMSO-^{35}S, Kolb *et al. (1)* evaluated absorption and distribution of DMSO in lower animals and man. Ten minutes after cutaneous application in the rat, radioactivity was measured in the blood. In man radioactivity appeared in the blood 5 min after cutaneous application. Kolb further demonstrated distribution of DMSO, using whole-body radioautograms in rats following cutaneous application. One hour after application of DMSO to the skin, radioactivity could be detected in bones.

Denko *(2)* and his associates applied ^{35}S-labeled DMSO to the skin of rats. Within 2 hr a wide range of radioactivity was distributed in all organs studied. The highest values occurred in decreasing order in the following soft tissues: spleen, stomach, lung, vitreous humor, thymus, brain, kidney, sclera, colon, heart, skeletal muscle, skin, liver, aorta, adrenal, lens of eye, cartilage.

Rammler and Zaffaroni *(3)* have reviewed the chemical properties of DMSO and suggest that the rapid movement of this molecule through the skin, a protein barrier, depends on a reversible configurational change of the protein occurring when DMSO substitutes for water.

B. Membrane Transport

Nonionized molecules of low molecular weight are transported through the skin with DMSO. Substances of high molecular weight

such as insulin do not pass through the skin to any significant extent. Recent work in our laboratory has revealed that a 90% concentration of DMSO is optimal for the passage of morphine sulfate dissolved in DMSO *(4)*. It would have been expected that 100% would provide better transport than 90%, and the reason for an optimal effect at 90% DMSO remains unexplained. It is of course well known that 70% alcohol has a higher phenol coefficient than 100% alcohol.

Elfbaum and Laden *(5)* conducted an *in vitro* skin penetration study employing guinea pig skin as the membrane. They concluded that the passage of picrate ion through this membrane in the presence of DMSO was a passive diffusion process which adhered to Fick's first law of diffusion. It was demonstrated by diffusion and isotope studies that the absolute rate constant for the penetration of DMSO was approximately 100 times greater than that for the picrate ion. Thus, the two substances were transferred through the skin independently of each other. The exact mechanisms involved in the membrane penetrant action of DMSO have yet to be elucidated.

Studies on membrane penetration and carrier effect have been carried out in agriculture, basic biology, animals, and man. In field tests with severely diseased fruit, Keil *(6)* demonstrated that oxytetracycline satisfactorily controlled bacterial spot in peaches. Control was significantly enhanced by adding DMSO to the antibiotic spray. DMSO was applied at 0.25 and 0.5% with 66 ppm of oxytetracycline. This application gave control of the disease similar to that produced alone by 132 ppm of oxytetracycline and suggested the possibility of diluting the high-priced antibiotic with relatively inexpensive DMSO. There is no good evidence in animals that 0.5% DMSO has significant carrier effects. It could well be that Keil's results were attributable to a carrier effect, however, the possibility should always be considered that when DMSO is combined with another substance a new compound results which can then exert a greater or a lesser influence on a given process.

Leonard *(7)* studied different concentrations of several water-soluble iron sources applied as foliage sprays to orange and grapefruit trees whose leaves showed visible signs of iron deficiency. The application of iron in DMSO as a spray was followed by a rapid and extensive greening of the leaves, with a higher concentration of chlorophyll.

Amstey and Parkman *(8)* evaluated the influence of DMSO on the infectivity of viral nucleic acid. It was found that DMSO enhanced polio RNA infectivity in kidney cells from the monkey. Enhancement occurred with all DMSO concentrations from 5 to 80%, and was optimal at 40% DMSO, with a 20-min absorption period at room

temperature. A significant percentage of nucleic acid infection was absorbed within the first 2 min.

Cochran and his associates *(9)* recorded the effect of DMSO on tobacco mosaic virus (TMV), concluding that concentrations of DMSO below 20% did not influence the infectivity of this virus or the viral RNA. With concentrations between 20 and 60%, the infectivity of TMV and TMV RNA varied inversely with the DMSO concentration.

Nadel and co-workers *(10)* suggested that DMSO enhanced the penetration of the infectious agent in experimental leukemia of guinea pigs. Previously Schreck *et al. (11)* had demonstrated that DMSO was more toxic *in vitro* to lymphocytes from patients with lymphocytic leukemia than to lymphocytes from normal patients.

Djan and Gunberg *(12)* studied the percutaneous absorption of 17β-estradiol dissolved in DMSO in the immature female rat. These steroids were given in aqueous solutions subcutaneously or were applied topically in DMSO. Vaginal and uterine weight increases resulting from estrogen in DMSO administered topically were comparable to results obtained in animals in which the drugs were administered in pure form subcutaneously.

Smith and Hegre *(13)* showed that antibodies to bovine serum albumin developed when a mixture of DMSO and bovine serum albumin was applied to the skin of rabbits. Smith *(14)* has also reported that a mixture of DMSO and diphtheria toxoid applied frequently to the backs of rabbits causes a reduction of the inflammation produced by the Schick test, indicating that a partial immunity to diphtheria has been produced.

Finney and his associates *(15)* studied the influence of DMSO and DMSO–hydrogen peroxide on pig myocardium after acute coronary ligation with subsequent myocardial infarction. The authors ligated approximately 50% of the inferior descending branch of the left coronary artery and root of the right coronary artery. The addition of DMSO to a hydrogen peroxide perfusion system facilitated the diffusion of oxygen into the ischemic myocardium.

Maddock *et al. (16)* designed experiments to determine the usefulness of DMSO as a carrier for antitumor agents. The agents were dissolved in 85–100% concentrations of DMSO. One of the tumors studied was the L1210 leukemia. Survival time without treatment was approximately 8 days. The standard method of employing Cytoxan intraperitoneally produced a survival time of 15.5 days. When Cytoxan was

applied topically in water, the survival time was 12.6 days, and topical Cytoxan dissolved in DMSO resulted in a survival time of 15.3 days.

The possibility of altering the blood-brain diffusion barrier with DMSO needs additional exploration. Brink and Stein *(17)* employed pemoline-^{14}C dissolved in DMSO and injected intraperitoneally into rats. It was found in larger amounts in the brain than was a similar dose given in 0.3% tragacanth suspension. The authors postulated that DMSO resulted in a partial breakdown of the blood-brain diffusion barrier *in vivo.*

Maibach and Feldmann *(18)* studied the percutaneous penetration of hydrocortisone and testosterone in DMSO. Maximal excretion of both steroids occurred in man within 36 hr. The authors concluded that there was a threefold increase in dermal penetration by these steroids when they were dissolved in DMSO.

Sulzberger and his coworkers *(19)* evaluated the penetration of DMSO into human skin employing methylene blue, iodine, and iron dyes as visual tracers. Biopsies showed that the stratum corneum was completely stained with each tracer applied to the skin surface in DMSO. There was little or no staining below this layer. The authors concluded that DMSO carried substances rapidly and deeply into the horny layer and suggested the usefulness of DMSO as a vehicle for therapeutic agents in inflammatory dermatoses and superficial skin infections such as pyodermas.

Perlman and Wolfe *(20)* demonstrated that allergens of low molecular weight such as penicillin G potassium, mixed in 90% DMSO, were readily carried through intact human skin. Allergens having molecular weights of 3000 or more dissolved in DMSO did not penetrate human skin in these studies. Smith and Hegre *(13)* had previously recorded that antibodies to bovine serum albumin developed when a mixture of DMSO and bovine serum albumin was applied to the skin of rabbits.

Turco and Canada *(21)* have studied the influence of DMSO on lowering electrical skin resistance in man. In combination with 9% sodium chloride in distilled water, 40% DMSO decreased resistance by 100%. It was postulated that DMSO in combination with electrolytes reduced the electrical resistance of the skin by facilitating the absorption of these electrolytes while it was itself being absorbed.

DMSO in some instances will carry substances such as hydrocortisone or hexachlorophene into the deeper layers of the stratum corneum, producing a reservoir *(22)*. This reservoir remains for 16 days and

resists depletion by washing of the skin surface with soap, water, or alcohol *(23)*.

C. Effect on Collagen

Mayer and associates *(24)* compared the effects of DMSO, DMSO with cortisone acetate, cortisone acetate alone, and saline solutions on the incidence of adhesions following vigorous serosal abrasions of the terminal ileum of Wistar rats. Their technique had developed adhesions in 100% of control animals in 35 days. The treatments were administered daily as postoperative intraperitoneal injections for 35 days. The incidence of adhesions in different groups was: DMSO alone, 20%; DMSO–cortisone, 80%; cortisone alone, 100%; saline solution, 100%.

It has been observed in serial biopsy specimens taken from the skin of patients with scleroderma that there is a dissolution of collagen, the elastic fibers remaining intact *(25)*. Gries *et al. (26)* studied rabbit skin before and after 24-hr *in vitro* exposure to 100% DMSO. After immersion in DMSO the collagen fraction extractable with neutral salt solution was significantly decreased. The authors recorded that topical DMSO in man exerted a significant effect on the pathological deposition of collagen in human postirradiation subcutaneous fibrosis but did not appear to change the equilibrium of collagen metabolism in normal tissue. Urinary hydroxyproline levels are increased in scleroderma patients treated with topical DMSO *(25)*. Keloids biopsied in man before and after DMSO therapy show histological improvement toward normalcy *(27)*.

D. Antiinflammation

Berliner and Ruhmann *(28)* found that DMSO inhibited fibroblastic proliferation *in vitro*. Ashley *et al. (29)* reported that DMSO was ineffective in edema following thermal burns of the limbs of rabbits. Formanek and Kovac *(30)* showed that topically applied DMSO inhibited traumatic edema induced by intrapedal injection of autologous blood in the leg of a rat.

DMSO showed no antiinflammatory effect when studied in experimental inflammation induced in the rabbit eye by mustard oil and in the rat ear by croton oil *(31)*.

Gorog and Kovacs *(32)* demonstrated that DMSO exerted minimal

antiinflammation effects on edema induced by carrageenin. These authors also studied the antiinflammatory potential of DMSO in adjuvant-induced polyarthritis of rats. Topical DMSO showed potent antiinflammatory properties in this model. Gorog and Kovacs *(33)* have also studied the antiinflammatory activity of topical DMSO in contact dermatitis, allergic eczema, and calcification of the skin of the rat, using 70% DMSO to treat the experimental inflammation. All these reactions were significantly inhibited.

The study of Weismann *et al. (34)* deserves mention in discussing the antiinflammatory effects of DMSO. Lysosomes can be stabilized against a variety of injurious agents by cortisone, and the concentration of the agent necessary to stabilize lysosomes is reduced 10- to 1000-fold by DMSO. The possibility was suggested that DMSO might render steroids more available to their targets within tissues (membranes of cells or their organelles).

Suckert *(35)* has demonstrated antiinflammatory effects with intraarticular DMSO in rabbits following the creation of experimental (croton oil) arthritis.

E. Nerve Blockade (Analgesia)

Immersion of the sciatic nerve in 6% DMSO decreases the conduction velocity by 40%. This effect is totally reversed by washing the nerve in a buffer for 1 hr *(36)*. Shealy *(37)* studied peripheral small fiber after-discharge in the cat. Concentrations of 5–10% DMSO eliminated the activity of C fibers within 1 min; activity of the fibers returned after the DMSO was washed away.

DMSO injected subcutaneously in 10% concentration into cats produced a total loss of the central pain response. Two ml of 50% DMSO injected into the cerebrospinal fluid lead to total anesthesia of the animal for 30 min. Complete recovery of the animal occurred without apparent ill effects *(38)*.

F. Bacteriostasis

DMSO exerts a marked inhibitory effect on a wide range of bacteria and fungi, including at least one parasite, at concentrations (30–50%) likely to be encountered in antimicrobial testing programs in industry *(39)*.

DMSO at 80% concentration inactivated viruses tested by Chan and Gadenbusch. These viruses included four RNA viruses, influenza A virus, influenza A-2 virus, Newcastle disease virus, Semliki Forest virus, and DNA viruses *(40)*.

Seibert and co-workers *(41)* studied the highly pleomorphic bacteria regularly isolated from human tumors and leukemic blood. DMSO in 12.5–25% concentration caused complete inhibition of growth *in vitro* of 27 such organisms without affecting the intact blood cells.

Among the intriguing possibilities for the use of DMSO is its ability to alter bacterial resistance. Pottz and associates *(42)* presented evidence that the tubercle bacillus, resistant to 2000 μg of streptomycin or isoniazide, became sensitive to 10 μg of either drug after pretreatment with 0.5–5% DMSO.

Kamiya *et al. (43)* evaluated the effect of 5% DMSO on the increased sensitivity of bacteria to antibiotics. In this study a difference of more than 2 ml of growth-inhibitory circle between DMSO and the control medium was regarded as an increase in sensitivity. DMSO restored and increased the sensitivity of antibiotic-resistant strains. Restoration of sensitivity was found in some strains of *Pseudomonas* and in *Escherichia coli.* In particular, the sensitivity of *Pseudomonas* to colistin was restored when the medium contained 5% DMSO. All four strains of colistin-resistant *Pseudomonas* then became sensitive. The authors recorded that antibiotics not effective against certain bacteria, such as penicillin to *E. coli,* showed growth-inhibitory effects when the medium contained DMSO.

Ghajar and Harmon *(44)* studied the influence of DMSO on the permeability of *Staphylococcus aureus,* demonstrating that DMSO increased the oxygen uptake but reduced the rate of glycine transport. They could not define the exact mechanism by which DMSO produced its bacteriostatic effect.

Gillchriest and Nelson *(45)* have suggested that bacteriostasis from DMSO occurs due to a loss of RNA conformational structure required for protein synthesis.

G. Diuresis

Formanek and Suckert *(46)* studied the diuretic effects of DMSO administered topically to rats five times daily in a dosage of 0.5 ml of 90% DMSO per animal. The urine volume was increased 10-fold, and

with the increase in urine volume, there was an increase in sodium and potassium excretion.

H. Enhancement or Reduction of Concomitant Drug Action

Rosen and associates *(47)* employed aqueous DMSO to alter the LD_{50} in rats and mice when oral quaternary ammonium salts were used as test compounds. In rats, the toxicity of pentolineum tartrate and hexamethonium bitartrate was increased by DMSO, while the toxicity of hexamethonium iodide was decreased.

Male *(48)* has shown that DMSO concentrations of upward to 10% lead to a decided increase in the effectiveness of griseofulvin.

Melville and co-workers *(49)* have studied the potentiating action of DMSO on cardioactive glycosides in cats, including the fact that DMSO potentiates the action of digitoxin. This effect, however, does not appear to involve any change in the rate of uptake (influx) or the rate of loss (efflux) of glycosides in the heart.

I. Cholinesterase Inhibition

Sams *et al. (50)* studied the effects of DMSO on skeletal, smooth, and cardiac muscle, employing concentrations of 0.6–6%. DMSO strikingly depressed the response of the diaphragm to both direct (muscle) and indirect (nerve) electrical stimulation, and caused spontaneous skeletal muscle fasciculations. DMSO increased the response of the smooth muscle of the stomach to both muscle and nerve stimulation. The vagal threshold was lowered 50% by 6% DMSO. Cholinesterase inhibition could reasonably explain fasciculation of skeletal muscle, increased tone of smooth muscle, and the lower vagal threshold observed in these experiments. *In vitro* assays show that 0.8–8% DMSO inhibits bovine erythrocyte cholinesterase 16–18%.

J. Nonspecific Enhancement of Resistance

In a study on antigen–antibody reactions, Raettig *(51)* showed that DMSO did not disturb the immune response. In fact, the oral administration of DMSO to mice for 10 days prior to an oral infection with murine typhus produced a leukocytosis and an enhanced resistance to the bacterial infection.

K. Vasodilation

Adamson and his co-workers *(52)* applied DMSO to a 3-to-1 pedicle flap raised on the back of rats. The anticipated slough was decreased by 70%. The authors suggested that the primary action of DMSO on pedicle flap circulation was to provoke a histamine-like response. Roth *(53)* has also evaluated the effects of DMSO on pedicle flap blood flow and survival, concluding that DMSO does indeed increase pedicle flap survival, but postulating that this increase takes place by some mechanism other than augmentation of perfusion. Kligman *(54,55)* had previously demonstrated that DMSO possesses potent histamine-liberating properties.

Leon *(56)* has studied the influence of DMSO on experimental myocardial necrosis, evaluating 240 rats given isoproterenol subcutaneously on 2 consecutive days. The 177 survivors were divided into three groups: (1) no treatment; (2) DMSO, 0.5 ml subcutaneously daily; (3) water, 0.5 ml subcutaneously daily. Myocardial lesions were present on gross and histological examination in all animals. DMSO therapy effected a distinct modification with less myocardial fiber necrosis and reduced residual myocardial fibrosis. The author reported that neither myocardial rupture nor aneurysm occurred in the group treated with DMSO.

L. Muscle Relaxation

DMSO applied topically to the skin of patients produces electromyographic evidence of muscle relaxation 1 hr after application *(57)*.

M. Antagonism to Platelet Aggregation

Deutsch *(58)* has presented experimental data showing that 5% DMSO lessens the adhesiveness of blood platelets *in vitro*. Gorog *(59)* has shown that DMSO is a good antagonist to platelet aggregation as well as thrombus formation *in vivo*. Gorog evaluated this in the hamster cheek pouch model.

N. Influence on Serum Cholesterol in Experimental Hypercholesteremia

Rabbits given a high cholesterol diet with 1% DMSO showed one-half as much hypercholesteremia as control *(60)* animals.

O. Radioprotective Action

(See Chapter 5).

III. SUMMARY

A wide range of primary pharmacological actions of DMSO has been documented in laboratory studies: membrane penetration, membrane transport, effects on connective tissue, antiinflammation, nerve blockade (analgesia), bacteriostasis, diuresis, enhancement or reduction of the effectiveness of other drugs, cholinesterase inhibition, nonspecific enhancement of resistance to infection, vasodilation, muscle relaxation, antagonism to platelet aggregation, and influence on serum cholesterol in experimental hypercholesteremia. DMSO also has prophylactic radioprotective properties.

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Chapter 3

Toxicology of DMSO in Animals

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I. INTRODUCTION

Dimethyl sulfoxide (DMSO) is a compound with remarkable solvent properties and relatively low toxicity. Since all chemical compounds have toxicity to some degree, it is appropriate that the basis for the estimation of toxicity be briefly reviewed.

Toxicology is the science of determining the lethal and morbid effects of drugs. To explain the lethal or morbid effects, the histopathological effects as well as systems derangements are investigated. These studies can be further pursued to see whether the changes can reverse themselves through accommodation, through cessation of medication, or through the use of some ameliorating factor. The initial efforts in toxicological investigations involve determination of (1) the amount of a drug that will cause lethality in 50% of the treated subjects, (2) which organs are primarily affected, and (3) the levels of medication that the organism can tolerate.

To establish the tolerance limits or levels for DMSO alone, or in combination with other substances, the multitude of variables that may affect the use of DMSO must be considered carefully. To emphasize the conditions that influence the tolerance levels for DMSO, the following factors are listed: (1) Total amount of DMSO used; (2) time for administration (single, repeated, rapid or slow); (3) route (dermal, oral, intravenous, intramuscular, subcutaneous, and so forth); (4) concentration; (5) temperature (of drug and of recipient); (6) solubility or rate of release; (7) physical form (liquid, ointment, tablet, capsule, and so forth); (8) total volume administered; (9) excretion interference; (10) species differences; (11) age differences; (12) sex differences; (13) genetic enzymic differences; (14) induced enzymic differences; (15) tolerated variations attributable to altered physiological or pathological conditions; (16) tolerance variations resulting from hypersensitivity or allergies.

In this discussion only the administration of DMSO as a pure compound will be considered. It must be repeatedly stated that DMSO is of such low toxicity that grams per kilogram are commonly used rather than milligrams per kilogram as in the case of most other drugs. It therefore becomes important that the rate of administration and the concentration of this compound be carefully considered in each reported value. We have seen a dog succumb to a 5-ml rapid intravenous injection of pure DMSO, but could give a similar animal 20 times the dose merely by infusing the DMSO over a 4-hr period. This point might be emphasized by noting that the demise of a dog can be caused by the intravenous administration of physiological saline given too rapidly and in too great a quantity.

II. MEASUREMENT OF DMSO TOXICITY

The toxicity of DMSO will be considered in four categories: (1) lethality, (2) general morbidity, (3) cutaneous toxicity, and (4) maximum tolerated dose.

A. Lethal Doses of DMSO

From Table 1 it can be seen that if species differences exist, they are less than a one-half log dose. We have found that cats are not usually

TABLE 1

Single dose LD_{50}[a]

Species	Route of administration				
	Intravenous	Intraperitoneal	Subcutaneous	Oral	Dermal
Mouse	3.8-8.9	14.6-20.6	13.9-25.6	16.5-24.6	44
Rat	5.2-8.1	5.5-13.6	13.7	17.4-28.3	40-50
Rabbit	6-8	–	>14.	>14.	>MDA[b]
Dog	2.5-8	–	–	>10	>MDA
Monkey	4-8	–	–	>4	>MDA

[a]In grams per kilogram.
[b]MDA, Maximum dose applicable.

susceptible as has been reported elsewhere *(1,2)*. A further refinement in the assessment of comparative toxicity would be to compute the toxicity as a function of body surface rather than on the basis of weight. There are certainly species differences as to modes and speeds of metabolism and excretion, but they have not radically altered the overall view of low toxicity by all routes.

In recent work by Benville and co-workers *(3)*, the intraperitoneal toxicity of DMSO in salmon and trout was carefully determined, and the LD_{50} was found to range from 12 to 17 g/kg. This is in conformity with that of mammalian toxicity. When the fish were totally immersed in DMSO, different percentages of the compound were tolerated for the amounts of time indicated in the accompanying tabulation.

DMSO, %	Exposure time, hr
7.2	24
5.5	48
4.9	72
4.6	96

Exposures to 2% DMSO solution for 100 days resulted in no toxicity, with good appetite and normal weight gains. When the concentration approached 4%, however, lesions became evident in the gills.

The ability of DMSO to be absorbed and spread through the body rapidly when taken orally or injected is so great that, as far as can be

determined, there is no increased toxicity with repeated daily dosing. It is assumed that the repeated daily dose will not exceed the single maximum tolerated dose. The one factor modifying this statement is the concentration of DMSO used. Repeated intravenous administration of concentrations greater than 50% resulted in injury to the internal tissue of injected vessels, and fibrosis developed in direct proportion to the concentration and the number of injections. The result of continued insult was a narrowing of the lumen to the point at which reentry was impractical. Along with the reaction of the intima, evidences of hemoglobinuria and bilirubinuria were observed as a result of direct damage to some erythrocytes (RBC) in which rupture of the cell membrane and release of hemoglobin occurred. These changes reach significant levels only at or above the single maximum tolerated dose. None of the findings can be directly related to lethality and more properly belong in the next category.

B. General Morbidity

1. Single-Dose Observations

Regardless of how high the dose given and regardless of the route, if the animal survived for 24 hr all signs and symptoms of toxicity eventually disappeared. The most transitory effects were those on the central nervous system. Reaction of the liver and kidney to the stress of hemolyzed blood required several days for rectification. Gastrointestinal tract injury was overcome rapidly by replacement of damaged cells. The more lasting lesions were local tissue reactions in blood vessels or in tissues into which undiluted DMSO had been injected. Here the damaged tissue responded with a typical inflammatory pattern, but in 1 week the plasma cells, mast cells, and macrophages disappeared, being replaced by small indurations of fibrous tissue. In time even these disappeared, leaving negligible traces of tissue damage.

2. Repeated Sublethal Doses

Neither DMSO nor its metabolites accumulate in the tissues, and there is no typical delayed toxicity. Any pathology observed is the result of many small local injuries or the sum of the insult of hemolyzed blood. These changes are found only at doses very close to the LD_{50}, and there only if the concentrations are above 50% DMSO.

However, it is the duty of the toxicologist to point out the histological and biochemical changes that accompany the morbidity seen when repeated excessive doses of DMSO are given.

3. Gross and Histopathological Changes Caused by DMSO

a. Thickening and Occlusion of Injected Vessels. These changes are directly proportional to the concentration of DMSO and the number of repeated injections. The initial damage is very rapid and usually occurs when the concentration of DMSO is above 80%. Whenever DMSO is mixed with an aqueous system, some heat of reaction is evolved; the higher the concentration, the more rapid and intense the reaction *(4)*. Undiluted DMSO is the most common offender. Since a large part of the usefulness of DMSO rests on its activity as a solvent, undiluted DMSO is often used. Its potential as a good solvent decreases rapidly as it is used in a more dilute form. Intravenous irritation is therefore a minor but troublesome side effect. Willson *et al. (5)* described perivascular inflammatory reactions and intravascular thrombi. The thrombi are organized and canalized and there are numerous round cells, macrophages, and plasma cells in their vicinity. It is surprising, however, that no necrosis or sloughing takes place and that the original irritation or thrombus disappears in time. Johnson *et al. (6)* have shown that intraarterial injection of *100% DMSO* produces large, rigid masses of agglutinated red blood cells and marked injury to endothelium.

b. Local Tissue Reaction to Subcutaneous or Intramuscular Injections of DMSO. Rosenkrantz *et al. (4),* Sommers and Tauberger *(2),* and Smith *et al. (7)* all reported a hemorrhagic, gelatinous, edematous reaction resulting from the injection of DMSO. The intensity of the hemorrhage and inflammatory reaction was proportional to the concentration and total amount of DMSO injected. It is to be noted, however, that no instances of abscess formation, necrosis, or sloughing were reported. Walters *et al. (8)* injected 0.2 ml of 33% DMSO directly into the cremaster muscle of the rat. Light and electron microscope studies revealed alterations of the postcapillary venules with adherence of platelets to the affected intima, opening of the intercellular junctions, and progressive alteration of the endothelium very soon after the injection. These changes take place prior to the inflammatory response. The basement membrane remains intact, and particulate matter is not allowed passage.

Willoughby *et al. (9)* investigated the action of undiluted DMSO when 0.1 ml was injected intradermally. They observed intense local vasoconstriction followed by hemorrhage and necrosis. DMSO diluted to 10% produced a significant leukocyte emigration within 45 min which lasted for more than 24 hr.

They also injected 25% DMSO into the rat pleura, causing a rapid formation of exudate within 15 min which lasted for 3 hr; thereafter it fell rapidly and disappeared within 12 hr. By the use of colloidal carbon injected intravenously, it was determined that the carbon leakage had taken place in the venules and corresponded in timing to the release of exudate into the pleural cavity. The early fluid is a transudate, being low in protein, while the later exudate has a protein concentration as high as 5 g/100 ml.

c. Hepatotoxicity. Most liver damage reports involving DMSO concern cases in which the animal has succumbed because of very high doses of DMSO. Caujolle *et al. (10)* gave doses as high as 5 g/kg of 50% DMSO for 45 days and only caused slight weight loss. There were evidences of degeneration in the hepatocytes and inflammation in the postat spaces. The degree of toxicity was not indicated. Brown *et al. (11)* state that after large repeated interperitoneal doses of DMSO a rounding of the liver margin occurred, and strands of fibrin formed within the peritoneum.

Rosenkrantz *et al. (4)* noted two types of liver changes. The first was related to slight centrolobular fatty changes which occurred after 33 days of intravenous injection of sublethal doses in dogs. The second was observed in rats in which massive subcutaneous doses of DMSO were used. In these cases the DMSO and the edema followed the tissue planes, migrating down the back and around and through the abdominal muscles to cause a fibrinous percipitate between the peritoneum and the liver.

Wood *et al. (12)* treated rabbits with dermal and oral DMSO at 10 g/kg/day for 12 weeks and found no gross or microscopic histopathology of the liver.

Shilkin *et al. (13)* administered 0.4 ml of DMSO (100%) intravenously to rats (about 2.0 g/kg) and examined liver biopsies in the electron microscope 12 min later. He found the hepatic sinusoids to be filled with hemolyzed RBC and cytoplasmic fragmentation of the Kupfer cells, some of which were vacuolated. The hepatocytes had prominent organules, poor cytoplasmic projections, enlarged mitochondria, and free ribosomes. He suggested that the DMSO had acted on the cell surfaces to alter their sensitivity.

Willson *et al. (5)* noted cloudy swelling and granularity of the parenchymal cytoplasm in dogs. They report that this was present at all dose levels. In view of the absence of dose response and the minimal damage reported, however, this observation is perhaps an insignificant finding.

It is significant that accumulation of hemosiderin granules has been observed by several workers in the tissue spaces of the liver, spleen, and kidney. This accumulation probably occurs most frequently when concentrated DMSO is administered intravenously and there is a resultant hemolysis.

d. Kidney. The changes seen in the kidney usually involve a mild tubular nephrosis and are ordinarily related to the breakdown products of hemolysis incidental to high concentrations of intravenous DMSO. This observation is supported by the finding of iron-positive pigment granules in the tubular epithelium, and Cajoulle has noted this mild nephrosis.

e. Lung. Changes in the lung may occur either after a single slowly administered lethal dose or after repeated increasing doses of DMSO. There is usually pulmonary edema attributable to the seepage of fluid into the tissue spaces of the alveolar wall, probably as a result of the decreased heart rate, decreased blood pressure, vascular distention, and stasis of blood. Pulmonary edema resulting from DMSO developed only after the administration of very large doses of DMSO given at a critical rate designed to bring about death very slowly.

4. Teratogenic Effects

Caujolle *et al. (10)* reported teratogenic effects of DMSO in rabbits, chickens, mice, and rats administered very high dosages. Eighty-three rabbit embryos were obtained from 10 DMSO-treated rabbits. The only abnormality was a single fetus showing celosomia. The mean number of embryos was remarkedly reduced in the oral DMSO-treated rabbits.

This was not the case in chickens, in which the survivors of an LD_{50} trial showed a greater number of malformations. At the 96-hr stage there were 436 surviving embryos and 113, or 25.9%, were deformed.

Malformations of the limbs	21.8%
Malformations of the beak	4.6%
Malformations of the eye	3.9%
Anourous embryos	3.7%
Celosomia	5.5%

The time at which the embryo is subjected to DMSO treatment often determines the type of deformity. Treatment at an earlier stage (72 hr) changed the ratio of malformation, i.e., 16.9% anophthalmia and 11.4% malformations of the beak were observed. The dose of DMSO used to cause death of some chick embryos and the malformation of many of the survivors varied from 1.0 mg per embryo at the 72-hr stage and 2.5 mg at the 96-hr stage.

To mice, rats, and rabbits Cajoulle gave doses of 5–12 g/kg orally or intraperitoneally, and in fact killed a considerable number of the dams and embryos. Observations of teratogenicity were made on the survivors. In the mouse trials, 7 out of 100 fetuses were malformed.

Montuori and Adler *(14)* injected DMSO directly into chick embryos and yolk sacs. They observed no increase in teratogenic effects in the yolk sac group, but the embryos showed high teratogenic activity and a high mortality rate. The authors conclude that teratogenesis of DMSO in the chicken embryo depends upon the route of administration. They also state that injection directly into an embryo might not be of great significance in as much as many substances that are relatively chemically inert are teratogenic when introduced as foreign bodies, e.g., talc, animal carbon, pieces of skull, and so forth.

Ferm's *(15)* observations on the teratogenicity of DMSO are interesting in that he was able to inject up to 1.5 ml intraperitoneally in pregnant golden hamsters (about 10–15 g/kg). This dose of 100% DMSO is the maximum tolerated dose for mice and rats. No deaths occurred in the dams but there was a mortality of 28% among the embryos at the highest dosage. In the surviving embryos there was 100% gross abnormality with various degrees of exencephaly and anencephaly. There seemed to be dose response in that doses down to 2.5 g/kg caused decreasing abnormalities as indicated in the accompanying tabulation.

Approximate DMSO dose, g/kg	Gross abnormal embryos, %
7-15	100
7-10	95
10	25
2.5-5	26
2.5-5	8
2.5	0

This system seems exquisitely sensitive in detecting embryo toxicity and teratogenic potential. The use of sublethal doses of 100% DMSO intraperitoneally is so artificial a system that correlations as to its danger in clinical use is to be avoided.

5. Biochemical Changes Caused by DMSO in Animals

Rosenkrantz *et al. (4),* Brown *et al. (11),* Cajoulle *et al. (10),* and Willson *et al. (5)* all concur that animals dying from the toxic effects of DMSO show no characteristic lesions. The question is repeatedly presented "What kills animals given lethal doses of DMSO?" The answer is not entirely clear, but the damage done is certainly on a generalized molecular basis and is not confined to a single system or organ. In support of this contention is the work of Panuska *et al. (16)* in which they state that "DMSO had no effect on the heart rate during the cooling process and also did not change the body temperature at which cardiac arrest occurred." These hypothermia experiments in rats are questioned by the isolated tissue work done by Sams *et al. (17)* in which they note that the observed fasciculation of the skeletal muscle, the increased tone of the stomach muscle, and the lowering of the vagal threshold by DMSO could all be attributable to cholinesterase inhibition.

Weiss and Orzel *(18)* investigated DMSO as a pesticide solvent and gave it to rats orally and intraperitoneally. They showed that it had no effect on brain cholinesterase. However, they felt that the depressant effect of DMSO was not on the central nervous system and did not increase hexobarbital sleeping time. This supports the contention of Smith *et al. (19)* that DMSO has little toxic effect on the central nervous system when administered slowly via the cisternal route.

A number of changes have been seen by our clinical laboratory staff in animals heavily and repeatedly dosed with DMSO. Most of them relate to hemolysis and include: hemoglobinuria, decreased hemoglobin in the circulatory blood, lowered hematocrit value, bilirubinuria, and anemia. They are all related to direct hemolysis when high concentrations and large amounts of DMSO are given intravenously, or when doses of oral DMSO are so large and toxic as to cause gastroenteritis and hemorrhage from the bowels. These changes are well documented in the works of Smith *et al. (7),* Brown *et al. (11),* Willson *et al. (5),* and Cajoulle *et al. (10).* As a result of the red blood cell damage, reticulocytosis and increased erythoid activity in the spleen and bone marrow are often found.

Altland and co-workers *(20–21)* and Willson *et al. (5)* have shown that animals treated with DMSO show slight serum transaminase increases while lactic dehydrogenase may be slightly decreased. None of the changes cited are dramatic or characteristic enough to be used in describing the action of DMSO. There is far more evidence for the preservative action of DMSO on enzyme systems than there is of its inhibitory powers. A case in point is the study by Weissman *et al. (22)* in which DMSO enhanced the stabilizing effect of cortisone on the lysosomes of rabbit liver.

6. Ocular Changes

Of all the changes related to the toxicity of DMSO, changes in the lenses of dogs, rabbits, and swine were held to be of the greatest importance. It is, therefore, essential that an effort be made to review and summarize the findings of many workers [Rubin and Barnett *(23),* Wood *et al. (12),* Kleberger *(24),* Smith *et al. (19)*].

It is of the utmost importance to point out that changes in lenses were consistently seen only when high doses were repeatedly used over an extended period. The routes used to elicit changes in lenses were oral and dermal. It is also noteworthy that in none of the reports on eye changes was there mention of alteration in the visual acuity of the subject. Dogs never showed difficulty in running, jumping, or avoiding obstacles, despite a marked change in lens refraction.

Rubin and Barnett *(23)* reported on the results of their trials using DMSO orally and dermally in dogs, rabbits, and swine. The eye changes were as follows:

(1) Refractoriness to the normal mydriatic action of 0.5% tropicamide. This change was unequivocal at doses of 5 and 10 g/kg/day and was noted after 4 weeks of treatment in dogs.

(2) Relucency of the cental and peripheral lens zone changed so that there was a sharp demarcation as the central zone became more myopic. A lens of −20 diopters was needed to correct this difference. Sometimes a second band formed at this junction. The lens change is best seen with the slit-lamp microscope, and once the condition is well established it can be seen by retroillumination. These changes occurred in dogs after 9 weeks of treatment using doses of 5 g/kg/day or more. Slight changes were noted after 13 and 18 weeks at doses of 2.5 g/kg/day given orally. Once established, the changes persist for a long time.

To date, no reports of complete disappearance have been seen, however, regression of lens change has been reported in swine *(25)*.

The lens changes in rabbits occurred when 100% DMSO was applied to the skin for 90 days at a dose level of 4 ml/kg/day. In another trial using 90% DMSO, 6 out of 10 rabbits developed lens changes after 105 days when 9 ml/kg/day was given. At a lower level of 3 ml/kg/day, it took 140 days to see changes in 2 out of 12 rabbits.

Lens changes in swine were reported to be similar but less pronounced than those in the dog and the rabbit. These subtle changes were found after 90 days of treatment during which 90% DMSO was applied to the skin twice daily at a level of 9 ml/kg/day.

Regardless of the species, the lowest dose that would elicit lens changes was more than 1 ml/kg/day, and the shortest time was 68 days. Dogs and rabbits seem to be the most sensitive test animals, and the most effective route was oral.

(3) Equatorial opacities in lenses were reported by Rubin and Barnett *(23)* who found that after 120 days of treatment, 7 out of 10 dogs underwent these changes when given DMSO in doses of 4.5 g/kg/day. On day 132 (12 days cessation of treatment) 5 out of the 7 had no visible opacity; the other 2 dogs had either shown regression, or only one eye was affected.

Wood *et al. (12)* made an intensive study of lens changes in the rabbit eye after DMSO was given orally and topically in doses of 10 g/kg/day. His report is well illustrated and shows the concentric reflex bands that developed in the lenses of treated animals (Fig. 1). Myopia developed with further treatment. Changes in the lenses were observed as early as 2–3 weeks, and the nuclei of the lenses were so dense that detailed retinal observation became difficult. No opacities developed from the treatment and the lenses were clear at autopsy. Weight gains were less when DMSO was given orally. Since DMSO is readily absorbed through the skin, and weight gains increased when equivalent doses were administered topically, it may be inferred that this level of DMSO (10 g/kg/day) interfered with digestion to some degree. Wood and Wirth *(26)* found the DMSO effect in rabbit lenses to be dose dependent. The fact that large oral dosing brings about the most rapid development changes in the lenses argues strongly for the involvement of some metabolic disturbance. Wood feels that changes in refractive index and the appearance of lines of discontinuity in the cortex might

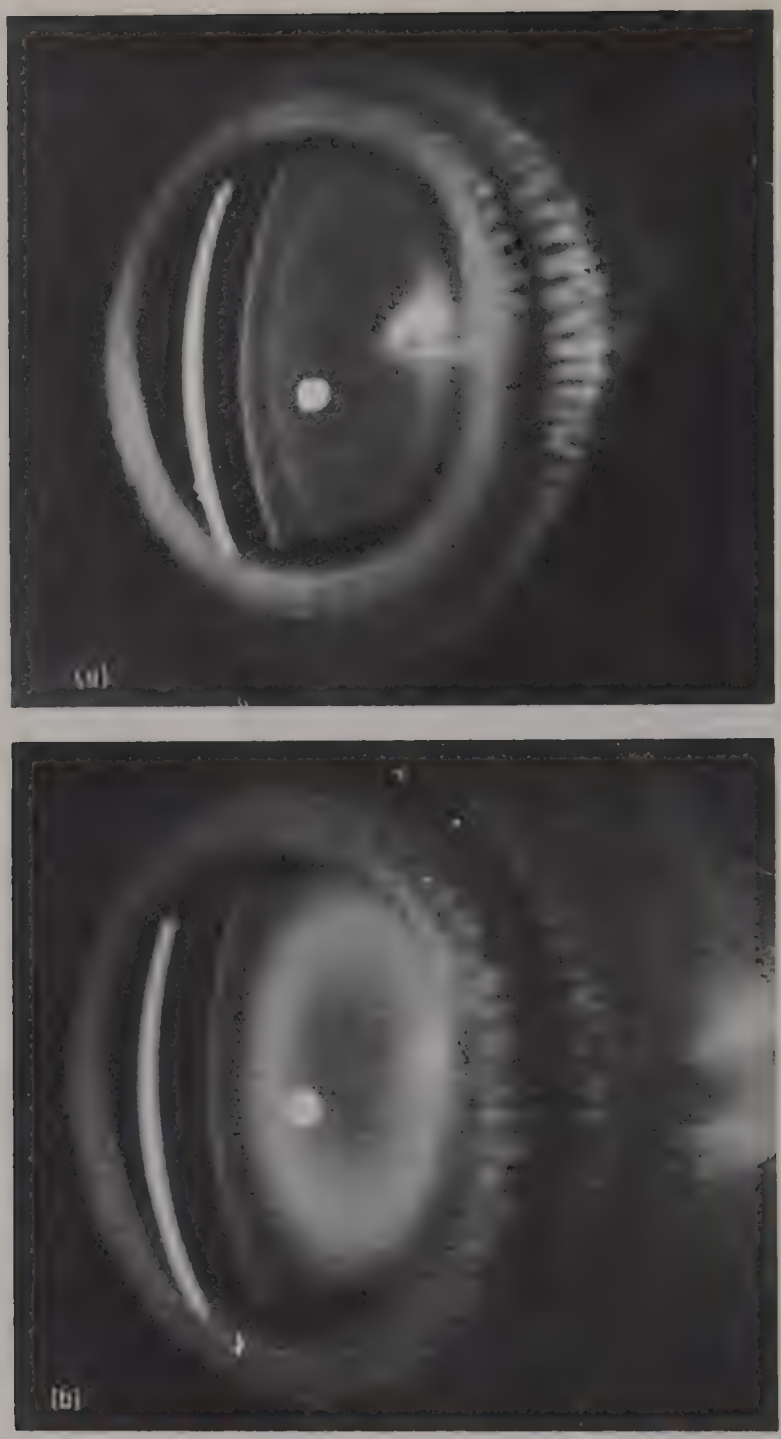
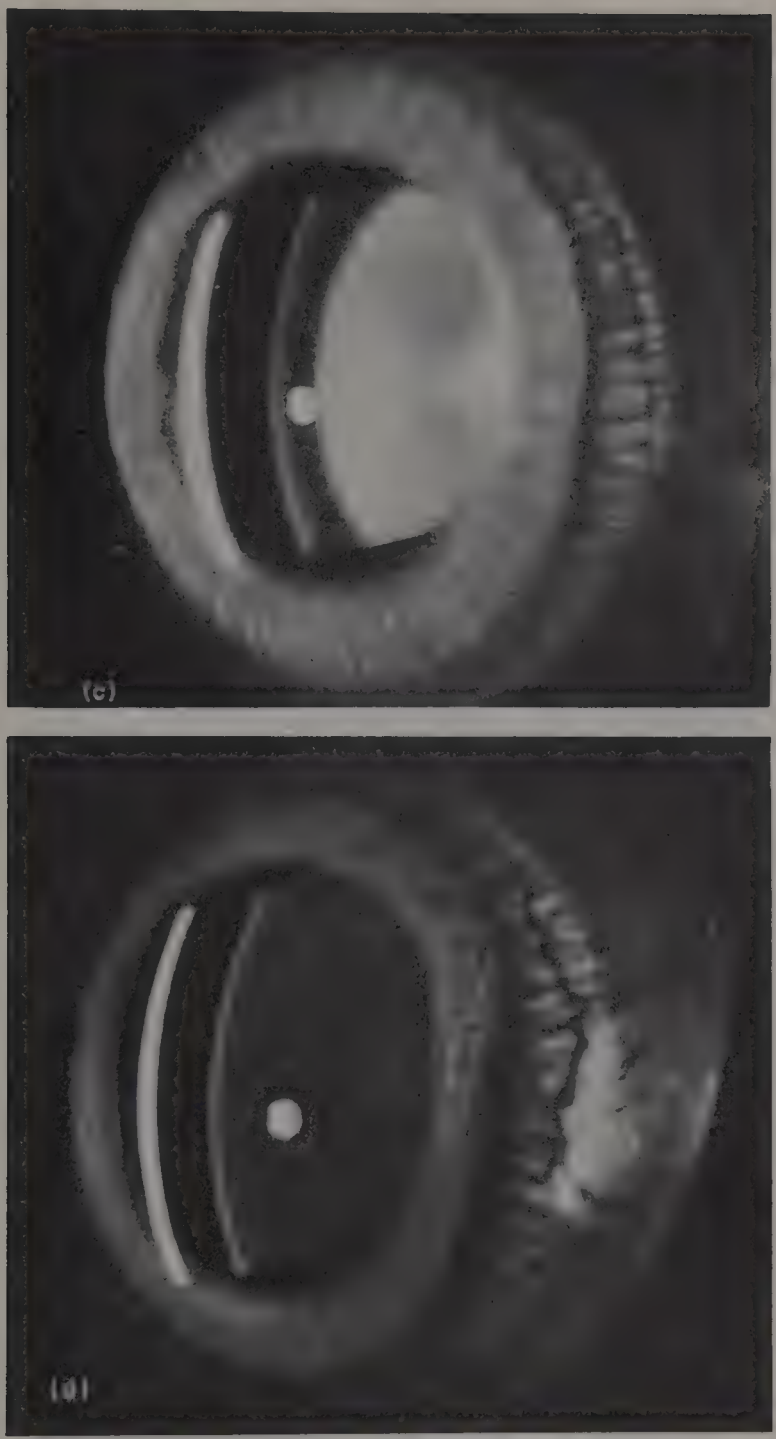


Fig. 1. Biomicroscopic view of lenses from rabbits that had consumed an equivalent of 10 g of 100% DMSO/kg/day for (a) 2 weeks, (b) 12 weeks, and (c) 26 weeks, as compared with (d) untreated animals.


(c)
(d)

be explained by the loss of soluble lens protein with a corresponding increase in the insoluble components.

Kleberger *(24)* treated beagle dogs with 50% DMSO in doses of 9 ml/kg/day, checked their eyes with a slit lamp microscope and direct and indirect ophthalmoscopy, and determined the refraction of the lenses.

Dioptric changes in the direction of myopia were detected as early as 3 days; all animals showed these changes at 7 days. After 14 weeks of treatment there was a difference of 15–20 diopter between the center and periphery of the lens. No opacities developed, but there was a slight opalescence in the nucleus of the lens. Although conjectures are made as to hypothetical mechanisms, no simple explanation for the lens change has been forthcoming.

Smith *et al. (19)* reported on DMSO given intravenously, orally, and dermally to dogs. The highest intravenous dose was 4.8 g/kg/day for 28 days. There were no opacities, no histological changes, and no alteration in visual acuity. However, in these early studies a slit lamp microscope was not utilized. In other studies DMSO was given as a 50% oral solution to a total dose of 10 g/kg/day. After 28 days there was distinct myopia to −2.25 diopters, and at 110 days the myopia had developed to −8.0 diopters; nuclear haze developed at 50–60 days.

Dogs were treated dermally with doses as high as 11 g/kg/day. A progressive and marked myopia developed in 8 weeks and a nuclear haze after 10 weeks. Every instance of nuclear haze or cortical banding was preceded by marked myopic changes by at least 1 month. It is still unknown whether or not the visible lens changes would have occurred if DMSO treatment had been suspended after myopia had developed.

Later another study was carried out with 24 monkeys at the Mason Research Institute in Worcester, Massachusetts. The range of dermally applied DMSO (90%) was 1.1–11 g/kg/day. Eight monkeys ranging from 2 to 4.5 years were given the highest dosage. During the 168–175 days of treatment, no refractive change greater than 1 diopter was observed. The average change seen for the eight monkeys was 0.66 diopters. None showed any myopia and only one showed a trace of cortical banding. These findings were repeatedly and carefully checked by slit lamp microscopy.

While this chapter deals exclusively with animal toxicology, the significance of the lens alterations becomes extremely important when the use of DMSO in humans is considered. For this reason one carefully designed human study should be referred to here. Hull *et al.*

(27) recently reported ophthalmological findings obtained in a study of human volunteers treated dermally with 1 g/kg DMSO daily for 12 weeks. Thirty-eight treated and 18 control patients were evaluated for accommodation, near point of convergence, ocular tension, refraction, visual acuity, biomicroscopy, ophthalmoscopy, pupillary mydriasis, and peripheral and central visual field examinations. Treatment failed to show any changes in the parameters described above. Patients were also examined at varying periods up to 1 year after treatment was discontinued. The follow-up examinations likewise revealed no significant ocular changes.

The sharp demarcation between the lens changes seen in dogs, rabbits, and swine and the absence of these changes in monkeys and man leaves the research door wide open for numerous investigations using various approaches.

C. Cutaneous Toxicity of DMSO

The dermal route of administration of DMSO is the least toxic (see Table 1). McDermott *et al. (28)* have calculated that more than 90% of the DMSO applied to the clipped ventral surface of a restrained rat was absorbed. This level of absorption was achieved between 45 and 60 min. Stoughton and Fritsch *(29)* comment that they are unaware of any agent other than water that influences percutaneous absorption to the extent that DMSO does without causing any obvious or prolonged damage to the skin other than transient erythema.

It is not within the scope of this chapter to discuss the mechanism of skin penetration or how it is influenced by the combination of DMSO with other drugs. There is no doubt that DMSO passes through the skin fairly rapidly and causes vasodilation and erythema in proportion to its concentration *(30)*. Reports on changes in guinea pig skin by Montes *et al. (31)* and those in human skin by Wahlberg and Skog *(32)* are excellent in describing the early and transient changes seen. It must be stated that despite intense and repeated treatment of the skin of animals with undiluted DMSO there is an absence of pathology. Indeed, the evidence points to an abatement of all but a slight furfuraceous desquamation.

In support of these observations is the experimental work of Brown and associates *(11)* in which undiluted DMSO applied to the clipped heads of guinea pigs for 28 days produced no gross or microscopic evidences of injury.

At our laboratory we have done extensive trials using mice, rats, dogs, and monkeys. We have found that dipping rats into 100% DMSO produced death in almost every instance, whereas dipping them into 80% DMSO did not. By weighing rats before and after dipping, the difference being the adherent DMSO, it was possible to estimate the LD_{50} by this route to be about 40 g/kg (Table 2).

TABLE 2

Cutaneous Toxicity of DMSO in Rats

Concentration of DMSO, %	No. of rats	Mean body weight				Maximum dose of DMSO, g/kg	24-hr Mortality
		Before, g	After, g	Change, g	Change, g/kg		
100	4	136	141	+5	+37	37	4/4
	5 M	169	178	9	53	53	5/5
	5 F	167	174	7	42	42	4/5
80	4	171	178	7	41	33	0/4
60	4	224	229	5	22	13	0/4
40	4	171	172	1	6	2	0/4
20	4	151	151	0	0	0	0/4

A similar determination has also been made in mice. Female mice were dipped into 100, 80, 60, and 40% DMSO in distilled water, being weighed before and after dipping. The results are summarized in Table 3. All animals dipped into 100% DMSO died; three of the four animals dipped into 80% DMSO died; none of the animals dipped into 60 and 40% DMSO died. The LD_{50} was estimated to be about 50 g/kg.

TABLE 3

Cutaneous Toxicity of DMSO in Mice

Concentration of DMSO, %	No. of mice	Mean body weight				Maximum dose of DMSO, g/kg	24-hr Mortality
		Before, g	After, g	Change, g	Change, g/kg		
100	5	23.1	25.2	2.1	90.9	90.9	5/5
80	4	24.4	25.9	1.5	61.5	49.2	3/4
60	6	17.9	19.0	1.1	61.4	36.8	0/6
40	6	19.7	20.8	1.1	55.8	22.3	0/6

Six dogs and 24 monkeys were divided into three groups of 2 dogs and 8 monkeys each and were given daily dermal treatment for 6

months with 1.1, 3.3, and 11 g/kg DMSO as a 90% solution in distilled water. In both species, DMSO produced an odor on the breath and desquamation at the site of application. Other than a slight hyperkeratinization there was no histological change that could not be seen in the range of normal variation. It is of interest to note that Brown *et al. (11)* gave a course of intradermal injections of 0.1 ml of 10% DMSO on alternate days for 3 weeks. Two weeks after the last intradermal injection, a challenge dose of intradermal DMSO was given. No signs of sensitization were noticed although the test was repeated.

Kligman *(33,34)* used 90% DMSO in five, 48-hr occlusive patches on 25 human subjects and found no reaction when these subjects were challenged with 50% DMSO after 2 weeks. Occlusive bandaging over DMSO is to be avoided, especially if high concentrations of DMSO are used. Kligman describes a papulovesicular reaction that arises in 1 hour in which the vesicles that arise are independent of the sweat glands. If continued, death of the epidermis ensues and an inflammatory reaction takes place. If the condition is not relieved, a slough takes place, with parakeratotic scaling followed by complete healing.

D. Maximum Tolerated Doses of DMSO

At best, the maximum tolerated dose (MTD) is a compromise and should be considered as a working approximation. The figures listed in Table 4 should be altered in terms of concentration of DMSO used and

TABLE 4

Maximum Tolerated Dose[a]

Species	Route of administration				
	Intravenous	Intra-peritoneal	Subcutaneous	Oral	Dermal
Mouse	2.7 × 4	14.0 × 1 2.5 × 35	2.2 × 1 2.0 × 20	14.0 × 1 2.5 × 35	37.0 × 180
Rat	6.0 × 4 >2.5 × 14	11.1 × 1 7.5 × 24	10.0 × 15	13.7 × 1 11.0 × 10	33.0 × 180
Rabbit	–	–	>4.0 × 28	10.0 × 60	–
Dog	4.8 × 60	–	–	9.0 × 100	11.0 × 180
Monkey	4.0 × 69	–	–	4.0 × 5	11.0 × 180
Horse	–	–	–	–	>1.0 × 13
Pig	–	–	–	4.5 × 5	>4.5 × 30

[a]In grams per kilogram per day × days.

rapidity of administration. The toxicology of domesticated or farm animals has not been described in the literature in any great detail as has been done with the laboratory animals.

Ziv *(35)* tested a number of dyes and antibiotics dissolved in DMSO in the treatment of mastitis in ewes and cows and found that by the intramammary route sheep tolerated up to 7.5 ml of 90% DMSO, while cows could take 45 ml.

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Chapter 4

Fate and Metabolism of DMSO

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I. INTRODUCTION

Many interesting physicochemical and pharmacological properties of dimethyl sulfoxide (DMSO) make it an intriguing chemical to study in biological systems. Unlike most solvents, DMSO readily penetrates skin in nearly all concentrations and has been studied both as a carrier for drugs as well as a therapeutic agent in a number of clinical conditions. The latter use creates the need for a clear understanding of its fate and metabolism in animals and man.

Prior to the first clinical reports on DMSO *(1)*, metabolic activity of some sulfoxides and sulfones had already been described. In 1935

Medes *(2)* suggested that cystine sulfoxide was an intermediary metabolite of cystine. Bennett *(3)* reported that *l*-cystine disulfoxide sustained growth in animals nearly as favorably as cystine, and later *(4)* observed essentially the same growth rates for rats fed *dl*-methionine sulfoxide as for those consuming *dl*-methionine. Rose and Spinks *(5)* and later Snow *(6)* suggested that the oxidation of a sulfide *in vivo* proceeded through a sulfoxide intermediate, and further that sulfoxides were oxidized to sulfones.

Black *et al. (7)* isolated two enzymes from yeast that demonstrated individual specificity for the reduction of one of the two stereoisomeric forms in which methionine sulfoxide can exist. Later Henry *et al. (8)* isolated methionine sulfoxide in the cockroach and suggested that it played a role in maintaining biological oxidation–reduction reactions.

Dent *(9)* reported that human urine collected from a case of Fanconi syndrome which had received methionine orally contained increased levels of α-aminobutyric acid, methionine, and methionine sulfoxide as compared with normal urine. He concluded that methionine was metabolized to α-aminobutyric acid, but did not attach biological significance to the elevated methionine sulfoxide levels.

S-methyl cysteine sulfoxide, a homolog of methionine sulfoxide, has been found in plants *(10–13)*. Doney and Thompson *(14)* reported that plant tissues contained enzymes capable of oxidative conversion of methyl cysteine to methyl cysteine sulfoxide. Furthermore, the plant system investigated was able to reduce *S*-methyl-L-cysteine sulfoxide to methyl cysteine and methionine sulfoxide to methionine.

Gaitonde and Gaull *(15)* isolated and identified a number of sulfur derivatives including sulfoxides and sulfones from animal organs following administration of methionine-^{35}S, i.e., methionine sulfoxide, L-cystathionine sulfoxide and sulfone, and *S*-adenosylhomocystine sulfoxide.

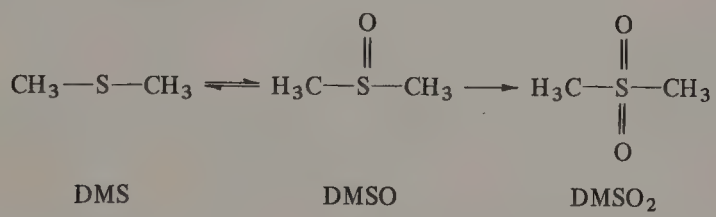
It is of particular interest that $DMSO_2$ has been isolated and identified from biological systems as an apparent natural constituent. In 1934 Pfiffner and Vars *(16)* isolated it from beef adrenals. It was later found in beef blood *(17)* and normal human urine *(18)*. Both dimethyl sulfide (DMS) *(19)* and $DMSO_2$ *(20)* have been found in cows' milk.

II. METABOLISM OF DMSO

DiStefano and Borgstedt *(21)*, using gas chromatography and spectrographic techniques, first identified the presence of DMS in the

expired breath of cats previously treated with DMSO. Later Hucker *et al. (22–25),* Gerhards *et al. (26,27),* Kolb *et al. (28,29),* Williams *et al. (30,31),* and Denko *et al. (32)* made valuable contributions toward our understanding of the metabolism and fate of DMSO in animals and man. Isotope-labeled DMSO is rapidly metabolized *in vivo* and can be largely recovered in blood, organs, stool, and urine as unaltered DMSO and $DMSO_2$. A small percentage of an original dose is reduced to DMS and exhaled in the breath. These studies generally indicate, at least in animals and man, that DMSO can be either oxidized to $DMSO_2$ or reduced to DMS.

Williams *(31)* reported that subcutaneous injection of DMS or DMSO into rabbits resulted in excretion of DMSO and $DMSO_2$ in urine. The animals also had a breath odor which was believed to indicate the presence of DMS. When $DMSO_2$ was administered, however, no DMS or DMSO was found. A simplified chemical expression summarizing these observations is shown.



Gerhards and Gibian *(27)* studied cofactor and cellular particulate fractions necessary to convert DMSO to $DMSO_2$ in rat liver. The oxidation required microsomal enzymes found in the supernatant following 10,000 × *g* centrifugation. Supernatant from a 100,000 × *g* preparation was unable to catalyze the reaction. The oxidation required the presence of $NADPH_2$ or $NADH_2$ and molecular oxygen. The reaction failed to take place in a nitrogen atmosphere.

Ando *et al. (33)* demonstrated the presence of a DMSO reductase enzyme in many Enterobacteriaceae species after having tested more than 300 stock and routine cultures. A few other strains of microorganisms were also studied. *Bacillus subtilis, B. megatherium, B. mesentericus, Staphylococcus aureus,* and *Streptococcus faecalis* were all negative. *Serratia marcescens, Pseudomonas fluorescens,* and *Aerobacter aerogenes* reduced DMSO.

Rammler and Zaffaroni *(34)* studied the metabolism of DMSO, $DMSO_2$, and DMS using an *A. aerogenes* (ATCC-9621) strain which was capable of utilizing each of these compounds as its sole sulfur source. The microorganism reduced DMSO and $DMSO_2$ to DMS, but

the reaction apparently was not reversible because when DMS was used as the sulfur source no DMSO or $DMSO_2$ could be detected. When DMSO-^{14}C was added as the only sulfur source, 80% of the radioactivity was detected as labeled carbon dioxide. At least in this microorganism the carbon structure of DMSO was related to a major oxidative metabolic pathway. The authors found that a small part of the radioactivity remained in the cells and could only be extracted in hot trichloroacetic acid, suggesting its incorporation into nucleic acids. The radioactivity was lost after hydrolysis with potassium hydroxide, thus indicating that the DMSO-^{14}C carbon had been incorporated into ribonucleic acid.

Oae *et al. (35)* and Rammler and Zaffaroni *(34)* have published clever schemes suggesting a hypothetical metabolic pathway for DMSO in biological material. Both systems relate to other known biological oxidation–reduction reactions utilizing methyl group transfer or the oxidation of a methyl group with an activated acyl derivative. The system created by Rammler and Zaffaroni *(34)* suggests sulfate as one of the possible end products of oxidation.

Studies in animals and humans have not yet identified such oxidative systems as those described above, however, it is of interest that Gerhards and Gibian *(27)* reported that an intravenous injection of DMSO-^{35}S in rats produced a trace amount (0.02%) of the total radioactivity in the sulfate fractions of urine after precipitation with barium chloride. However, Maw *(36)* reported that oral or injected DMS did not increase urinary sulfate levels in rats.

III. FATE

A. Absorption

Hucker *et al. (22,23)* treated rats with DMSO-^{35}S by oral, cutaneous, and intraperitoneal routes. The animals were sacrificed at various time periods and radioactivity content of blood and organs was measured. Regardless of the route of administration, a remarkable amount of radioactivity was found in the plasma after only 30 min. The maximal level in blood following cutaneous application was reached in 2 hr, however, following oral or intraperitoneal treatment optimal levels of radioactivity were found after 0.5–1 hr. Half of the peak blood level

was lost in 6 hr, and only 5–10% remained after 24 hr. These investigators observed that DMSO-^{35}S rapidly penetrated the skin. In rats 63% of the original dose was found at the site of application after 30 min, and 19% remained in the skin after 1 hr. In rabbits 85% remained at the application site after 30 min, and 11% after 4 hr. After 24 hr skin at the point of DMSO-^{35}S application contained the same number of counts as the surrounding skin.

Kolb *et al. (28,29)* likewise observed rapid cutaneous absorption of radioactive DMSO in rats and dogs. More than 90% of a given dose of DMSO-^{35}S penetrated dog skin within the first 24 hr. After 14 days only 0.3% of the applied dose was found in the original area of application. The authors reported a near-linear decline of radioactivity in the skin for 2 weeks. They found the half-time of DMSO in blood to be greater than 10 hr. McDermot *et al. (37)* reported that rats treated cutaneously with DMSO absorbed 37% of the applied dose in 15 min, 67% in 30 min, 90% in 45 min, and 94% in 60 min. Peak blood levels were reached in 1.5–2 hr.

In man *(29)* radioactivity was found in the blood 5 min after cutaneous application of DMSO-^{35}S. A maximal level was reached after 4–6 hr and remained at a plateau for 1.5–3 days. Hucker *et al. (25)* also reported rapid dermal absorption of DMSO in man, with peak blood levels after 4–8 hr. Radioactivity was detected in serum for as long as 400 hr.

Kolb *et al. (29)* scraped the dermis of male volunteers 8 hr following application of 2 g of DMSO. The radioactivity accounted for approximately 20% of the initial dose.

B. Urine Excretion

Urinary excretion rates for DMSO and its metabolites have been studied in small animals and man *(22,23,25,28,29,38)*.

1. Rats

Approximately 65–75% of a total cutaneous DMSO-^{35}S dose appeared in the urine of rats after 24 hr *(23,28)*. When the dose was administered intravenously, 80% of the radioactivity was collected in a 24-hr urine *(28)*. Kolb *et al. (28,29)* studied the excretion rate when different initial concentrations of DMSO were applied to the skin. When 50% DMSO was administered to rats, 64% of the radioactivity

was excreted in the urine within the first 24 hr; however, when 10% DMSO was applied, only 35% of the original dose was eliminated in the same amount of time.

2. Rabbits

Following cutaneous application of DMSO-^{35}S to rabbits, 24-hr urines were reported to contain 30% *(23)* and, in another study, 70% *(28,29)* of a total radioactive dose. After 48 hr approximately 80% was recovered, and 90% was collected during a period of 10 days *(28,29)*.

3. Guinea Pigs

DMSO-^{35}S was administered to guinea pigs by intraperitoneal injection, and 52% of the radioactivity was recovered in a 24-hr urine collection *(23)*. Malinin *et al. (38)* recovered 30% of a DMSO-^{14}C intraperitoneal dose in a 24-hr urine.

4. Beagles

Approximately 50% of a total radioactive DMSO dose given to beagles was collected in 36 hr whether administered by oral or intravenous routes. However, when DMSO-^{35}S was applied cutaneously the urine contained 12–25% of the total dose after 24 hr, 37–48% in 7 days, 60% in 60 days, 75% in 80 days, and approximately 83% in 115 days *(28,29)*.

5. Man

Hucker *et al. (25)* recovered 30.8% of a total cutaneous DMSO-^{35}S dose from human urine in 19 days. Kolb *et al. (28,29)* recovered 80% of the original radioactivity in 8 days when the drug was administered intravenously and approximately 50% in 10–15 days when it was administered cutaneously.

C. Feces Excretion

Radioactivity has been found in the feces of animals treated with labeled DMSO. Hucker *et al. (25)* found 10% of the total dose in rat feces after 24 hr when the rat was treated orally and 4% was recovered following intraperitoneal and dermal treatment. Kolb *et al. (29)*

reported that 0.5–2% of the dose in rabbits and dogs, irrespective of the route of therapy, was excreted in feces. No remarkable radioactivity has been observed in human feces *(22,28)*.

D. Bile Excretion

Kolb *et al. (29)* observed that 8% of an intravenous DMSO dose given to rats was excreted with the bile in 24 hr. They suggest that a large part of the radioactivity is reabsorbed in the gastrointestinal tract.

E. Breath Excretion

Approximately 3.5% of a total intravenous dose was recovered as DMS in rats after 12 hr of continuous sampling *(28)*. Hucker *et al. (23)* recovered 6% in 24 hr from rats treated cutaneously, and a rabbit treated intraperitoneally respired less than 1% of a single dose in 3 hr.

F. Distribution of DMSO and $DMSO_2$ in Tissues and Urine

The distribution of DMSO in some rat tissues was first reported by Ashwood-Smith *(39)*. He observed rapid accumulations of radioactivity in blood, testes, spleen, liver, kidney, and brain. In 1965 Hucker *et al. (22)* found radioactivity well distributed in the soft tissues of rats treated with DMSO-^{35}S. Later *(23)* they compared the organ distribution of radioactivity as a factor of time in rats and rabbits that had received DMSO-^{35}S administered by oral and cutaneous routes. Rat and rabbit organ levels of radioactivity were remarkably high 30 min. after treatment regardless of therapy route. The peak level was measured after 4 hr and the total concentration of radioactivity was ordinarily less in animals treated cutaneously as compared with those given oral doses. Total organ radioactivity dropped off remarkably in both rats and rabbits after 24 hr. The rate of disappearance from organs was found to be faster in rats than in rabbits.

McDermot *et al. (37)* found peak concentrations of DMSO in rat tissues, following cutaneous application, 1.5–2 hr after treatment. The highest concentrations were found in kidney and the lowest in lung. The authors suggest that the kidney contained high levels of drug because it was the organ of excretion. After 6 hr organ DMSO levels were reduced and by 24 hr none could be detected.

$DMSO_2$ and DMSO concentrations in rat tissues 4 hr after an oral dosing of DMSO-^{35}S yielded values that were essentially constant for liver, testes, kidney, spleen, small intestine, heart, and plasma. The mean ratio value of $DMSO_2$ to DMSO was 6.5%, indicating that most of the radioactivity was DMSO *(23)*.

The ratio of $DMSO_2$ to DMSO following cutaneous administration of DMSO-^{35}S to rabbits was 11.6% for the following tissues: testes, brain, plasma, bile aqueous humor, lens, vitreous humor, and skin at the site of application. Rabbit liver and kidney had a higher ratio of 21% *(23)*.

The 24-hr urine collections from rats that had received an intraperitoneal injection of DMSO-^{35}S contained about 12.8% of the original dose as $DMSO_2$ *(23)*.

Gerhards *et al. (26)* and Gerhards and Gibian *(27)* studied recoveries of DMSO and $DMSO_2$ in human urine following cutaneous or intravenous administration of DMSO-^{35}S in humans as a factor of time. The urine collection after 6 hr contained approximately 82% DMSO and 18% $DMSO_2$, after 24 hr, 28% DMSO and 72% $DMSO_2$, and after 48 hr, 4% DMSO and 96% $DMSO_2$.

Hucker *et al. (25)* also reported changes in blood and urine levels after cutaneous and oral dosings of DMSO-^{35}S in man as a factor of time. Cutaneous administration of DMSO-^{35}S produced peak serum levels of DMSO in about 4 hr. No DMSO could be detected in the serum 30–48 hr after dosing. In the same patients serum $DMSO_2$ levels reached their peak value after 36–72 hr and had essentially disappeared after 312 hr. DMSO was measured in the urine of these patients shortly after the drug was administered and in 48 hr essentially all of the detectable DMSO had been excreted. However, $DMSO_2$ was observed after 8 hr and was measured in the urine for 480 hr, after which it was no longer detected. In this study 13.0% of the original dose was recovered in urine as DMSO and approximately 17.8% as $DMSO_2$. Urine recovery of DMSO and $DMSO_2$ when the drug was administered orally was 50.8% and 22.0%, respectively. Kolb *et al. (28)* reported nearly complete urine recoveries of total radioactivity when the isotope was injected intravenously as compared to cutaneous treatment.

Kolb *et al. (28,29)* employed whole-body radioautography for studies in rats 1, 4, and 24 hr following a single cutaneous dose of DMSO-^{35}S. After 1 hr the dermal site of application was very distinct and radioactivity was well distributed throughout the animals, including all

bones and connective tissue. In 4 hr all organs showed radioactivity, but with less contrast, and the area of cutaneous application showed little if any residual isotope. The 24-hr sections had no remarkable accumulation of radioactivity in brain, spinal cord, vertebral discs, fatty tissues, or adrenal.

Malinin *et al. (38)* used radioautography in studying pieces of tissue from guinea pigs previously injected intraperitoneally with DMSO-^{14}C. They found DMSO to be associated primarily with interstitial spaces in tissues with orientation of structural components. Where tissue planar orientation did not exist they observed DMSO to be located at the surface of cell membranes, suggesting that the membrane was acting as a barrier. Most of the radioactivity in the liver was related to the vascular system and liver sinusoids. Lungs concentrated label in the lumina of bronchi and alveolar spaces. Most of the activity in the kidney related to the lumina of collecting tubules and interstitial spaces. DMSO in myocardial and striated skeletal muscle was essentially limited to interstitial spaces.

The distribution of radioactivity in components of the eye was determined following cutaneous treatment of rats and rabbits with DMSO-^{3}H *(28,29)*. Treated rabbits showed larger amounts of radioactivity in the cornea than in plasma or in any other part of the eye. Maximum concentration was reached about 2 hr after treatment and the radioactivity of cornea was 4 times greater than that of the lens. Iris and cilliary body were also rich in isotope. Similar isotope distributions were found in rat eye components treated in the same manner, however, optimum concentrations were reached somewhat later and were retained for a longer time. Rat cornea reached its optimum concentrations in 4 hr, as compared with 2 hr for rabbits, and at that time it contained 2.5 times more radioactivity than aqueous or vitreous humor, and 4 times that found in the lens. These data suggest a rapid peak accumulation of radioactivity in the cornea at approximately 4 hr, followed by a progressive loss. The aqueous humor and lens reached peak concentrations after 24 hr, in contrast to other rat tissues discussed previously which reached a radioactivity peak in 2 hr. After 72 hr all rat eye components demonstrated decreasing isotope levels and by 8 days no radioactivity was detected.

Denko *et al. (32)* evaluated the distribution of DMSO-^{35}S in hard and soft tissues of rats. They also observed a wide distribution of radioactivity after 2 hr in all tissues examined. Hard tissues, i.e., lens and bone, accumulated approximately one-sixth the radioactivity that

most of the soft tissues accumulated in the same period of time. Denko *et al. (32)* also found more radioactivity in the tissues from rats treated with intraperitoneal DMSO-^{35}S as compared with the cutaneous route. They state that on the first day, when most of the drug was excreted, radioactivity values were comparable in both groups. When twice as much isotope was administered higher levels of radioactivity were found in most tissues, however, the ratio did not double. These workers observed that tissues having the highest activity demonstrated the shortest drug half-life.

Gerhards *et al. (26)* and Gerhards and Gibian *(27)* dialyzed DMSO-^{35}S with tissues *in vitro* to evaluate binding potential. Their results indicated a remarkable capacity for binding with plasma, cornea, skin, liver, and diaphragm. Human plasma proteins were shown to bind DMSO-^{35}S. They reported that 25% of the DMSO present in blood was found in the sediment. Denko *et al. (32)* separated the albumin and globulin from serum of rats previously treated with DMSO-^{35}S, using acrylamide gel electrophoresis and by sodium sulfate fractionation. They found that 96% of the radioactivity bound to serum proteins was in the albumin fraction. Malinin *et al. (38)* did not find radioactivity associated with serum proteins in guinea pigs previously treated with DMSO-^{14}C.

IV. CONCLUSIONS

The above-mentioned studies indicate that DMSO rapidly penetrates the skin of all species tested, most rapidly in the rat and most slowly in humans. There appeared to be a general distribution of DMSO or its metabolites in all tissues, reaching peak levels within 2–6 hr. Serum levels were generally lower when the drug was administered cutaneously than when it was given either orally or intravenously. It was of particular interest that tissues rich in lipid content, such as the brain, contained as much DMSO as those with a low lipid content. An explanation for this observation has not been documented. Denko *et al. (32)* pointed out that vascularity or metabolic activity of the organ cannot account for this fact completely, because avascular tissues such as vitreous humor contained more DMSO during the same test period than brain, kidney, or liver.

DMSO was metabolized in animals and man to form $DMSO_2$ and DMS. Approximately 3–6% was exhaled as DMS. Both DMSO and

$DMSO_2$ were present in all tissues examined, with unchanged DMSO being in remarkable excess. A trace amount was found as sulfate in urine and 3–4% was isolated in feces of small animals but not in man.

It seems likely that most tissues can metabolize DMSO, although this has not been demonstrated. Borgstedt and DiStefano *(40)* observed that hepatectomized cats metabolized DMSO and suggested that the reaction might be nonenzymatic.

Failure to identify metabolic products other than $DMSO_2$ and DMS in animals and man indicates that a simple oxidation–reduction system exists. This, however, is not true for microorganisms in that some are apparently capable of metabolizing the entire DMSO molecule.

In man the total urine recovery after approximately 20 days for a dose applied cutaneously was 35–50%, orally 71%, and intravenously 80–98%. This remarkable difference, associated with the route of therapy, is not as great in other species tested. In all of the species DMSO appeared in the urine earlier than $DMSO_2$. Hucker *et al. (25)* suggested that this might simply indicate differences in renal clearance or that $DMSO_2$ was bound to tissues and slowly liberated. They further speculated that these differences could occur if DMSO were bound and slowly released so that the conversion to $DMSO_2$ was complete before excretion in the urine.

Perhaps the most perplexing problem to those who have studied the fate and metabolism of DMSO is the fact that 100% recovery of the initial drug has not been achieved. Studies in the rat have come close to a complete yield, however, in dogs and man the recovery is considerably less. Poorest recovery yields have resulted from studies in which the drug was applied cutaneously, while the best recoveries followed intravenous injection. A good explanation of this observation awaits further investigation.

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Chapter 5

Radioprotective and Cryoprotective Properties of DMSO

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I. INTRODUCTION

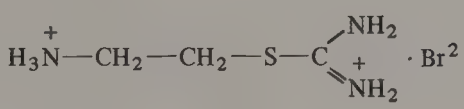
A large number of compounds have been discovered that possess radioprotective properties when present before and during exposure of whole animals or various bacterial and mammalian systems to γ- or x-irradiation. The mode of action of these substances has been adequately reviewed in several books *(1,2)*. A smaller and much less diversified number of chemicals have cryoprotective properties; the reader is referred to an excellent account of the chemical nature of cryophylactic agents by Nash *(3)*. Dimethyl sulfoxide (DMSO) has at least two well-documented and very important biological properties which in the last decade have been extensively studied *(4,5)*. One of the important properties of DMSO is its radioprotective action. When DMSO is present before and during exposure of rodents and a variety of mammalian or bacterial cells to x-irradiation, the damage sustained by these systems is considerably reduced. Another important property possessed by DMSO is its ability to protect a number of different mammalian and nonmammalian cells from the damaging effects of freezing, freeze-storage, and thawing from very low temperatures. This latter property of DMSO is of practical importance, as DMSO has largely replaced glycerol as an alternative cryophylactic agent in the preservation of animal cells grown in tissue culture. Are these two important biological effects of DMSO in any way related? It is proposed in this chapter to discuss in some detail these two characteristics and then attempt to answer this question.

II. GENERAL CONSIDERATIONS REGARDING RADIOPROTECTION

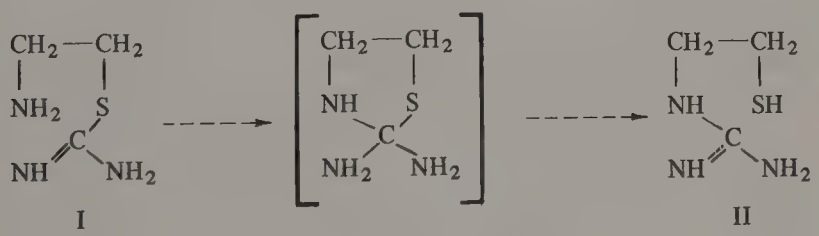
The radioprotective action of DMSO in modifying damage caused to cells by ionizing radiation is less well known than its cryoprotective action. However, a number of reports from different laboratories throughout the world have confirmed and extended the original observations on the radioprotective action of DMSO and other related sulfoxides *(6)*. A drug that could decrease the damage caused to radiosensitive tissues by x- or γ-radiation would be of interest to the military. Such thoughts stimulated much extensive and largely abortive

work which continued in many lands from the end of World War II until about the beginning of the 1960's. Although a practical antiradiation drug was not found, many interesting facts became known about the possible mechanisms of radiation damage in small animals and in numerous different cell types. A large amount of basic information was collected on the pharmacological action of some of these compounds.

When mice are injected with a solution of cysteine before exposure to a lethal dose of x-rays, a high proportion of the mice survive. The discovery of this effect by Patt *et al. (7)* stimulated an immense amount of research into other and perhaps more potent substances than cysteine. Bacq and Herve *(8)* demonstrated the protective action of β-mercaptoethylamine ($HSCH_2CH_2NH_2$, cysteamine), and it was shown that the oxidized form of this compound $[(SCH_2CH_2NH_2)_2]$ was also effective in protecting mice from radiation-induced death. Perhaps the chemical that received the greatest attention was *S*(2-aminoethyl)isothiuronium bromide hydrobromide (AET)



This compound was discovered by Doherty and Burnett *(9)* and was subsequently shown to undergo an interesting molecular rearrangement in solution at neutral pH. AET in solution gave an immediate and strong nitroprusside test indicative of a free SH group. It was also observed that the same solution gave a positive Sakaguchi reaction, evidence of a guanido group. These results suggested that, at physiological pH, AET (I) could intratransguanidate to form II, mercaptoethyl guanidine (MEG). This intramolecular rearrangement probably takes place through the formation of a cyclic intermediate. These reactions may be summarized as shown.



I II

It was suggested that for high protective activity a thiol and a guanido group must be together in one molecule, separated by not more

than two or three carbon atoms. It was also suggested that only those mercaptoalkylguanidines capable of forming five- or six-membered cyclic intermediates would show the most radiation-protective action. Various theories have been postulated to explain the protective action of AET. Doherty and co-workers *(10)* advanced the hypothesis that AET reacts directly with free radicals to form resonance-stabilized hybrids which then react not with key biochemical targets but with other less important radicals. Evidence has been presented that AET inhibits a number of important SH enzymes of the rat *in vitro (11). In vivo* inhibition of pyruvate and α-ketoglutarate oxidation after the injection of sublethal doses of AET has also been reported.

Hope *(12)* discovered that the simplest member of a series of *S*-alkylisothiuronium salts, the *S*-methyl compound, had protective properties. Later work in our laboratory indicated that the *S*-ethyl and *S*-propyl compounds were also active *(13)*. The results were surprising, as molecular rearrangement in these simple thiuronium compounds is not possible. There are at least two possibilities which could explain their action. It was demonstrated that liver contains a system capable of catalyzing the formation of the alkyl thiol from the thiuronium compound and the thiol thus formed might be expected to show protective activity. The amounts of free thiol produced, however, were quite small, and it seems unlikely that this reaction could account for the protective activity of these simple isothiuronium compounds. A more probable explanation would be that the compounds are hypertensive agents and that they protect *in vivo* by producing local anoxia in radiosensitive tissues. Further investigational work has failed to reveal protective activity in other isothiuronium salts, and it is probable that the activity of these compounds is limited to the *S*-methyl, *S*-ethyl, and *S*-propyl derivatives, all of which possess the ability to raise blood pressure when injected.

It was known that tryptamine, β-phenylethylamine, histamine, and adrenaline are capable of protecting lethally irradiated mice when injected before irradiation. Alexander *et al. (14)* used an *in vitro* polymer system with aromatic amines to protect the system from the effects of x-irradiation. They suggested that *in vivo* protection with aromatic amines could be explained as a result of amine–free radical combination. Such an explanation excludes from consideration the physiological results that follow the administration of such pharmacologically active chemicals. Injection of aromatic amines is usually followed by changes in blood pressure and in the rate of blood flow

through organs of the body. In some organs an amine will produce vasodilation with a concomitant increase in blood flow, in others, vasoconstriction and a decrease in blood flow. A restricted blood supply leads to a limited oxygen tension, with an increased resistance to radiation damage, so that any process that lowers oxygen tension in bone marrow, spleen, or the gastrointestinal tract is likely to increase an animal's chance of survival. Evidence has been produced that many of these amines do in fact decrease spleen oxygen tension *in vivo.* The cardiovascular changes that occur in the whole animal after an aromatic amine has been injected are complex, and arguments used in the past to counter the pharmacological rather than the direct chemical radical combination theory have tended to oversimplify the issue. Histamine produces a general fall in blood pressure, and tryptamine has hardly any vasoconstriction properties; yet both of these compounds protect animals, as do many other amines, most of which increase blood pressure when injected.

Until recently there was no direct evidence linking the pharmacological properties of an amine with its radiation protective ability. It was known that the injection of specific antagonists destroyed the protective action of the amine. Van der Meer and van Bekkum *(15)* have published a study of the effect of various amines on oxygen tension in the spleen of mice. The spleen, an important organ in the hematopoietic system, is radiosensitive, and damage to this organ as well as to other blood-forming organs is one of the characteristic changes that occur in irradiated mice. By means of a polarographic technique, it was possible, with a platinum electrode implanted in the spleen of an unanesthetized mouse, to measure oxygen tension. The results of these experiments demonstrated that histamine, adrenaline, tryptamine, and β-phenylethylamine but not noradrenaline produced a considerable fall in oxygen tension which could be correlated with the protective ability of the injected amine. Noradrenaline, which possesses no radioprotective action, had little effect on oxygen tension. Van der Meer and van Bekkum noted that the protective action of a number of these amines could be virtually abolished by the preadministration of the requisite pharmacological antagonist.

The hydroxyl derivative of tryptamine, 5-hydroxytryptamine (serotonin), is a powerful radioprotective amine *in vivo.* It was suggested that, in a way similar to tryptamine, serotonin owed its protective action more to the fact that it combined with free radicals than to the fact that it exerted a specific pharmacological action. There is evidence that

serotonin plays an important part in the physiology of the cardiovascular and central nervous systems. One of the properties of serotonin is its ability to constrict smooth muscle and elevate blood pressure. Reports by Hope *(16)* and van der Brenk and Elliot *(17)* on the protective action of serotonin indicate that it protects through a physiological mechanism. These authors showed that the administration of two antimetabolites, *d*-lysergic acid diethylamide and its brominated derivative, provided no protective action, but when these compounds were injected together with serotonin the protective action of serotonin was abolished.

Langendorff and associates *(18)* maintain that serotonin is a better protective agent than either cysteamine or AET. Nearly all protective chemicals have to be injected in doses approaching toxic proportions before good protection is obtained. Serotonin is an exception because the dose that gives the best protection against x-rays is considerably lower than the toxic dose; in fact, the protective action tends to fall off as the dose of serotonin is increased above a certain level. It is possible that this effect may be the result of the action of rather complex cardiovascular compensating mechanisms.

Drugs therapeutically useful in preventing radiation death have not been found. All the drugs thus far reported have three major disadvantages:

(1) The therapeutic index is very low (i.e., the ratio between the LD_{50} of the drug and an effective antiradiation dose). Effective doses are often as high as one-third to one-half the LD_{50} of the compound. At these doses severe side effects are nearly always seen.

(2) Antiradiation drugs must be present in the tissue at the time of exposure to radiation. In practice this means that administration of compounds must take place about 1/2 hr before radiation; administration after radiation is useless.

(3) The most effective drugs in animal experiments, namely cysteamine, AET, and serotonin, are either ineffective or only slightly effective against neutron irradiation.

There have been two major reasons why research into antiradiation drugs has continued during the last few years even though it had become apparent by the beginning of 1960 that a therapeutically useful drug was becoming more and more elusive.

First, it was hoped that a breakthrough might occur, and this kept

many laboratories active. Published results of really useful antiradiation drugs have yet to appear. The second reason has more scientific merit, namely, that an appreciation of the mechanism of action of antiradiation drugs would contribute to an understanding of the basic mechanisms of radiosensitivity both in the whole animal and at the cellular level. It has been partly as a result of research with antiradiation chemicals that some of the radiobiological effects of x-rays are better understood. The concept that substances such as cysteine and cysteamine could "mop up" free radicals produced in aqueous systems as a result of x-irradiation has been a productive and helpful concept in radiobiology even if it is an oversimplification of the facts. This radical-scavenging theory, as it became known, helped to explain why cysteamine and cysteine were largely ineffective in protecting systems from the radiation of neutrons and other densely ionizing radiations. The idea that chemical protective agents that contain SH groups owed their activity to an ability to react in a reversible manner with sensitive SH target enzymes was proposed and developed into a most interesting and stimulating idea by Eldjarn and Pihl *(19)* in Norway.

III. RADIOPROTECTIVE PROPERTIES OF DMSO

In 1959 we were engaged in a screening program for antiradiation drugs. DMSO, which had been introduced into biological research by Lovelock and Bishop *(20)* as a cryophylactic agent for bull spermatozoa, was studied as a possible carrier or solvent chemical for antiradiation chemicals that were not very soluble in water. Preliminary toxicity tests of DMSO in mice indicated that an approximate LD_{50} of 12,500 mg/kg by intraperitoneal (I.P.) injection, although low, was still too high when compared to arachis oil and ethyl palmitate which were then used as solvents. The possibility occurred to us that a simple, water-soluble chemical of low toxicity containing a sulfur atom might possess radioprotective properties. Since sulfoxides had not previously been tested for an antiradiation properties, DMSO was included in a routine screen test. The initial test consisted of injecting groups of 10 mice with doses of the test compound corresponding to about one-fourth to one-third of the acute LD_{50}, 1/2 hr before exposing them to a lethal dose of x-rays (LD_{99} at 30 days = 1007 rads with our mice in 1959–1961 at Harwell). Control groups of mice which had been randomized with the

treated mice were injected with buffered saline and were all dead by the end of the 30-day test period, the 15th postirradiation day being the peak for death. Any substance that showed good results in the screening test was investigated in more detail. The results of the experiments with DMSO indicated that 70% of the mice given a lethal dose of x-rays but injected with 4500 mg/kg I.P. of DMSO 30 min before x-irradiation were still alive and well at the finish of the 30-day test period. This figure of 70% was rarely achieved with our mice and under our irradiation conditions. AET and cysteamine gave 100% protection to mice under similar conditions, and the amount by which these compounds increased the LD_{50} to x-rays (DRF = dose reduction factor) was 1.6. DMSO gave a DRF of 1.45.

About 18 other sulfoxides and derivatives of sulfoxides were tested for antiradiation properties, and the results of these experiments are shown in Table 1. DMSO was the only sulfoxide to possess marked radioprotective properties. The reduction product of DMSO, dimethyl sulfide, and the oxidation product, dimethyl sulfone, were without activity. These results suggested that the action of DMSO was probably not mediated by either of its two most likely metabolic products. The percent survival of mice treated with different doses of DMSO is shown in Table 2, and in Table 3 the time course of the protective action can be seen. DMSO given after x-irradiation was without effect, and treatment of mice 4 hr before x-ray exposure was ineffective. When supralethal doses of x-rays were delivered to mice injected with DMSO, the results shown in Table 4 were obtained. Doses of x-rays greater than 1300 rads were lethal to all mice.

At this stage in the investigations, it was apparent that DMSO was a reasonably powerful radioprotective compound of a novel type, although it was not as effective as either AET or cysteamine. A further series of studies was undertaken to study its mode of action. The distribution of DMSO in rat tissues after an I.P. injection of a radioprotective dose was investigated. In 1959 radioactive ^{35}S-labeled DMSO was not available commercially so that studies on DMSO in tissues had to be based on not very accurate methods. Deproteinized tissue extracts were separated by chromatography. The DMSO position on the paper chromatogram was compared with known amounts. The DMSO position on the paper was visualized by treatment with Toennies reagent. By using these methods it was demonstrated that 80% of the injected DMSO appeared unchanged in the urine within 48 hr (these results were also confirmed by infrared spectroscopy; see Fig. 1).

The pattern of DMSO distribution in the tissues of the rat is shown in Fig. 2. Nearly all the tissues examined showed similar concentrations of DMSO. The amounts of DMSO in the testis, which is normally a very sensitive organ to radiation damage, suggested that it might be protected from radiation damage by I.P. injection of DMSO before x-irradiation. This, however, was not true. Analysis of testis weight and histology 28 days after irradiation gave no indication of protection. AET also proved to be ineffective *(21)*. These results are shown in Table 5. Although the bone marrow had not been analyzed for DMSO content, the hematological response of irradiated mice injected with DMSO was good (Fig. 3), thus indicating that sufficient amounts of DMSO must have been present in the cellular marrow to protect a small number of stem cells from radiation death. The spleen is an important organ of hematopoeisis in the rat and mouse, and large amounts of DMSO could be detected in this organ after I.P. injection.

DMSO mixed with cysteamine or AET was administered to mice before x-irradiation. These experiments *(22)* demonstrated a synergistic action of DMSO and are summarized in Table 6.

IV. MECHANISMS OF THE RADIOPROTECTIVE ACTION OF DMSO

The action of DMSO in protecting an animal from radiation death may be different from its action in preventing the death of bacteria or cells in tissue culture. In the initial part of this chapter, reference was made to the protective action of various biological amines such as histamine and serotonin. These compounds have marked radioprotective properties in mice but their effectiveness appears to rest on their pressor action and can be abolished by injection of the appropriate pharmacological antagonist. DMSO is known to have an appreciable effect on increasing blood pressure when injected I.P. into mice *(4)*, an observation confirmed by van der Meer *et al. (23)*. These workers were also able to show that injection of a radioprotective dose of DMSO produced a marked fall in the oxygen tension of the spleen and have suggested that the lowered oxygen tension in that organ, which would certainly cause a marked decrease in radiosensitivity, is probably the reason for the protective effect of DMSO in mice. There is evidence that DMSO can protect a variety of isolated mammalian and bacterial cells. Thus, pharmacological mechanisms of radioprotection are not the

TABLE 1

Radioprotective Action of Various Sulfoxides against a Lethal Dose of x-Irradiation (1007 rads) in Mice

Compound	Formula	LD_{50}		Dose administered		Solvent	30-Day survival (%)
		mg/kg	mmoles/kg	mg/kg	mmoles/kg		
Dimethyl sulfoxide	CH_3SCH_3 ‖ O	11,000	140	4,500	58	Water	70
Methyl ethyl sulfoxide	$CH_3SCH_2CH_3$ ‖ O	3,500	38	3,000	33	Water	10
Diethyl sulfoxide	$CH_3CH_2SCH_2CH_3$ ‖ O	2,500	24	1,700	16	Water	0
β-β^1-Dihydroxydiethyl sulfoxide	$HOCH_2CH_2SCH_2CH_2OH$ ‖ O	3,000	22	2,250	16	Water	0
Di-*n*-propyl sulfoxide	$CH_3CH_2CH_2SCH_2CH_2CH_3$ ‖ O	900	6.7	600	4.5	Water	0

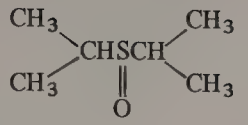
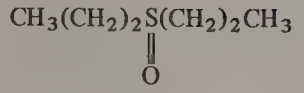
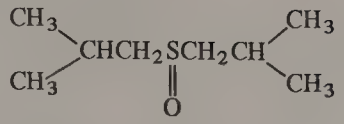
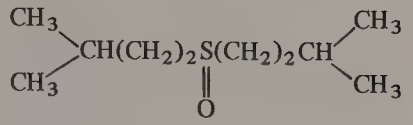
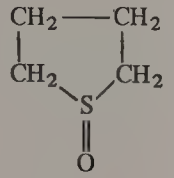
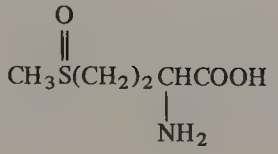
Diisopropyl sulfoxide		1,750	13.4	1,000	7.5	Water	0
N-Dibutyl sulfoxide		450	2.8	350	2.2	Ethyl palmitate	0
Diisobutyl sulfoxide		1,250	7.4	1,000	6.2	Ethyl palmitate	0
Diisoamyl sulfoxide		500	2.6	225	1.2	Ethyl palmitate	0
Tetramethylene sulfoxide		3,500	34	2,500	24	Water	30
dl-methionine sulfoxide		4,000	24	3,250	20	Water	20

TABLE 1 (continued)

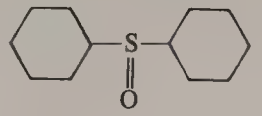
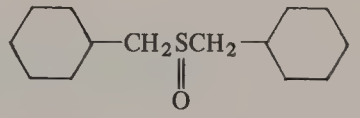
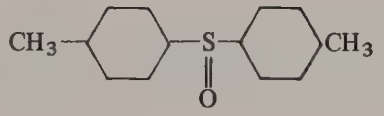
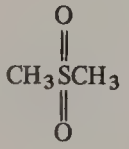
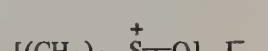
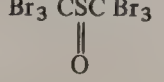
Compound	Formula	LD_{50} mg/kg	LD_{50} mmoles/kg	Dose administered mg/kg	Dose administered mmoles/kg	Solvent	30-Day survival (%)
Diphenyl sulfoxide		750	3.7	200	1.0	Ethyl palmitate	30
Dibenzyl sulfoxide		600	2.6	400	1.7	Ethyl palmitate	0
Ditolyl sulfoxide		600	2.6	400	1.7	Ethyl palmitate	0
Derivatives of dimethyl sulfoxide							
Dimethyl sulfone		10,000	104	4,500	52	Water	0
Dimethyl sulfide	CH_3SCH_3	8,000	130	5,000	80	Ethyl palmitate	20
Trimethyl sulfoxonium iodide		900	4.1	400	1.8	Water	0
Bistribromomethyl sulfoxide		40	0.053	25	0.025	Ethyl palmitate	20

TABLE 2

Protective Action of Different Doses of DMSO Administered to R Mice Exposed to Lethal x-Irradiation (1007 rads)

Dose, mg/kg I.P.	Time before x-rays, min	Number of mice living/ number of mice used	Percent survival
Controls	30	0/10	0
2250	30	4/10	40
4500	30	14/20	70
8100	30	14/20	70

TABLE 3

Protective Action of DMSO Administered to R Mice at Different Times before a Lethal Dose (1007 rads) of x-Rays

Time before x-rays	Dose, mg/kg I.P.	Number of mice living/ number of mice used	Percent survival
5 min	4500	7/10	70
15 min	4500	7/10	70
30 min	4500	7/10	70
1 hr	4500	7/10	70
2 hr	4500	3/10	30
4 hr	4500	0/10	0
Immediately after	4500	0/10	0
30 min (Controls)	Saline	0/35	0

TABLE 4

Protective Action of DMSO against High Doses of x-Rays

x-Ray dose (rads)	Number of mice living/ number of mice used	Percent survival
1100	9/20	45
1200	5/20	25
1300	0/20	0
825 (Controls)	19/40	47.5

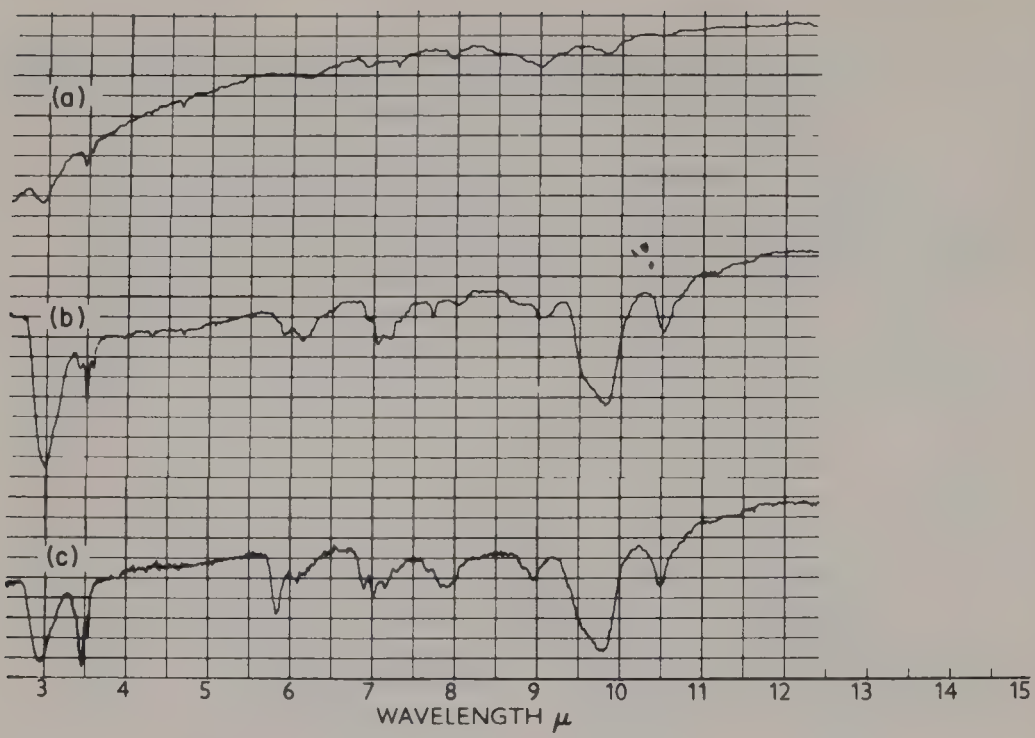


Fig. 1. Infrared spectra of urine samples from a rat before and after the administration of DMSO (after elution from two-dimensional chromatograms). (a) Control: urine before DMSO treatment. (b) Control: urine plus a standard of DMSO (500 μg). (c) Urine from rat treated with DMSO.

only ones to be considered. Mouse bone marrow cells irradiated *in vitro* in the presence of DMSO are protected. Injection of irradiated protected cells into lethally x-irradiated mice resulted in survival of some of the mice (Table 7). Vos and Kaalen *(24),* using a well-established cell line of human kidney cells and the Puck cloning technique, demonstrated a linear response between the concentration of DMSO present during x-irradiation and the DRF. At 1% DMSO the DRF was 1.2, at 10% approximately 2, and at 30%, 2.4. These authors also demonstrated a lack of protective action in the oxidation product of DMSO, dimethyl sulfone. Bridges *(25)* has reported that DMSO at a concentration of 15% (approximately 2 *M*) protected a bacterium (*Pseudomonas* sp.) against γ-radiation under anoxic conditions. This was an important observation as it indicated that DMSO was acting, at least in this test system, by a mechanism independent of the well-known radiobiological oxygen effect. The DRF for DMSO under anoxic conditions was about 2.0. Other well-known protective substances such as thiourea, glycerol, and cysteine protected *Pseudomonas* to a similar extent although thiourea, with a DRF of 2.7, was best.

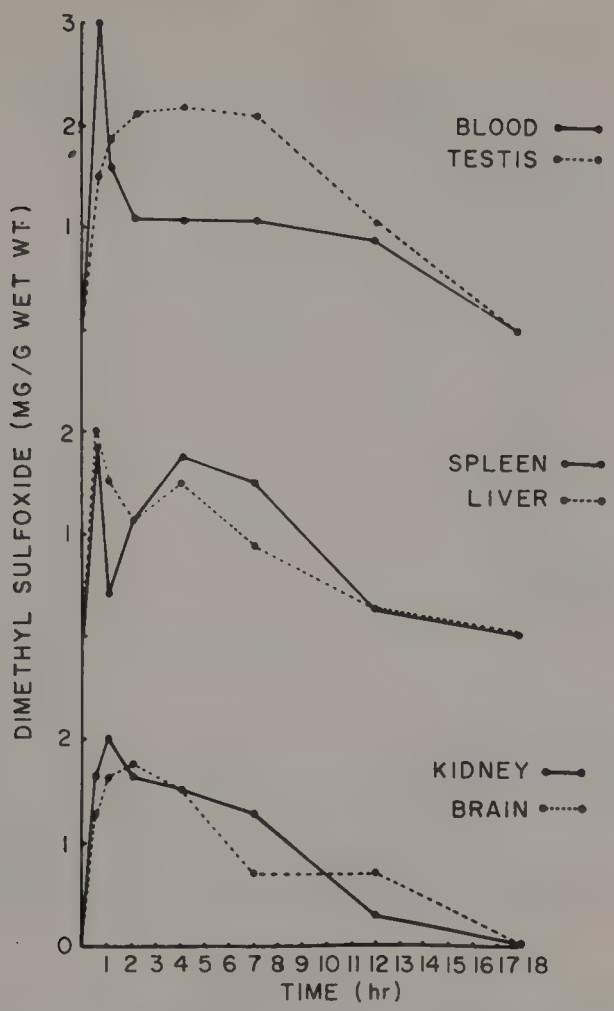


Fig. 2. Concentration of I.P. dose of 4500 mg/kg DMSO in rat tissues measured after chromatographic separation of homogenates.

TABLE 5

Effect of DMSO and AET on the Weight of Mouse Testis Irradiated with x-Rays[a]

x-Ray dose, rads	Control group (0.5 ml saline)	DMSO-treated	AET-treated
0	214.9 ± 9.5 (*9*)	216.7 ± 14.1 (*9*)	222.0 ± 7.8 (*10*)
100	138.6 ± 4.7 (*9*)	142.5 ± 8.8 (*10*)	140.3 ± 4.2 (*10*)
300	84.2 ± 4.2 (*10*)	93.4 ± 2.2 (*9*)	84.0 ± 2.2 (*9*)
500	72.6 ± 1.2 (*10*)	75.4 ± 2.3 (*9*)	68.3 ± 1.7 (*10*)
800	59.8 ± 4.6 (*8*)	57.0 ± 3.2 (*10*)	53.5 ± 3.3 (*8*)

[a]Mean testicular weight (milligrams ± S.E.) 28 days after irradiation.

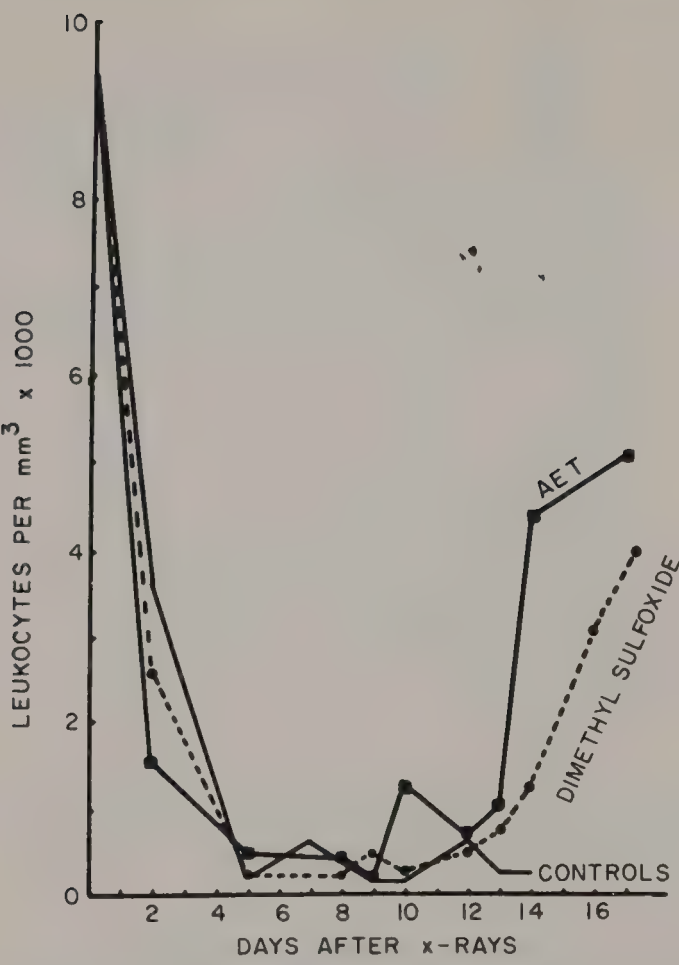


Fig. 3. Recovery of peripheral leukocytes in lethally x-irradiated mice (groups of 10) injected I.P. 30 min before x-irradiation with AET (225 mg/kg) or DMSO (4.5 g/kg).

Combinations of DMSO with these substances never resulted in DRF's greater than 2.7. Bridges suggested these substances were therefore protecting a similar radiosensitive target. Dewey *(26,27),* working with the bacterium *Serratia marcescens,* has reported that glycerol, ethylene glycol, and ethanol possess radioprotective action. Analysis of Dewey's results indicates that the amount of protective chemical required on a molar basis was similar to that reported for DMSO in studies already mentioned. The amount of DMSO in the tissues of mice after the radioprotective dose (4500 mg/kg I.P.) is sufficiently high to be within the lower limits needed to offer a small degree of protection to cells irradiated *in vitro.* It has been suggested by Vos and Kaalen *(24)* that the mechanism of protection with glycerol, ethanol, and perhaps DMSO *in vivo* and *in vitro* may be the same.

The radioprotective action of DMSO on other cellular systems has

TABLE 6

Radioprotective Effect of Combinations of AET or Cysteamine with DMSO against High Doses of x-Irradiation in Female Mice

Treatment[a]	Percent 30-day survival (20 mice per group)				
	1007 rads	1100 rads	1200 rads	1300 rads	1400 rads
AET (250 mg/kg)	100	56	45	5	0
DMSO (4.5 g/kg)	70	40	0	0	0
DMSO (4.5 g/kg) plus AET (250 mg/kg)	100	94	74	77	0
Cysteamine (175 mg/kg)	–	–	–	5	0
DMSO (4.5 g/kg) plus cysteamine (175 mg/kg)	–	–	–	55	0

[a]I.P. injection 30 min before x-irradiation.

been studied. Lohman *et al. (28)* have produced evidence to suggest that protection of the enzyme catalase from irradiation may be a result of complexing of the sulfoxide with the enzyme through Fe^{3+} which is its active site. Lohman and co-workers *(29)* have also found that DMSO protects lactate dehydrogenase from radiation damage.

TABLE 7

30-Day Survival of CBA Mice Irradiated with 1000 rads of x-Rays and Injected Immediately after x-Irradiation with 5×10^6 Syngeneic Bone Marrow Cells[a]

Treatment[b]	Number of mice surviving 30 days after x-rays/ number of mice used	Percent survival
No cells	0/20	0
5×10^6 Cells	18/20	90
5×10^6 Cells irradiated *in vitro* with 800 rads	0/20	0
5×10^6 Cells irradiated *in vitro* with 800 rads in presence of 10% DMSO	6/20	30
5×10^6 Cells irradiated *in vitro* with 800 rads in presence of 15% DMSO	2/20	10

[a]Cells irradiated *in vitro* in presence or absence of DMSO.

[b]Syngeneic marrow cells injected into mice irradiated with 1000 rads (LD_{90}) of x-rays.

Whatever the basic mechanism of radioprotection may be at the cellular level, there are several profound physiological changes that occur when DMSO is injected into mice or rats. These changes must not be overlooked when discussing protective action. It is probable that DMSO protects against lethal effects of whole-body radiation through several mechanisms. It has already been mentioned that large doses of DMSO injected into mice and rats produce hypertension and splenic anoxia *(23)*. A commonly observed phenomenon is hypothermia *(6)*, the time course of which is shown in Fig. 4. It is apparent from an examination of this figure that the magnitude of radioprotection afforded by DMSO is constant at a value of 70% during the first hour after injection, during which time the hypothermia varies widely. However, as the body temperature increases, there is a fall in the degree of protection afforded by the injected DMSO. It is well known that the extent of hypothermia produced by DMSO and other antiradiation drugs is unlikely to provide any protective effect per se. Hope *(30)* reported that radioprotective compounds (injected into mice) produced a moderate fall (4–5 °C) in whole-body temperature. He also showed that the radioprotective, sedative, and hypothermic actions of serotonin were all abolished by administration of its specific pharmacological antagonist, bromolysergic acid diethylamide. Hope was most careful to point out, however, that although all radioprotective compounds were capable of producing hypothermia when injected into animals, chemicals with hypothermic properties were not necessarily radioprotective. An example of this is shown in Fig. 5. Nicontinamide, which induces marked hypothermia in mice, has no radioprotective action.

An experiment in which the hypothermic effect of AET injected into groups of mice was abolished by keeping them at an ambient temperature of 37 °C demonstrated that AET still produced a maximum protective effect *(5)*. The hypothermia associated with the administration of radioprotective drugs was not associated with radioprotection. Bacq and co-workers *(31)* also reported that the hypothermia induced by antiradiation compounds was not linked to radioprotective action. Why do these compounds produce hypothermia? Several workers have demonstrated that the enzymic activities of a number of cellular systems such as DNA synthesis, lipid snythesis, and protein synthesis are reversibly inhibited *in vitro* when DMSO or glycerol is present at concentrations greater than 3–5% *(4)*. The effect of DMSO and glycerol on protein synthesis as measured by the uptake of glycine-^{14}C into protein is shown in Fig. 6. This fact must not be overlooked when

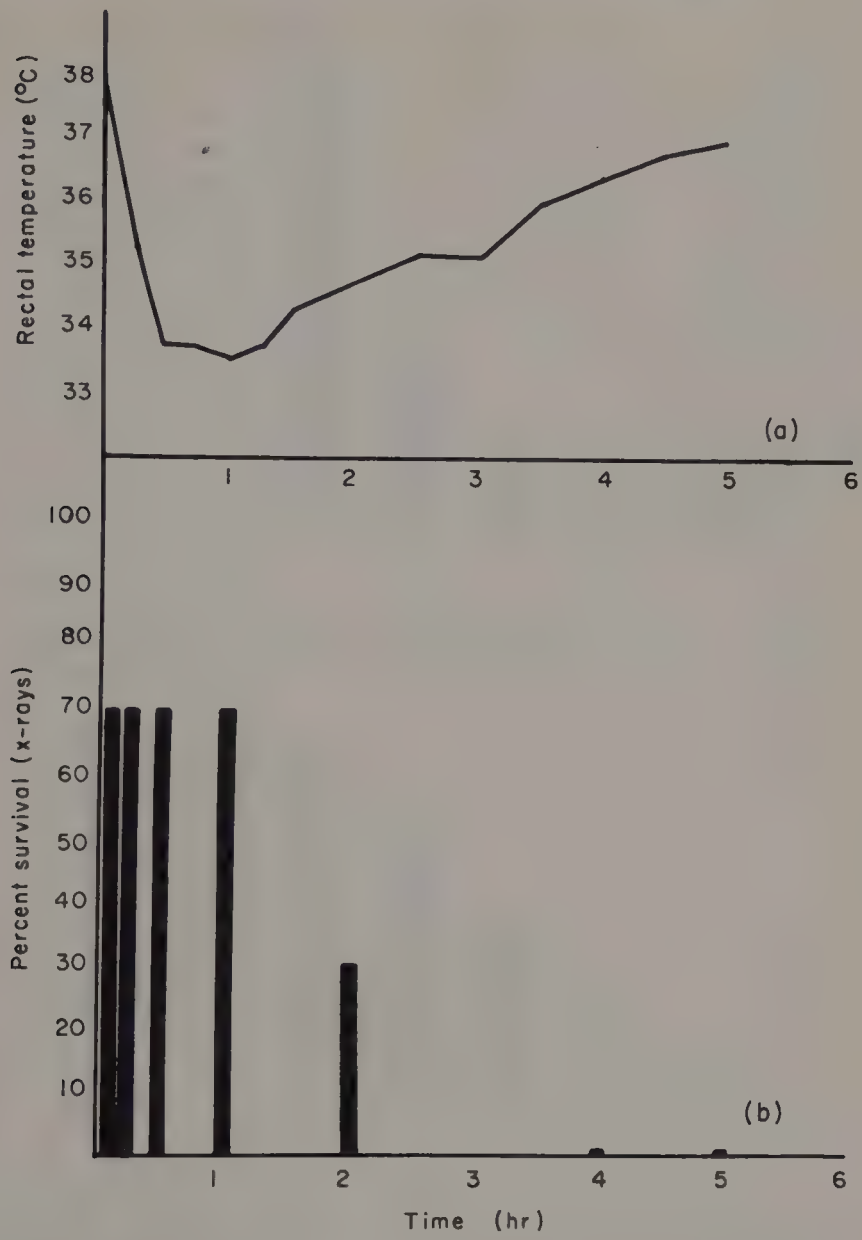


Fig. 4. Effect of DMSO (4.5 g/kg) on body temperature and radiation sensitivity of mice. (a) Hypothermic effect; (b) radiation protective effect.

seeking an explanation of the basis of cellular radiosensitivity when these compounds are present. Also, hypothermia produced by DMSO might be related to a general decrease of cellular metabolism as a result of a partial generalized inhibition of enzyme systems.

Discovery of the membrane penetrant action of DMSO has recently

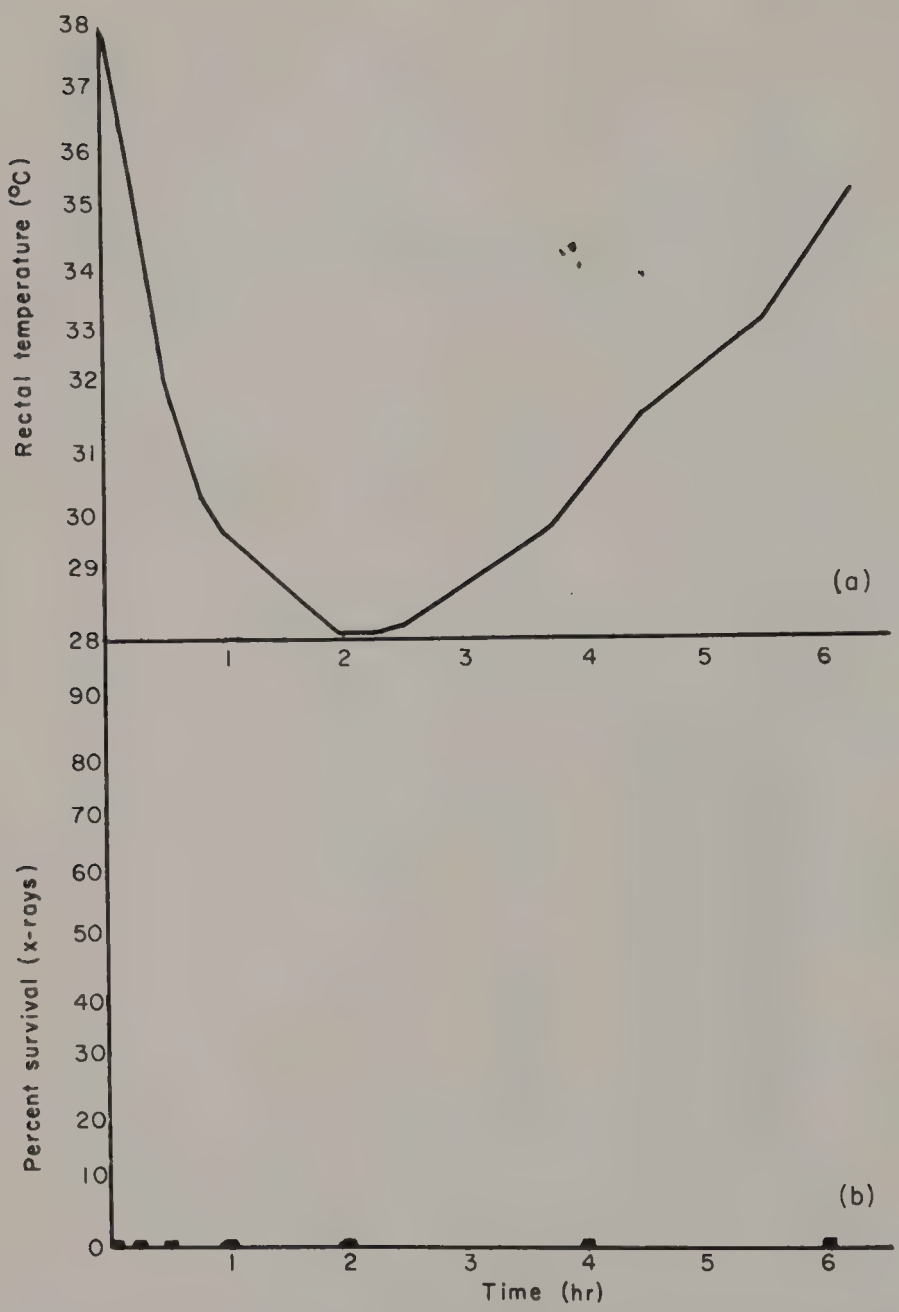


Fig. 5. Effect of nicotinamide (1200 mg/kg I.P.) on body temperature and radiation sensitivity in mice. (a) Hypothermic effect; (b) radiation protective effect.

led to studies of topical protection against irradiation damage by DMSO. Dod and Scheiwell *(32)* have shown that 20-min topical pretreatment of the limb of a rat protects against x-ray-induced epilation.

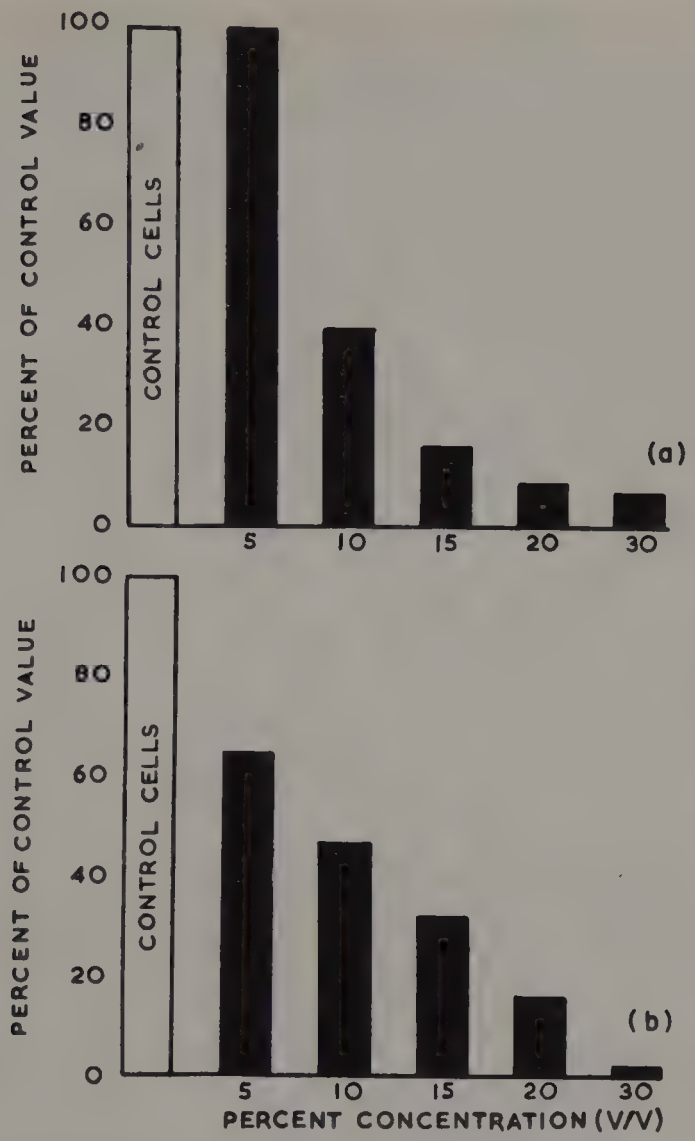


Fig. 6. Effect of DMSO and glycerol on the protein synthesis of mouse bone marrow *in vitro.* (a) DMSO; (b) glycerol.

V. CRYOPHYLACTIC PROPERTIES OF DMSO

There is now a vast literature on the subject of low-temperature preservation of cells. Excellent books on this subject by Smith *(33),* Meryman *(34),* and Luyet and Gehenio *(35)* cover much of the earlier work on effects of low temperature on biological systems and contain historical information.

Few vertebrate or invertebrate cells survive freezing and thawing.

Among the vast range of invertebrates there are a few specialized animals or stages in the life history of animals that possess a peculiar tolerance for exposure to low temperatures. Larvae of various insects, desiccated rotifers, tardigrades, and certain intertidal molluscs are examples of this type of adaptation. When most invertebrate and vertebrate mammalian cells are frozen and thawed, the majority are killed; however, under special circumstances a few may survive. For example, the survival rate of erythrocytes is extended when the water content and/or cooling rate is manipulated.

To store cells, tissues, and organs for prolonged periods of time, two approaches are possible. First, water can be removed by dehydration of freeze-drying as with plasma, enzymes, viruses, and bacteria. With a low residual moisture content these materials are relatively stable for extended periods at normal room temperature. Mammalian cells and tissues that are freeze-dried do not resume viability after suspension in water. Some day this may be possible, but as yet the reports that semen and red blood cells can be freeze-dried have been either recanted or unconfirmed. The second method, development of which has been largely empirical, depended on the chance discovery in 1949 by Polge *et al. (36)* that glycerol protected fowl spermatozoa against freezing and thawing damage. This method is the one routinely used by experimental biologists and clinicians to store blood, bone marrow, platelets, sperm, and perhaps even whole organs such as kidney.

The changes produced in cells when water turns to ice are profound. Most cells are destroyed by alterations associated with the phase shifts of water to ice. At lower temperatures perhaps the most damaging changes result from exposure of lipoprotein membranes and other subcellular components to the high concentrations of electrolytes, which have increased in the temperature region between the water/ice change and the eutectic temperatures of the various salts. If cells can be steered safely through this temperature region, which in practice extends over a limited range of about −1 to −35 °C, then temperatures lower than these seem in themselves not to be damaging and will lead to prolonged storage times. Substances such as glycerol, methanol, and DMSO enable cells to pass safely through these damaging temperature ranges.

VI. MECHANISMS OF CRYOPHYLACTIC PROTECTION

An excellent account of the chemical nature and physical properties of compounds that protect living cells against damage from freezing

and thawing has recently been reported by Nash *(37)*. The conventional and still the most reasonable view having much supportive experimental evidence is that substances such as glycerol act through their colligative properties. According to this concept, originally proposed by Lovelock, glycerol, DMSO, or other neutral and nontoxic solutes in the medium lower the concentration of electrolytes in equilibrium with ice at any temperature below freezing. This also applies to natural salts inside cells, provided the neutral solute can penetrate the cells. Lovelock suggested that if enough glycerol, DMSO, or neutral solute were present the electrolyte concentrations would not rise to a critically damaging level until the temperature was so low that the damage produced by the increased electrolyte levels was reduced sufficiently to be tolerated by the cells. Because DMSO penetrates cells readily, its protective action appears to be explained best by the concept suggested by Lovelock in reference to glycerol.

Nash believes *(37)* that the most important single property possessed by effective endocellular cryophylactic agents is the strong affinity they possess for water. He has studied the freezing and thawing of human red cells in DMSO. His results, which are shown in Fig. 7, are almost identical to those obtained with glycerol and some protective amides. They demonstrate that equivalent amounts of various protective substances give identical results. The solubility of salts is pure protective and nonprotective compounds and how these solubilities change when water is added to the system have been studied by Nash *(37)*. Results of several experiments are shown in Fig. 8. It is clear that the ability to dissolve salt is not correlated with protective ability either directly or inversely. Formamide, an excellent solvent for salt, does not protect, and acetone and acetonitrile, both nonprotective compounds, are poor salt solvents. The concept that high salt concentration is the principal cause of cellular death following freezing and thawing led Nash to suggest that dimethylformamide might remove the salt as a solid. A list of water solubilities and other physical properties of a variety of compounds as proposed by Nash appear in Table 8. The "required protective coefficient" Q has been derived from some of the physical values listed in this table. All known protective compounds, including DMSO, have a Q value as great as or greater than 1. Relationships between the Q value and other physical characteristics of a chemical, such as hydrogen donating and accepting properties, possession of lone pair electrons, and basicity have been explored in considerable detail by Nash. Possible connections between cryophylactic properties and germicidal properties are also discussed by Nash *(37)*. A word of

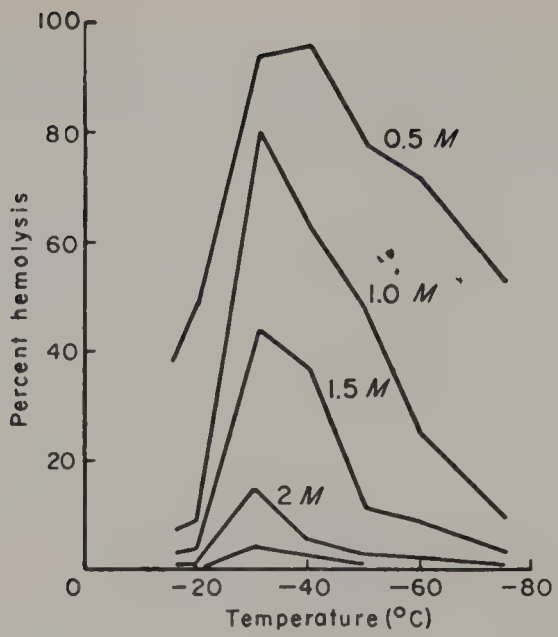


Fig. 7. Prevention of hemolysis of red blood cells by different concentrations of DMSO. Cells were frozen for 15 min at the indicated temperatures. At 2.5 *M* (lowest curve) the compound produced almost complete protection at all temperatures.

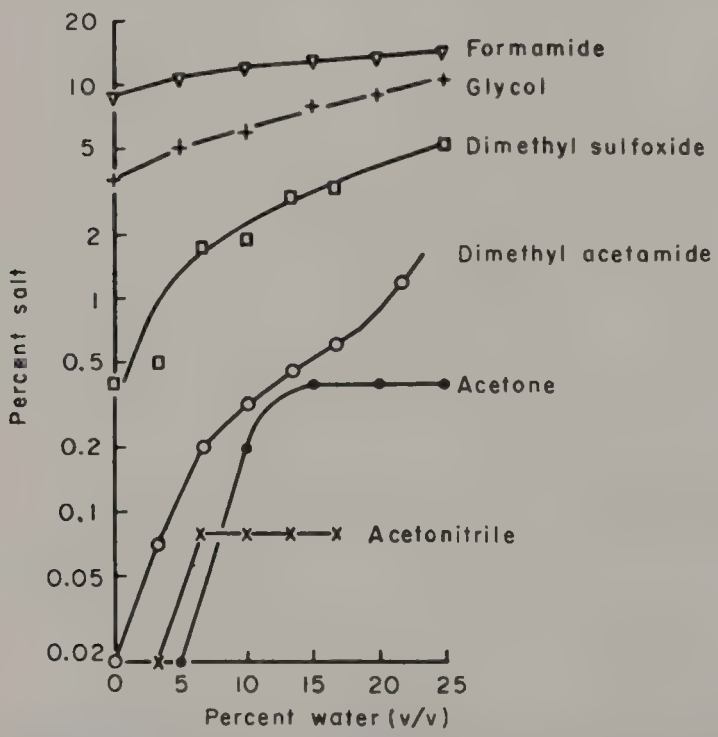


Fig. 8. Solubility of sodium chloride in different compounds, each containing up to 25% water (v/v) at 20 °C.

TABLE 8

Water Solubility and Other Properties of Various Compounds[a]

Compound	*P*	*P(s)*	*G(s)*	MSE	*P(v)*	*G(v)*	*Q*	Protection
$PhNH_2$	256	−25	230	−ve	420	165	−ve	Not tested
Me_3N	180	120	300	0.7	190	10	0.45	Toxic
Pyridine	195	100	295	0.5	300	105	0.5	Toxic
MeCN	106	60	165	0.55	250	145	0.5	None
Me_2O	128	80	210	0.6	130	0	0.3	Not tested
Acetone	156	60	215	0.4	225	70	0.3	None
Bu-lact.	190	80	270	0.4	460	270	0.6	None
Me_2SO	176	230	405	1.3	420	245	1.8	Complete
PyNO	212	>220	>430	>1.0	600	390	>2.0	Partial
Me_3PO	228	320	550	1.4	620	390	2.9	Not tested
Me_3NO	196	>480	Very high	Very high	Very high	Very high	Very high	None[b]
$MeNO_2$	123	15	140	0.15	285	160	0.1	Not tested
MeCOOMe	173	40	210	0.25	225	50	0.2	Not tested
$MeCONH_2$	145	115	260	0.8	495	(350)	1.4	Good
$MeCONMe_2$	225	170	395	0.75	380	155	1.0	Complete
Phenol	223	Nil	225	0.0	420	(195)	0.0	Toxic
Resorcinol	250	100	350	0.4	630	(380)	0.8	Toxic
Methanol	88	120	210	1.4	240	(150)	1.1	Partial
Glycol	146	220	365	1.5	440	(295)	2.2	Complete
Glycerol	205	320	525	1.6	680	(475)	3.3	Complete

[a] *P*, parachor of the simplest compound capable of bearing the group concerned; *P(s)*, parachor of the alkyl chain which has to be added in order that the solubility be 1 M, *P(s)/P*, molar solubility coefficient (MSE); *P(v)*, parachor of the normal paraffin whose boiling point is the same as that of the original compound; $Q = P(v) \times \text{MSE}/300$. $G(s) = P + P(s)$ and $G(v) = P(v) - P$ are group parameters. Except for *P*, figures are rounded off. Protection refers to protective action on red blood cells frozen down to −79°C and intermediate temperatures. Bu-lact., butyrolactone; PyNO, pyridine *N*-oxide.

[b] Does not penetrate cells.

caution, however, seems in order regarding the excellent theoretical account by Nash on cryophylactic compounds. Many of the experiments reported relate to the protection of red blood cells against freezing and thawing damage, and some of the observations may not be applicable to the mammalian nucleated cell.

Farrant and his co-workers *(38)* investigated two alternative procedures using DMSO with the purpose of preventing damage associated with a rise in electrolyte concentration during freezing. The test organ employed by these workers was the guinea pig uterus. The capacity of smooth muscle in this organ to respond to standard doses of histamine

was used to test function after different freeze-thaw procedures. Electron microscope studies were made on the smooth muscle preparations. Three comparative observations were carried out: (1) freezing and thawing uteri with added DMSO according to the standard procedures adopted for cell suspensions; (2) treatment of uteri with DMSO solutions at temperatures above the freezing point; (3) freezing in the absence of any protective substance such as DMSO. Uteri frozen at −79°C and thawed from this low temperature without DMSO do not respond to histamine. Neither do they contract spontaneously. Examination of these specimens by electron microscopy revealed changes, but the most conspicuous was extensive fracturing of the cell membrane and mitrochondrial envelopes. Uteri frozen in the presence of DMSO (1.4 *M*) according to standard procedures (i.e., similar to those used for sperm or tissue culture cells) showed partial recovery of uterine contractability after thawing from −79°C. However, in spite of the presence of 1.4 *M* DMSO the structural damage to the cell was comparable to that seen in the uteri frozen without DMSO, although Farrant noted that fewer cells had completely disintegrated. Clumping of nuclear chromatin was also observed. When the "new" method of freezing uteri with DMSO was used, results different from the two so far discussed were obtained. Uteri thawed with the new method quickly recovered response when challenged with histamine, and the abnormal slow contractions seen with other methods of freezing and thawing were absent. Additionally, the overall function of the muscle was comparable to that of untreated uteri. There was also considerably less structural damage, and the cell membranes were essentially unchanged. Although most subcellular components in the uteri frozen by this new method were in good condition. the mitochrondia were seen to have been altered. Many mitochondria had assumed the form of a long chain of alternating swollen and collapsed segments. These mitochrondria were, however, apparently intact.

What is the rationale of the new method developed by Farrant from theoretical considerations and then applied so successfully to a complex tissue such as the uterus?

Both Nash *(37)* and Farrant *(38,39)* have discussed the changes in electrolyte concentration and the osmotic effects associated with the slow freezing and thawing of mammalian cells. Farrant had demonstrated quantitatively that DMSO lowered the amount of ice in equilibrium with the remaining solution at any temperature during freezing (Fig. 9). It follows, then, that the increase in concentration of

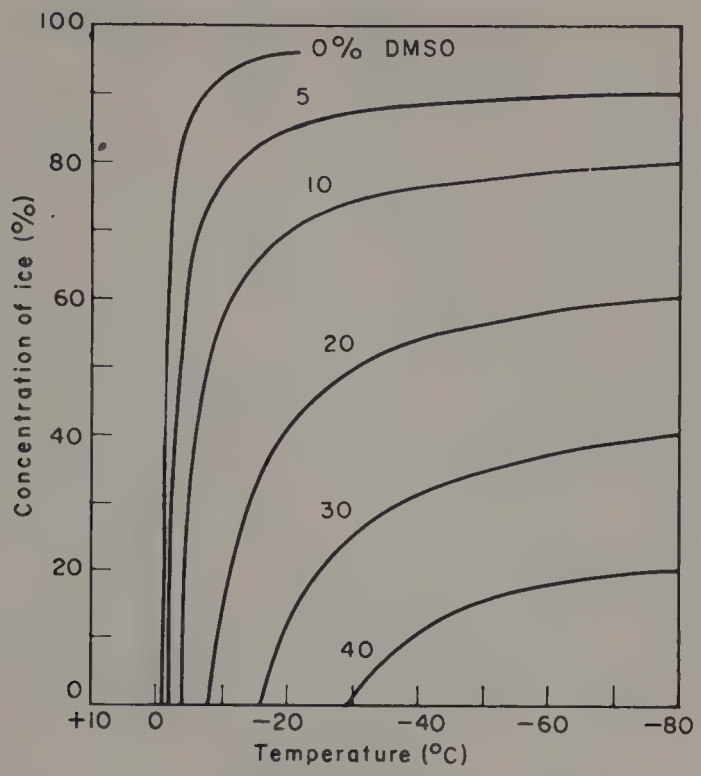


Fig. 9. Effect of initial concentration of DMSO (v/v) on percentage of ice formed at different temperatures. All solutions contained sodium chloride (0.154 *M*) initially.

electrolytes and other solutions is less marked at any temperature during the freezing process when DMSO is present (Fig. 10). The higher the initial concentration of DMSO, the less the damage to cells associated with a buildup of electrolytes during freezing and thawing. However, this ideal state cannot be realized because DMSO, although remarkably nontoxic, is not without damaging effects at very high concentrations (above 15%). At low temperatures, however, the toxic effects of large amounts of DMSO are less. The "new method" developed by Farrant is essentially based on the concept of replacing the volume of liquid converted into ice at any one temperature (see Fig. 11) by the addition during cooling of calculated volumes of solutions of DMSO in de-ionized water, the liquid volume being kept constant. This technique is shown in diagram form in Fig. 12. When a solution of volume V ml is cooled from T_1 to T_2°C and the concentration of DMSO has increased from C_1 to C_2 as a result of separation of ice, then the addition of $[1\text{-}(C_1/C_2)]V$ ml of DMSO (concentration C_2) in water will return the volume to its original value. It is necessary, in

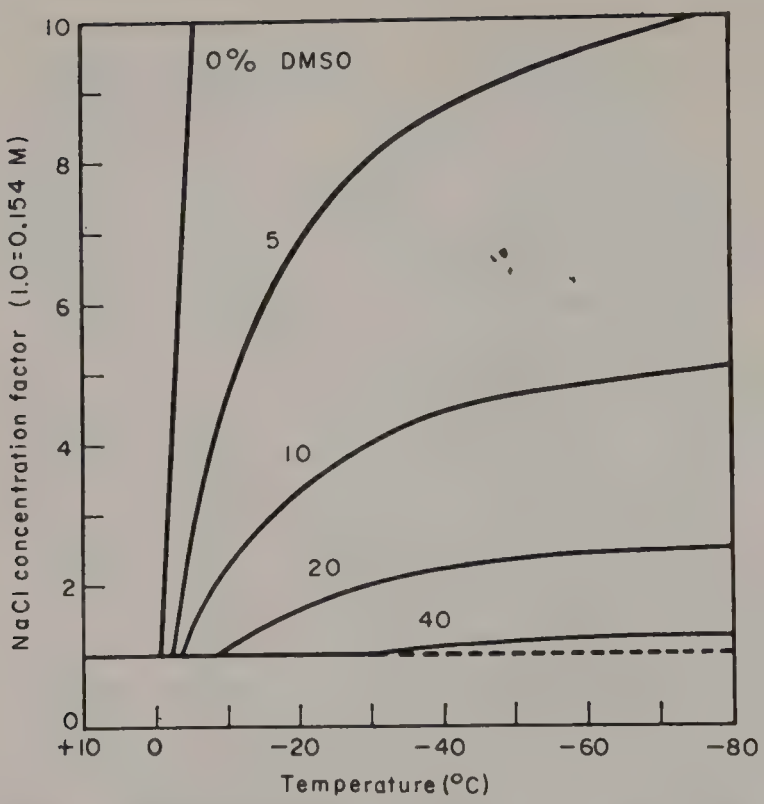


Fig. 10. Effect of initial concentration of DMSO (v/v) on concentration of sodium chloride in remaining liquid phase at different temperatures during freezing. All solutions contained sodium chloride (0.154 *M*) initially.

order to perform these techniques perfectly, to know the equilibration times for the distribution of electrolytes between the various components of the system, and this really requires for each organ or tissue quantitative data on diffusion times. Theoretically, thawing should take place at the same rate as cooling, but for various good reasons this was not done in the experiments with uteri reported by Farrant and his colleagues. The conclusion reached by Farrant *et al. (38)* in these studies are best quoted in their own words: "these experiments have shown that structural and functional damage to an organized tissue after thawing from −79°C can be greatly reduced if solute concentrations are kept close to normal levels during freezing." It would appear that methods based on Farrant's ideas are the most likely to succeed in preserving complex tissues and organs at low temperatures. The major difficulty, however, is the length of time required for diffusion of electrolytes and protective agent at the various temperature stages which such procedures employ. The larger the organ, the more difficult is this problem.

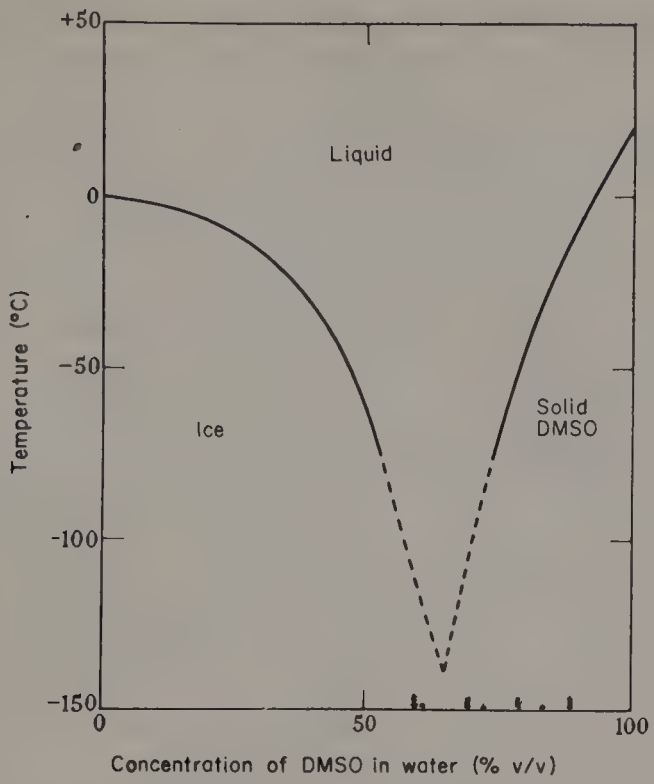


Fig. 11. Eutectic diagram of DMSO–water system.

DMSO has been employed for preservation of different cells and tissues. A selected list of readings provided merely as a guide to the subject, is given in Section VIII.

VII. CONCLUSIONS AND DISCUSSION

The possibility that the radioprotective and cryophylactic properties of DMSO are related to each other has been discussed *(5)*. Vos *et al. (40,41)*, who have made an extensive study of the radioprotective properties of compounds with cryoprotective properties, concluded that these substances could be separated into three groups.

(1) Substances that possess both radioprotective and cryoprotective properties such as DMSO, glycols, and glycerol.

(2) Substances with cryoprotective properties only; pyridine *N*-oxide is the best example.

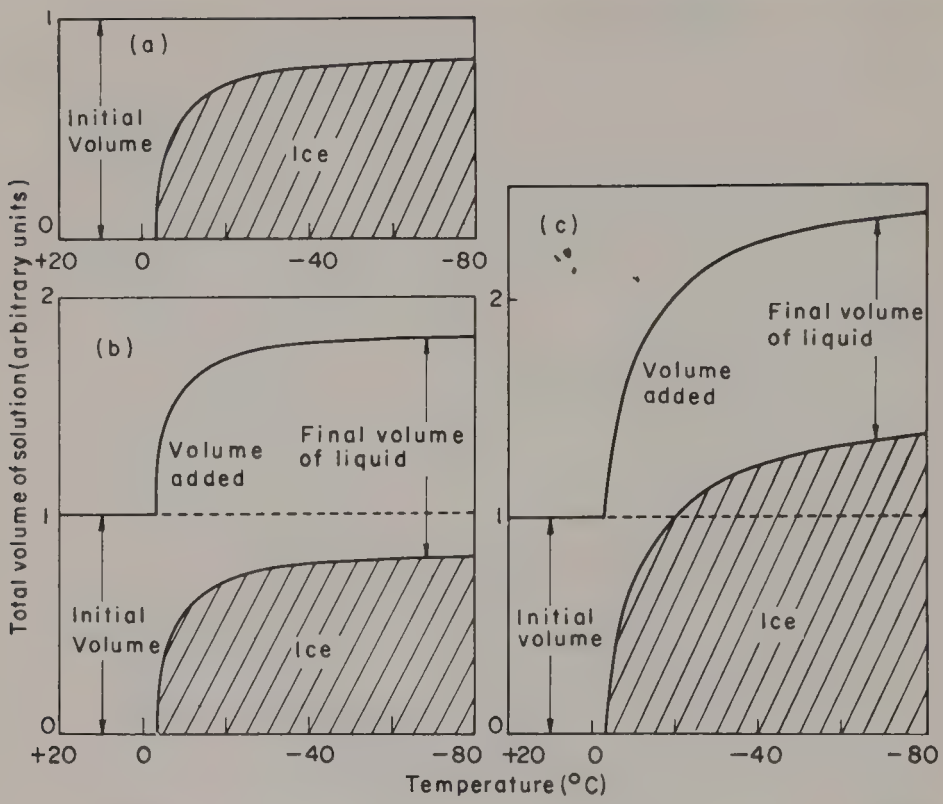


Fig. 12. Principle of cooling procedure used in group VI. The volumes are calculated for the model system of sodium chloride (0.154 *M*) plus DMSO (1.4 *M*) in water. (a) The DMSO and sodium chloride in solution become concentrated in the diminishing liquid phase as ice separates. (b) The volume of the liquid phase can be maintained at a constant value by the addition of amounts of DMSO in deionized water throughout freezing. (c) Since the extra volume added deposits ice on further cooling, the correct volumes to be added are shown in this diagram.

(3) Substances that have radioprotective properties but lack cryoprotective effects (ethanol, methanol, and resorcinol).

This classification is not completely accurate, however, since Lovelock *(42)* has demonstrated the cryoprotective action of methanol and ethanol with erythrocytes.

DMSO protects cells in tissue culture *(24,40)* and bacterial cells *(25,27)* against radiation damage under anoxic conditions. Thus, anoxia is probably not involved in the protective mechanism. The possibility that DMSO or glycerol functions in a manner similar to that of either

AET or cysteamine seems unlikely. A suggestion that glycerol acts as a radioprotective agent by dehydrating cells has been made *(43),* but it seems more likely that the presence of glycerol rather than the absence of water is more important. Vos *et al. (40)* theorized that DMSO, glycerol, and other chemicals of a similar nature have the ability to bind water in a "water cloud" around target molecules. The replacement of water in the vicinity of a target molecule may alter response to either radiation damage or freezing damage. AET, cysteamine, and cysteine bind hydrogen through donation of a proton from their SH groups. The sensitive targets for radiation damage and freezing damage are not the same. This may explain why many radioprotective drugs are not cryophylactic agents. However, lipoprotein membranes are sensitive to radiation and freeze-thaw damage. It is thus possible that DMSO and glycerol protect these structures without protecting DNA from radiation. AET and cysteamine protect DNA but not lipoproteins and thus are inactive against freezing damage.

In four well-documented systems, DMSO or glycerol can either prevent or modify destructive changes:

(1) A wide variety of vertebrate cells, invertebrate cells, and microorganisms can be protected from freeze-thaw damage and changes in electrolyte concentration by the addition of DMSO or glycerol.

(2) Enzymes sensitive to damage during freezing and thawing can be protected by DMSO. The formation of subunit hybrids when lactic acid dehydrogenase is frozen and thawed can be prevented by DMSO *(44).*

(3) Enzyme instability at temperatures just above 0C resulting from the formation of a series of low-molecular-weight subunits can be prevented by the presence of DMSO or glycerol *(45).*

(4) Animals, mammalian cells in tissue cultures, bacteria, yeasts, and proteins can be protected by DMSO from radiation damage. The protective factors range from 1.4 to 3.0. Protection is independent of oxygen and synergism as has been shown with AET or cysteamine in mice *(22).*

Are there any common features in this list? The most obvious one is the liability of hydrogen bonds to the imposed stresses in systems (1) to (3). The possibility exists that DMSO is involved in modifying effects that interfere with normal hydrogen bonding.

VIII. A SELECTION OF READINGS DEALING WITH THE CRYOPHYLACTIC PROPERTIES OF DMSO

A. Blood, Bone Marrow, Lymphocytes, Leukocytes, and Platelets

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5. M. J. Ashwood-Smith, The Preservation of Bone Marrow, *Cryobiology,* **1,** 61 (1964).
6. M. J. Ashwood-Smith, Low Temperature Preservation of Mouse Lymphocytes with Dimethyl Sulfoxide, *Blood,* **23,** 494 (1964).
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9. H. B. Barner, Frozen Storage of the Spleen, *Surg. Forum,* **16,** 83 (1965).
10. B. A. Bouroncle, Preservation of Human Normal and Leukemic Cells with Dimethyl Sulfoxide at −80°C, *Cryobiology,* **2,** 310 (1966). Abstract.
11. J. A. Cavins, S. Kasakura, E. D. Thomas, and J. W. Ferrebee, Recovery of Lethally Irradiated Dogs Following Infusion of Autologous Marrow Stored at Low Temperatures in Dimethyl Sulfoxide, *Blood,* **20,** 730 (1962).
12. J. A. Cavins, Preservation of Dog Bone Marrow at Low Temperature in Dimethyl Sulfoxide, *Blood,* **20,** 635 (1962).
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Chapter 6

Use of DMSO in Enzyme-Catalyzed Reactions

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I. INTRODUCTION

The natural solvent of a living system is water, and for this reason water has been the solvent used almost exclusively in studies of enzymic reactions. However, within the cell the nature of the enzyme's cellular environment can be expected to influence its catalytic function. Thus, in closely compartmentalized reactions such as those occurring within the mitochondrion, it is appropriate to consider the influence of solvent viscosity, dielectric constant, hydrogen bond-accepting and -donating capacity, and relative acidity and basicity, in addition to other factors *(1,2)*. In the study of isolated enzyme reactions, alteration of this type in the chemical properties of the reaction solvent can be achieved by using partially aqueous systems. Such studies can be of value in

estimating environmental effects on these reactions. For this kind of study it is apparent that the chemical nature and concentration of the organic solvent are critical, but in enzymology such solvent information is somewhat limited. In general this limitation is the result of the sensitivity of proteins to the denaturing effects of organic solvents, particularly protic solvents *(3)*. The organic solvent dimethyl sulfoxide (DMSO) has been used in high concentrations in enzyme reactions with little permanent damage to the enzyme protein as evidenced by the retention of biological activity *(4)*. It is the purpose of this chapter to review its use with a number of different enzyme systems.

DMSO is a dipolar aprotic solvent. In its polarized form (Fig. 1) its oxygen as well as its sulfur has unshared electron pairs. Its broad

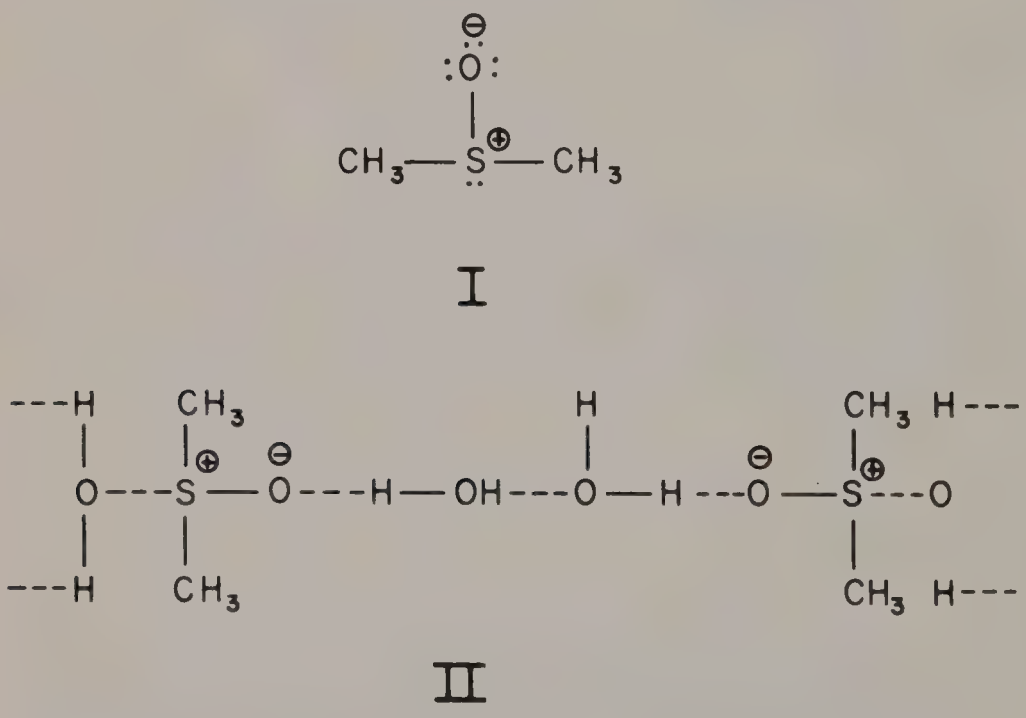

Fig. 1. Structure of dimethyl sulfoxide. Structure I represents a polarized form of DMSO. Structure II represents a possible hydrated form.

solvent characteristics are the result of an ability to form either stable solvates by dipole–dipole interactions or solvent–solute associations by hydrophobic interactions. It is hydroscopic and miscible with water in all proportions. With water it is probable that a 2:1 association complex is formed *(5)*. The formation of this hydrate is accompanied by the

evolution of heat. It has been suggested that the hydrogen bonds that exist between water and DMSO are stronger than the hydrogen bonds that exist between water molecules *(5)*.

II. PROTEINS AND NUCLEIC ACIDS

DMSO has been demonstrated to be an effective solvent for a wide variety of biological macromolecules such as proteins *(6–9)* and nucleic acids *(10)*. With proteins it has been shown to be a good structure perturbant *(11)*, modifying the protein's tertiary and possible secondary conformation *(9,11,12)*.

Direct evidence that DMSO can alter the conformation of proteins has been presented by Herskovits and Laskowski *(11)*. In protein topographical studies using the method of difference spectra, these authors have demonstrated that certain organic solvents induce shifts in the absorption maxima of tyrosine and tryptophan residues in the protein. This shift has been attributed to changes in the environment that surrounds these groups. Thus, in the absence of solvent these groups are buried within the native protein; with the addition of the organic solvent, protein conformational changes occur, with the resultant exposure of these chromophores. The extent of these spectral changes in different solvents is a measure of the exposure of these groups and can be related to solvent-induced conformational changes in the protein. In a study with bovine serum albumin comparing the efficacy of DMSO with ethylene glycol, polyethylene glycol, glycerol, and sucrose as a spectral perturbant, it was found that DMSO was most effective *(11)*. Despite the differences in the chemical structure of these substances, it was indicated that there is a correlation between the size of the solvent and its effectiveness as a perturbant with this protein. It was suggested that because of its comparatively small size DMSO is able to penetrate interior regions of the protein more readily than the other bulkier solvents. This effect depends upon the location of the chromophore and is not found for all proteins *(13)*.

DMSO has end absorption in the ultraviolet region of the spectrum. In high concentrations this absorption is significant and often presents problems in experiments that require ultraviolet spectrophotometry *(10)*. Most amino acids are asymmetric, and when covalently linked in protein their optical rotatory properties are influenced by their geomet-

rical position within the protein. Changes in the geometry or conformation of the protein are therefore reflected in changes in its optical rotation. Thus, the optical rotation of a protein or, more specifically, its optical rotatory dispersion, is uniquely suited for protein conformational studies. The latter technique measures changes in optical rotation as a function of wavelength. Using this technique, Hamaguchi *(9)* has studied the effect of increasing concentrations of DMSO on the enzyme lysozyme. In these studies it was observed that major configurational changes occurred with DMSO concentrations between 60 and 70 vol. %.

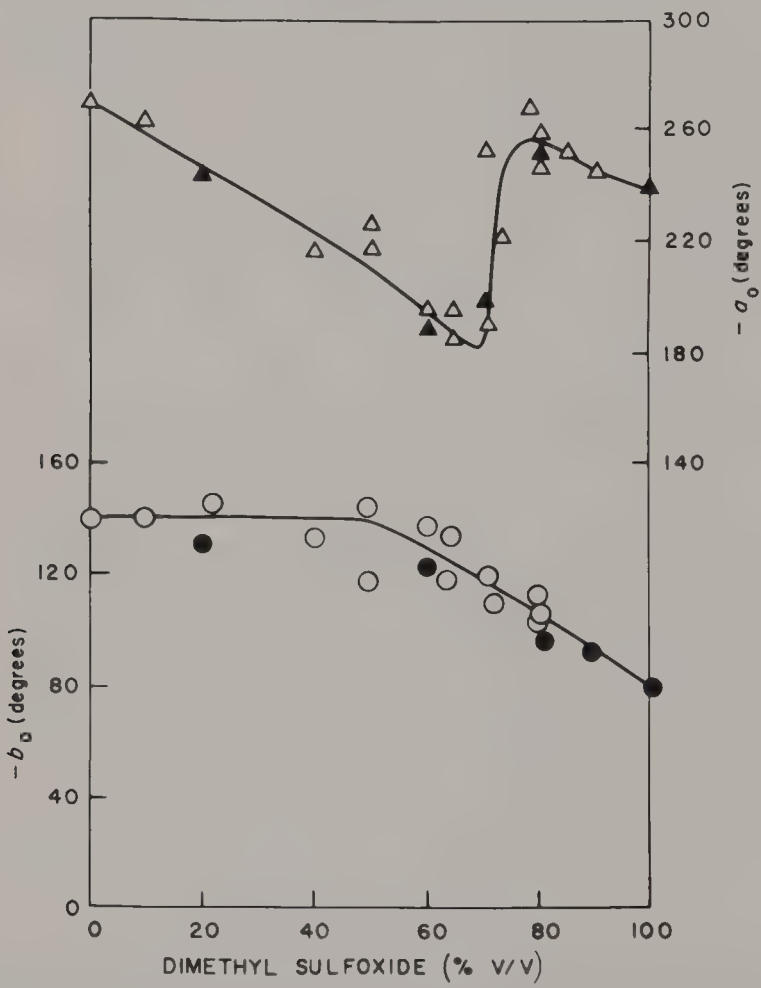

Fig. 2. Optical rotatory properties of lysozyme in DMSO–water mixtures. Variations of the value of a_0 (triangles) and b_0 (circles) with solvent composition in DMSO–water mixtures at 25°C. The open symbols represent solutions prepared by adding DMSO to aqueous lysozyme solutions. The closed symbols represent solutions prepared by adding water to DMSO solutions of lysozyme *(9)*.

This effect is clearly shown in Fig. 2. Here the constants a_0 and b_0 are derived from the curve fitting of experimental data using the equation of Moffitt and Yang *(14)*. The constant a_0 represents the intrinsic amino acid rotation and its interaction with the secondary structure of the protein. It should vary as the environment varies the intrinsic amino acid rotation. The constant b_0 is a function of the helical content of the protein. These conformational changes are completely reversible as measured by both physical and enzymic methods. Although conformational changes in proteins have been determined for the most part by measuring differences in some physical property of the protein *(15)*, enzyme activity can also be used for this purpose because the activity of an enzyme is generally greatly affected by changes in its protein structure *(8,16)*. Thus, organic solvent-induced changes in enzymic activity can be used as an indicator of conformational change. From the studies with lysozyme, it is clear that DMSO can cause directly measurable changes in protein conformation and, more importantly, from a number of other studies *(7,17)* it appears that enzymes can function in or be kept in high concentrations of DMSO with little loss in activity.

III. GLUTAMATE DEHYDROGENASE, AN ALLOSTERIC ENZYME

Glutamate dehydrogenase is an enzyme whose catalytic activity is reversibly affected by a variety of small molecules *(18,19)*. This modification of enzymic activity is postulated to be the result of specific conformational changes *(16)* in the enzyme protein produced by these substances *(18,20,21)*. This enzyme oxidatively deaminates the dicarboxylic amino acid glutamate, as shown in Eq. (1).

$$\text{L-Glutamate} + \text{DPN}^{2+} \underset{\rightleftharpoons}{\text{Enz}} \alpha\text{-Ketoglarate} + \text{NH}_4^+ + \text{DPNH}$$

It has also been shown to be capable of deaminating a monocarboxylic amino acid such as L-alanine, but at only 0.27% of the rate at which it utilizes L-glutamate *(22)*. In the presence of the steroid analog diethylstilbesterol, alanine activity is strongly stimulated, while glutamate dehydrogenase activity is coincidentally inhibited *(19)*. In investigations concerned with the effect of DMSO on a variety of enzyme systems

(23), and particularly on complex reactions of this type *(24),* it was observed that DMSO markedly affects the apparent specificity of glutamic dehydrogenase. The effects of varying concentrations of DMSO on the deamination of glutamate and alanine are shown in Fig. 3. It is apparent from this figure that the solvent affects these activities differently. With increasing concentrations of DMSO glutamate dehydrogenase activity decreases, while alanine dehydrogenase activity increases markedly. With saturating substrate and coenzyme concentrations DMSO has little apparent effect on the rate of deamination of glutamate up to a concentration of about 20%; however, further increases in its concentration lead to a drop in this rate. On the one hand, at 20% solvent concentration essentially all of the initial activity is retained, while a further increase of 10% in the solvent concentration reduces the rate by 40%. On the other hand, there is essentially an exponential increase in alanine dehydrogenase activity with increasing concentrations of DMSO, the rate at 40% solvent concentration being 14 times greater than the rate with no DMSO.

The effect of DMSO on the reverse reaction, that is, the amination of α-ketoglutarate or pyruvate at saturating substrate concentrations, is shown in Fig. 4. In general the effects of DMSO on these reactions are similar to the deamination reaction, that is, it stimulates the rate of amination of pyruvate to alanine and inhibits the amination of α-ketoglutarate. However, the amination of α-ketoglutarate is much more sensitive to the solvent than is the deamination of glutamate. This is indicated by the sharp fall in the rate even at low solvent concentrations. Thus, in 20% DMSO only about 8.5% of the initial activity remains.

The nucleotide derivative adenosine 5′-pyrophosphate (ADP) has been shown to affect glutamate dehydrogenase activity in a fashion which, superficially, is the reverse of the action of DMSO; it stimulates the deamination of glutamate *(18)* and inhibits the deamination of alanine *(19)*. Tompkins *et al.* demonstrated that this inhibition of alanine dehydrogenase activity can be reversed by the addition of diethylstilbesterol. The inhibition of alanine dehydrogenase activity by ADP is shown in Fig. 5a. The inhibition under the conditions of the assay is never total, but reaches approximately 38% in 1×10^{-4} *M* ADP. Increasing the concentration of ADP 10 times does not significantly increase this inhibition. The effect of dimethyl sulfoxide on this inhibition is recorded in Fig. 5b. It is clear that this solvent, similar to

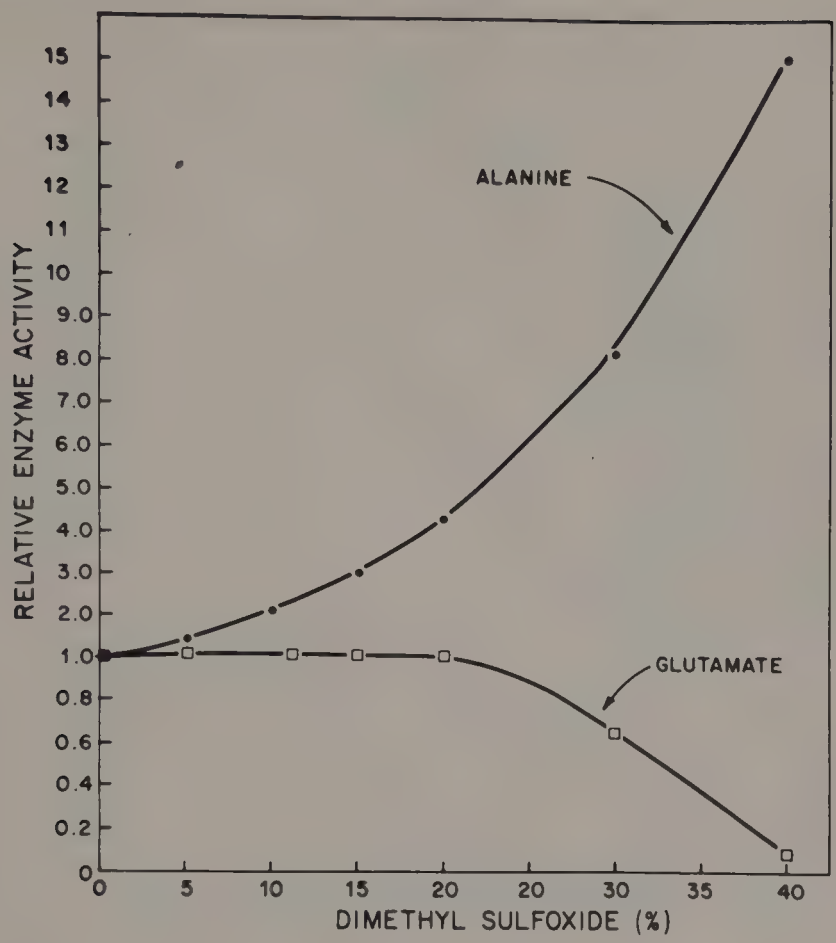

Fig. 3. The relative effect of DMSO on the oxidation of glutamate and alanine. The assay systems were as follows: glutamate (0.02 *M*), glutamic dehydrogenase (0.0025 mg, Boehringer Mannheim Corp.), tris-hydrochloride (0.025 *M*, pH 8.0), and diphosphopyridine nucleotide (DPN, 1×10^{-4} *M*); L-Alanine (0.025 *M*), DPN (2.8×10^{-4} *M*), glutamic dehydrogenase (1 mg), and tris-hydrochloride (0.025 *M*, pH 8.4). Both systems contained ethylenediaminetetraacetic acid (EDTA, 1×10^{-4} *M*) and were in a final volume of 1 ml. A value of 1 was taken to represent the change in optical density at 340 mμ per minute observed in the absence of DMSO. The amounts of DMSO were measured in volume percent. Before the start of the reaction all reagents except the enzyme and coenzyme were mixed and allowed to stand at room temperature for 15 min. Next the enzyme was added, and after 1 min the reaction was started by the addition of the coenzyme. The reaction was followed by the change in optical density at 340 mμ, using the interval between 0 and 30 sec for calculations of enzyme activity. The reaction was linear during this interval and was taken to represent the initial rate. In the oxidation reactions of glutamate and alanine this value corresponded to 0.054 and 0.009 optical density units, respectively. All assays were carried out at room temperature.

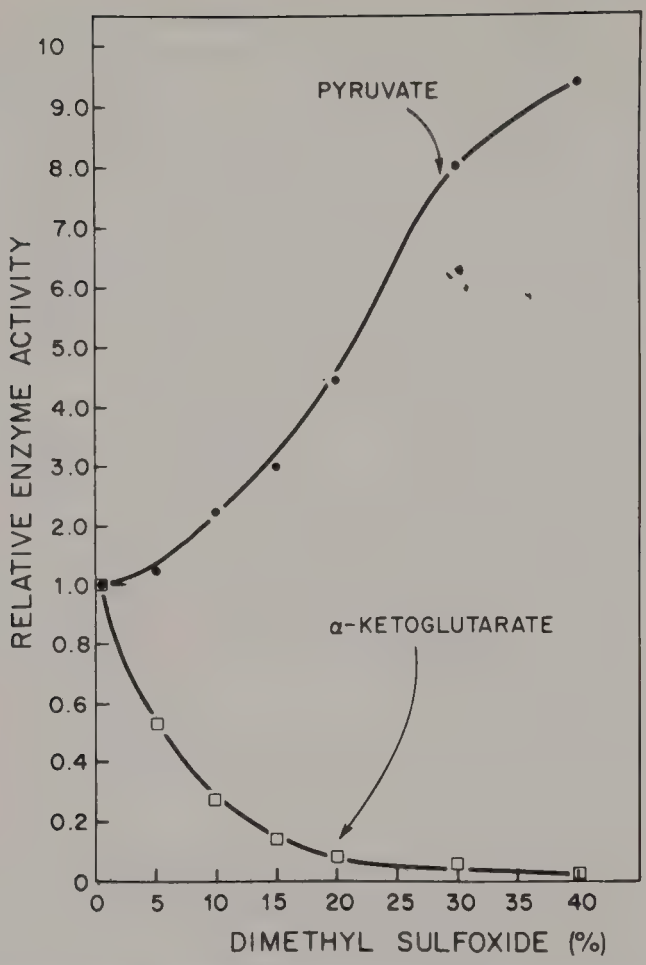

Fig. 4. The relative effect of DMSO on the amination of α-ketoglutarate and pyruvate. The assay system is the same as that described for glutamate oxidation (Fig. 3) except that the α-ketoglutarate and pyruvate concentrations were 0.02 *M* and the ammonium chloride concentration was 0.1 *M*. The amount of enzyme used for the amination of α-ketoglutarate was 0.001 mg/ml, and for pyruvate 0.05 mg/ml. The value of 1 is taken to represent the change in optical density at 340 mμ per minute observed in the absence of DMSO. In the amination reaction of α-ketoglutarate and pyruvate this value corresponded to 0.295 and 0.039 optical density units, respectively. The assays were carried out at room temperature.

diethylstilbesterol, reverses the effect of ADP; however, this reversal is never complete. The reversal of ADP inhibition by DMSO could possibly have been greater at a much lower ADP concentration; however, a high ADP concentration was used in order to minimize the effects of the altered polarity of the solution on the ionic form of this substance.

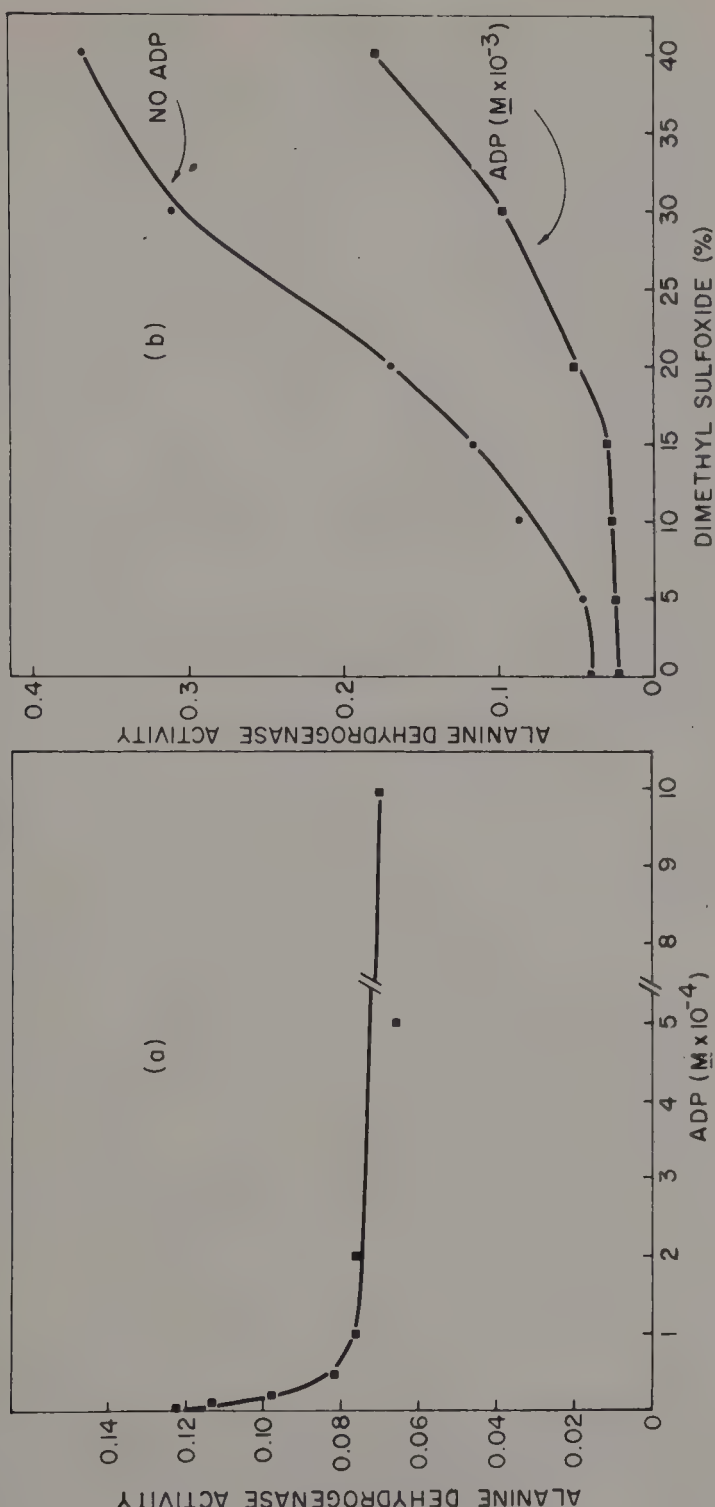

Fig. 5. The effect of DMSO on the Amination of pyruvate in the presence and absence of ADP. The assay system was similar to that described in Fig. 4 except that the enzyme concentration in Fig. 5a was 0.05 mg and in Fig. 5b 0.20 mg. In Fig. 5b the ADP concentration was 1×10^{-3} *M*. The value of 1 is taken to represent the change in optical density at 340 mμ per minute observed in the absence of both DMSO and ADP. This value was 0.039 optical density units for the rate shown in Fig. 5a, and 0.121 for Fig. 5b. All assays were carried out at room temperature.

A variety of proposals has been advanced to explain the effect of small molecules such as diethylstilbesterol and ADP on the specificity of glutamic dehydrogenase [for summarizing references see Bayley and Radda *(22)*], and in general these ideas have been concerned with alterations in conformation of the enzyme protein. It is clear that with a complex enzyme system such as the deamination of glutamate, an extensive kinetic analysis is required to limit the possible sites of action of DMSO; however, from these preliminary data it appears that this solvent, as a solvent, can mimic the specific effect of the steroid analog diethylstilbesterol. Whether this effect is the result of differences in the binding of the coenzyme, substrate, product, or a combination of effects, must await further investigation.

In these kinetic experiments there was a possibility that high concentrations of DMSO could cause an irreversible change in the glutamic dehydrogenase protein. Therefore, it was of interest to determine how much of the initial enzymic activity could be recovered after this solvent treatment. To test this, the enzyme (1 mg/ml) was kept in tris–hydrochloride buffer (0.1 *M,* pH 7.5) containing 35% DMSO at 37°C for 20 min. After this time a small aliquot (2.5 μg) of the enzyme was removed and assayed using the standard assay procedure. Under these conditions essentially all of the original activity could be recovered. Since most of the assays described in the kinetic section never exceeded more than 2 min at 22°C, it is probable that no invertible alteration in enzyme protein occurred during these assays. Furthermore, it was of interest to determine the effect of DMSO sulfoxide on the enzyme in the absence of buffer for longer periods of time. When the enzyme was kept in aqueous DMSO (35% v/v, nonbuffered) at 37°C for 20 min and assayed, about 50% of the original activity was retained, while about 25% remained in the control containing only water. After 10 hr no enzyme could be measured in the water-containing control. In the solution containing DMSO approximately 20% of the original activity remained.

IV. HYDROLYTIC ENZYMES

In addition to its effects on protein and coenzymes DMSO in high concentrations can affect the rate of enzyme-catalyzed hydrolytic reactions by reducing the concentration of one of the reactants, water. The effect of DMSO on several hydrolytic enzymes is shown in Table 1.

TABLE 1

The Effect of DMSO on Several Enzyme Systems

Enzyme[a]	Enzymic activity, % DMSO concentration, vol. %						
	0	1	10	20	30	40	50
Steroid sulfatase	100	79	82	68	52	26	16
Aerobacter aerogenes sulfatase	100	120	100	55	40	30	10
Escherichia coli β-galactosidase	100	91	85	72	44	5	–

[a]The incubation solution (1 ml) contained 100 μg of crude *Helix pomatia* extract or 5 μg of *A. aerogenes* sulfatase, 7.5 μmoles of *p*-nitrophenol sulfate, DMSO in the concentrations indicated, and tris-HCl (0.1 *M*, pH 7.8). The β-galactosidase assay solution (1 ml) contained 0.05 μg of β-galactosidase, 1.0 μmoles of *o*-nitrophenol-β-*D* galactoside, and tris-HCl (0.1 *M*, pH 7.4). All assays were carried out at 37° C.

Other than a slight stimulation (20%) of *Aerobacter aerogenes* sulfatase activity found at 1% DMSO, the patterns of inhibition for the steroid and *A. aerogenes* sulfatases are similar. β-Galactosidase is considerably more sensitive to high concentrations of this solvent. When β-galactosidase-induced *Escherichia coli* cells were assayed *(25)* in the presence of DMSO, the results indicated that the whole cells were more sensitive to the action of the solvent than the purified enzyme. Thus, at 10% DMSO the whole cells retained about 48% of their original activity while the purified enzyme retained 85%.

Trypsin behaved quite differently in DMSO. The relative rates of trypsin-catalyzed hydrolysis of *p*-toluene sulfonyl-L-arginine methyl ester (TAME, Worthington Biochemical, Freehold, New Jersey) are shown in Fig. 6. At every concentration there was a stimulation of enzymic activity, the major stimulation occurring around 20% DMSO. At concentrations of DMSO above 30%, a visible precipitation of buffer or substrate occurred.

Inagami and Sturtevant *(7)* have studied the effect of varying concentrations of DMSO on the trypsin-catalyzed hydrolysis of benzoyl-L-arginine ethyl ester (BAEE). Although a different trypsin substrate (TAME) and somewhat different assay conditions were used, our results are similar to theirs. They have suggested that the increase in the relative rate of trypsin-catalyzed BAEE hydrolysis in reaction solutions containing dioxane of DMSO can result from a variation in the dielectric constant of the reaction medium occurring with increasing concentration of organic solvents, this change affecting not only the

charge interactions of the substrate with the active site of the enzyme, but also the overall conformation of the enzyme protein. They also suggest that water can act as an inhibitor, this inhibition being relieved by increasing concentrations of the organic solvent. Indeed, DMSO stimulation of the several hydrolytic reactions as reported here suggests that one of its effects is to stabilize a favorable hydrolytic transition state complex by substituting for water in this complex. In a nonenzymic reaction this type of stabilization has been proposed for the methoxide ion-catalyzed racemization of (+)-2-methyl-3-phenylpropionitrile. Thus, Cram *et al. (26)* have shown that passing from methanol to 95% dimethyl sulfoxide increases the specific rate of this racemization by a factor of 5×10^7.

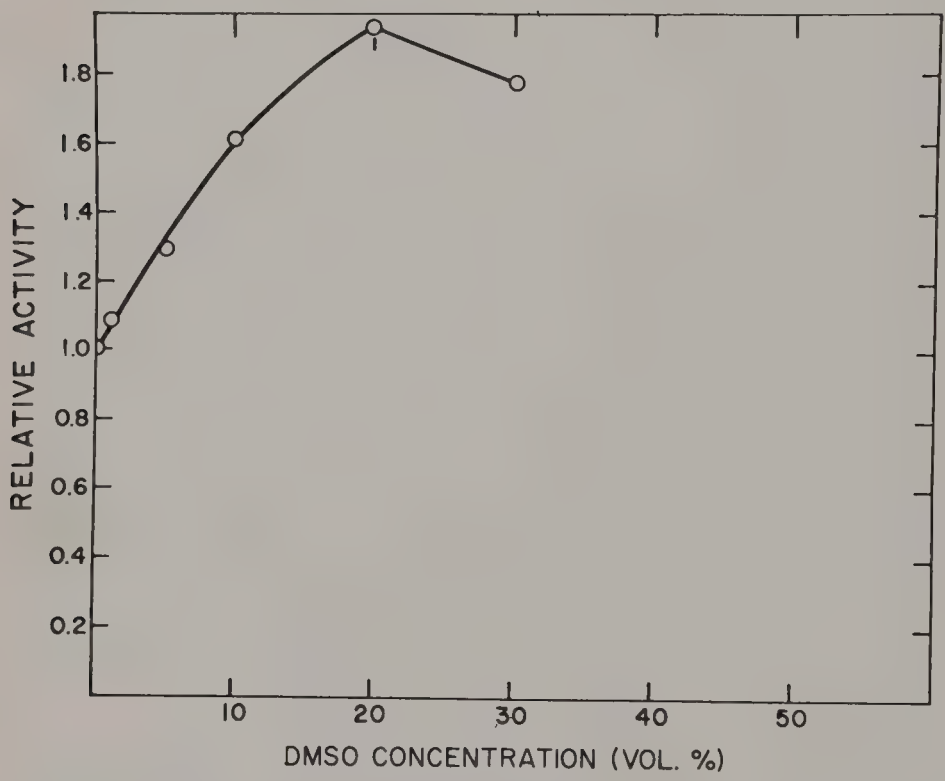

Fig. 6. The effect of DMSO on trypsin activity. The relative rate is the ratio of the rate in the indicated concentrations of DMSO to the rate in the absence of DMSO. The rate, at 23°C, is expressed as the change in optical density at 247 mμ per minute per microgram of protein. The enzymic reaction (1.2 ml) contained, in addition to DMSO, 0.8 μmoles of TAME, 1.2 μg of trypsin, and phosphate buffer (0.05 *M*, pH 8.0).

V. DIMETHYL SULFOXIDE AS A REDUCING AGENT

A number of enzymes are known to be sensitive to inactivation by oxygen or substances such as free radicals. Reducing agents such as 2-mercaptoethanol or cysteine are routinely used in attempts to stabilize such systems. Although less effective than most sulfhydryl reducing agents, DMSO can serve not only as a solvent but also as a reducing agent. It has been shown to be an effective agent for radiation protection of a variety of microorganisms and biological reactions [see Ashwood-Smith *(27)* for general references], and Barker *et al. (28)* have suggested that it protects sensitive biological systems against the damaging effects of free radicals by its ability to undergo oxidation. This property of DMSO, in addition to its solvent properties, most likely contributes to its demonstrated utility in the low-temperature stabilization of enzymes *(29,30)*, nucleic acids *(31)*, and living cells *(32)*, and suggests the possibility of its routine use in stabilizing a variety of partially purified and pure enzymes used routinely in the laboratory and kept at low temperatures. Another possible use of DMSO in enzymology is in the stabilization of enzymes during fractionations such as ion-exchange chromatography. Indeed, because of the effect of this solvent on enzyme conformation, variations in the chromatographic pattern of enzymes with and without this solvent can possibly be realized. An example of the chromatographic behavior of purified glutamic dehydrogenase on DEAE-cellulose in the presence of 35% v/v DMSO is shown in Fig. 7. It is apparent that the elution patterns in the presence and absence of DMSO are different, a second comment being partially resolved from the enzyme in the solution with the solvent.

The ability of DMSO to inhibit radical formation suggested that its use in enzymic reactions involving radical formation would be limited. The effect of varying concentrations of dimethyl sulfoxide on catalase *(33)* and peroxidase is shown in Table 2. It appears that their activity is more sensitive to this solvent than the hydrolytic enzymes tested (see Table 1). When these enzymes were kept in their respective complete reaction media containing DMSO (10% v/v) for several hours at room temperature, and each medium then assayed for dimethyl sulfone by vapor phase chromatography, each contained more dimethyl sulfone than the controls which did not contain the enzymes. Although no

quantitative figures were obtained, these enzyme proteins appear to be instrumental in oxidizing DMSO to dimethyl sulfone to a small degree.

VI. CONCLUDING REMARKS

DMSO in high concentrations has been shown to be extremely effective in destabilizing the secondary structure of nucleic acids. This destabilization presumably results from the disruption of hydrophobic interactions *(34)* between purine and/or pyrimidine bases.

The polymerization of guanosine 5′-pyrophosphate by polynucleotide phosphorylase into high-molecular-weight polyguanylic acid is difficult to achieve under conditions in which homopolymers containing adenosine, uridine, and cytidine are readily formed *(35)*. Polynucleotide phosphorylase is sensitive to the secondary structure of polymers *(36)*, and under the conditions normally used for its enzymic synthesis polyguanylic acid is a multistranded helix *(37)*. For this reason the preparation of polyguanylic acid is best accomplished under conditions in which it has the least secondary structure. Enzymic synthesis at elevated temperatures has been used to advantage in this regard *(38)*. In addition to temperature, other denaturants such as urea *(3,34)* have been shown to be useful in increasing the yields of enzymically synthesized polyribonucleotides *(39)*, this enhancement presumably resulting from urea-induced conformational changes in the enzyme protein. The effect of this structural change is to inhibit the back reaction, namely, the phosphorolysis *(40)* of the newly synthesized polymer. Because of the demonstrated effectiveness of DMSO as a perturbant for both proteins and nucleic acids, it was of interest to establish whether or not polymer synthesis catalyzed by this enzyme could occur in this solvent. In a preliminary experiment, it was shown that polyadenylic acid synthesis catalyzed by *Mycobacterium lysodiektikus* polynucleotide phosphorylase was possible in 20% v/v DMSO. Although the rate of synthesis was substantially reduced as compared with the control in the absence of DMSO, a polymer was synthesized. No attempt was made to characterize the polymer, but it is interesting to speculate that profitable use of this solvent can be made in enzyme-catalyzed reactions that lead to macromolecules such as nucleic acids.

An excellent demonstration of the ability of DMSO to increase cell membrane permeability to organic substances was performed using *E. coli* cells. The transport of isopropyl β-D-thiogalactoside (ITPG), a substance that induces the synthesis of the enzyme β-galactosidase in *E.*

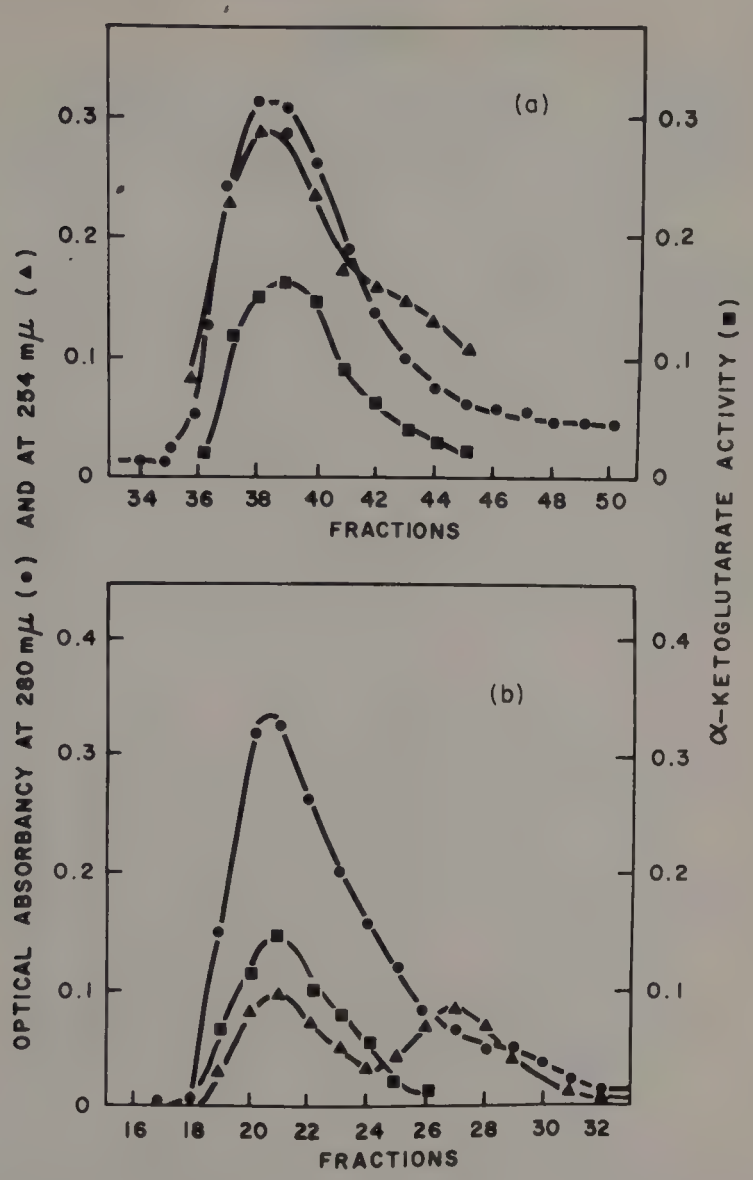

Fig. 7. Chromatography of glutamic dehydrogenase. (a) The elution pattern of the enzyme from DEAE Sephadex A-25. The enzyme (1 mg/ml) in 50 ml of tris–hydrochloride buffer (0.05 *M*, pH 7.5) was placed on a column (2.5 × 10 cm) of DEAE which had been washed and preequilibrated with the same buffer. The enzyme was eluted using a linear gradient of tris–hydrochloride (0.05 *M*, pH 7.5, 25 ml) in the mixing vessel and tris–hydrochloride (0.05 *M*, pH 7.5, 25 ml) containing potassium chloride (0.5 *M*) in the reservoir. The flow rate was 1 ml/min and 1-ml fractions were collected. (b) The elution pattern of the enzyme using the method described for Fig. 6a except that the column was equilibrated with buffer containing 20% DMSO and the elution solvents also contained 20% DMSO. All of the chromatography was carried out at 5°C. The assays used to determine α-ketoglutarate activity are described in the legend for Fig. 4. In addition to this analysis, glutamic dehydrogenase and pyruvate activity were also examined. Both of these activities were coincidental with the protein curve.

coli cells, requires a specific permease enzyme. Using a permeaseless mutant, Fowler and Zabin *(41)* were able to induce the synthesis of β-galactosidase with ITPG when the cells were treated with DMSO. These authors showed that a resting culture of *E. coli* ML 30 can survive with only a slight loss in viability after treatment with 20% DMSO for 3 hr at 37°C.

Despite the fact that a great deal of the data presented in this article is preliminary and cursory in nature, it is clearly demonstrated that DMSO can be used effectively in studies of enzyme-catalyzed reactions. It thus promises to find utility in enzymology for conformational studies of enzymes, in mechanistic studies, in assisting in solubilizing a variety of substrates, and in purification procedures. In particular, because of its pronounced and differential effect on proteins and nucleic acids *(7,10)*, its use in studying the association of these substances in biological material such as ribosomes *(42)* and viruses *(31,43)* should prove fruitful.

TABLE 2

The Effect of DMSO on Catalase and Peroxidase

	Enzymic activity, % DMSO concentration, vol. %				
Enzyme	0	1	5	10	20
Peroxidase[a]	100	110	71	46	11.5
Catalase[b]	100	106	91.3	2.8	–

[a] The incubation solution (3 ml) contained hydrogen peroxide (0.003 *M*), *o*-phenylenediamine (1% w/v), 1.5 μg of peroxidase, DMSO, and phosphate buffer (0.07 *M*, pH 7). The reaction was followed by measuring the change in absorbancy at 485 mμ at 23° C.

[b] The incubation solution (3 ml) contained hydrogen peroxide (0.012 *M*), catalase (1 μg), DMSO, and phosphate buffer (0.07 *M*, pH 7.0). The reaction was followed by measuring the change in absorbancy at 240 mμ. Because DMSO also absorbs in this region, relatively high concentrations could not be obtained.

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Chapter 7

Role of DMSO In Microbiology and Serology

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I. INTRODUCTION

Since the early use of dimethyl sulfoxide (DMSO) for protection of biological specimens against freezing damage, this chemical has been found to be useful in many other areas of clinical and laboratory medicine. The eventual impact of this compound on the entire field of medicine can only be theorized at this time. The amazing characteristics of DMSO, discussed extensively in other sections of this volume, have been instrumental in giving promise for the solution of numerous perplexing problems facing medicine today.

Jacob and co-workers *(1,2)* were early to report the basic medical characteristics of DMSO that have been the guideline for its very extensive experimental trial in the medical and veterinary field, as well as in agricultural investigation. The principal activities of DMSO, as listed by these workers, are: (1) penetrant carrier (with reversible effect on the biological membrane); (2) local analgesic agent; (3) antiinflammatory agent; (4) bacteriostatic agent; (5) diuretic; (6) tranquilizer; (7) potentiator of other compounds; and (8) growth stimulator.

Chemically, DMSO has been known for the past 100 years. It is a clear, colorless liquid which is practically odorless in the purified state. It is the lowest member of the group of alkyl sulfoxides, having a formula of CH_3SOCH_3 and a molecular weight of 78.13. As a highly polar organic liquid which is hygroscopic and miscible with water, it acts as a powerful solvent, dissolving most aromatic and unsaturated hydrocarbons, organic nitrogen compounds, and many other substances. When a 100% solution of DMSO is mixed with water, a heat of solution is evolved (60 cal/g at 20°C). DMSO has been used as a commercial solvent in the manufacture of synthetic fibers; consequently, clothing made of certain fabrics may be destroyed on contact *(3)*.

It becomes apparent that such a compound would attract the attention of workers in the field of microbiology and the related field of serology. Numerous researchers have made significant contributions to our ever-increasing fund of knowledge of the effect of DMSO on the morphology, physiology, growth rates, resistance to chemotherapeutic agents, and so forth, of microorganisms. This chapter is an attempt to review these significant findings and to reveal the enormous need and opportunity for additional research in this one aspect of the medical importance of DMSO.

Experience with DMSO in Microbiology

In the few years since the realization that DMSO possesses potential medical importance, considerable research has been done in the field of microbiology and associated areas. Krizek and Davis *(4)* reported on their early experiences with DMSO as a possible bacteriostatic agent. This bacteriostatic quality and bactericidal levels for DMSO were further studied and reported by Pottz *et al. (5)*. Tsuchiya and co-workers *(6)*, working in Japan in 1964, traced the formation of methyl methanethiosulfonate from DMSO and showed it to be antibacterial in nature. Pottz *et al. (7)* introduced in 1964 their widely accepted acid-fast staining technique using DMSO as a penetrant carrier. Pearce *(8)* recognized the role of DMSO as a carrier of iodine in treatment of fungal infections of the skin and nails. Yehle and Doi *(9)* found DMSO to be effective in the stabilization of *Bacillus subtilis* phage. Kligman *(10,11)* determined median lethal dose values for mice and reported further on the bacteriostatic qualities of DMSO. Belsky and Goldstein *(12)* investigated DMSO as a fungicidal compound on nonfilamentous marine *Phycomycetes*. Smith and Hegre *(13)* used DMSO in allergy skin-testing with mixed molds and other fungi. Stringer and Engle *(14)* found DMSO promising in the treatment of various nail infections. MacCallum and Juel-Jensen *(15)* reported successful treatment of herpes simplex with combined DMSO and idoxuridine. Goldman *et al. (16)* reported an extensive and complete study of the effects and promises of DMSO in the stabilization and sterilization of ophthalmic medication, and further developed data on the bacteriostatic qualities of the compound. Katz and Hood *(17)* combined thiabendazole and DMSO in the treatment of creeping eruption. Mitchell *(18)* introduced a simple method for the permanent staining of intestinal parasites using DMSO.

The above-mentioned investigations are only a few of the studies in the field of microbiology that have been undertaken to date. Much knowledge of this interesting compound has been added, and a portion of it has already passed into routine use. A great deal of basic research on the effects of DMSO on the various activities of bacteria, fungi, viruses, and animal parasites is underway in laboratories throughout the world. It is not overly optimistic to state that DMSO has already exerted, and will continue to exert, an astounding impact on laboratory methods of culture, diagnosis, and control of microorganisms.

This article will attempt to describe the development and uses of knowledge concerning DMSO from its introduction to the present time. The information will be essentially limited to the fields of microbiology and serology. It is the intention to give credit for all significant contributions in this field, however, it must follow that some current work or that published in some journals has possibly been overlooked. The presentation herein is organized according to subject matter and is not an attempt to provide a chronological record.

II. BACTERIOSTATIC AND BACTERICIDAL ASSESSMENT OF DMSO

Probably one of the most important characteristics of DMSO, at least from the microbiologist's standpoint, is its bacteriostatic quality. Future use of DMSO in many clinical conditions would conceivably be enhanced by the fact that it does have some degree of bacteriostatic and bactericidal effect against common wound and skin contaminants as well as generally recognized pathogenic microorganisms. Significant research to verify these two characteristics of DMSO has been completed.

Jacob *et al. (1)*, in their early review of DMSO as a new concept in pharmacotherapy, suggested the possibility that the compound might have bacteriostatic effects against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* in a 20% concentration and against *Mycobacterium tuberculosis* in a concentration as low as 5%. Sanders *(19)* felt that the bacteriostatic qualities of DMSO against such annoying agents as *Pseudomonas* alone would make this compound one of medical importance. The clinical microbiologist, with his experience in trying to control this microorganism, would be the first to agree. Rosenbaum and co-workers *(3,20)* also spoke of DMSO as a bacteriostatic agent against *Pseudomonas, Staphylococcus, E. coli,* and *M. tuberculosis.*

The minimal inhibitory concentration (MIC) of DMSO to the nearest 10% in nutrient broth was determined for two isolates each of *S. aureux, S. aureus var. albus (S. epidermidis),* beta hemolytic streptococci, *Corynebacterium acnes, Corynebacterium* spp. (normal skin residents), *Alcaligenes faecalis, E. coli,* and *Proteus* spp. by Kligman *(11)*. Twenty percent DMSO was found to be bacteriostatic to all of these organisms. Bactericidal concentrations were determined by

Kligman by subculture after 24 hr. For *S. aureus* the bactericidal concentration (50%) was 2.5 times the MIC; for the others it ranged from 30 to 40%, with the gram-negative bacteria being somewhat more susceptible. Kligman concluded that DMSO, by modern standards and as compared with antibiotics, is only a weakly active bactericide. The concentration required to kill all of the above microorganisms and 1-hour exposure to aqueous solutions of DMSO ranged from 65 to 75%.

A further series of human experiments by Kligman evaluated the ability of DMSO to reduce resident axillary flora composed mainly of aerobic staphylococci and diphtheroids. By applying 1.0 ml of 90% DMSO to the axilla of 10 male adults three times daily for 3 days, he showed that the normal population was reduced. Quantitative samples were obtained by scrubbing the skin surface manually within a 2.5-cm cup containing 2 ml of 0.1% nonionic detergent (Triton X-100) phosphate-buffered to pH 8.0. Quantitative counts were made and the mean control count was 6,088,300/cm^2 (range 550,000–11,000,000). After 3 days of treatment the mean was reduced to 583,000/ cm^2 (range 41,000–1,400,000). Kligman concluded that while DMSO was not as potent as certain antibiotics, 0.2% neomycin solution, for example, it effected about a 90% reduction in axillary flora when concentrated solutions were frequently applied for a number of days.

Seibert *et al. (21),* in their search for an agent to control the organisms isolated from the blood and tissue of patients with tumors and leukemia, reported well-controlled experiments using, among other compounds, lysozyme, DMSO, and *d*-ethambutol chloride. When lysozyme was added to various cultures of isolates, it caused complete growth inhibition over a period of 30 days in only 3 cultures, partial inhibition in 5, little or no inhibition in 13, and actually stimulated growth in 5 others. Against the same cultures it was found that neither 10 nor 100 μg of *d*-ethambutol appeared to have any effect on the normal growth of the organisms. Further study also showed that repeated exposures to the 10-μg dose of *d*-ethambutol seemed to increase progressively the lag phase during two successive exposures, but upon further exposure gave the opposite result, as if resistance to the drug were becoming evident.

Addition of a 12.5% concentration of DMSO to a few of these cultures used for growth-curve studies was striking in that all growth was completely inhibited for 30 days, while the accompanying control tubes showed marked growth. Cultures of all the isolates from blood

and tissues of patients having cancer or leukemia were similarly treated with the drug. It was clear that out of the 29 cultures tested, 9 were completely (100%) inhibited by 12.5% DMSO and 8 more were almost completely (75–90%) inhibited. The other 17 cultures, including 6 of the latter, were then treated with 25% DMSO. All were completely inhibited except two which showed only a trace of growth (90% inhibition or more). Thus, a single treatment with DMSO in a concentration of 25%, and even 12.5% in many cases, completely inhibited the growth of practically all of the organisms isolated from tumors and blood of cancer or leukemia patients. It was furthermore of interest that the addition of DMSO to fresh leukemic blood on a slide completely inhibited the "dancing motion" of particles free in the blood or attached to the periphery. At the same time, no apparent hemolysis or obvious damage seemed to occur to the normal red blood cells that previously appeared normal. Aggregations of immobilized crenated cells and platelets were seen.

In an extensive study of the effect of DMSO on the growth rate of a group of medically significant microorganisms, Rampey *(22,23)* noted an apparent effect on the lag phase as reported by Seibert. However, this appears to be more a question of the concentration of DMSO rather than an accelerated lag phase. In work with *P. aeruginosa, Salmonella paratyphi B,* beta hemolytic streptococci group A, *S. aureus* (coagulase positive), *Candida albicans, Streptococcus anginosus, Streptococcus faecalis, E. coli, Aerobacter gloacae,* and *Neisseria catarrhalis,* it was noted on plate counts that all the microorganisms except beta hemolytic streptococci showed equal or higher counts on exposure to 5–10% DMSO after 4 hr and after 24-hr incubation at 37°C than did the control plates. Growth rates for beta hemolytic streptococci were depressed at a 5% concentration and completely inhibited on exposure to a 10% concentration of DMSO. Subcultures from the above series showed conclusively that at given concentrations of DMSO the compound is not only bacteriostatic but also bactericidal. Repeated subcultures of *P. aeruginosa* showed no growth after an exposure to 20% DMSO. The concentrations at which DMSO inhibited growth in the other microorganisms are as follows: *S. paratyphi B,* 20%; *C. albicans,* 10%; beta hemolytic streptococci group A, 10%; *S. aureus,* 30%; *S. anginosus,* 25%; *S. faecalis,* 25%; *E. coli,* 25%; *A. cloacae,* 20%; and *N. catarrhalis,* 10%.

Little variation in the bacteriostatic or bactericidal levels was seen between growth on liquid media or on solid media. The majority of the

references given are based on data from growth in liquid media. Rampey checked his results from broth medium with the same series on trypticase soy agar by varying concentrations of DMSO from 0 to 60%. The bacteriostatic and bactericidal concentration on trypticase soy agar with DMSO was essentially that given for liquid media above. It is of interest, however, that there was a definite reduction in pigment produced by *Pseudomonas* on all media containing DMSO. Additional studies of the effect of DMSO on pigment production by microorganisms are being made at present.

Kamiya *et al. (24)* observed that eye drops containing 15–20% DMSO could be used for long periods without side effects. Their studies of the sterilizing and growth-inhibitory effect of DMSO on bacteria, such as *E. coli, S. aureus,* and *P. aeruginosa,* were the early experiments in this area. The effects of 5% DMSO on the restoration of sensitivity of antibiotic-resistant bacteria were also studied. Suspensions of bacteria from 18 to 20-hour broth cultures added to tubes containing 20% DMSO and to tubes containing 15% DMSO plus 1.0% sodium chloride and transferred to bouillon medium after 5, 10, 20, 30, and 60 min and 24 hr showed that DMSO had no sterilizing effect on *Pseudomonas, Staphylococcus,* and *E. coli.* In growth-inhibitory action studies, four strains of *Staphylococcus, Pseudomonas,* and *E. coli* were isolated from patients and cultured in bouillon for 18–20 hr. Suspensions of these bacteria were added to tubes containing 10 ml of 20% DMSO brain heart infusion and cultured for 24 hr. The growth of all strains was completely inhibited by 20% DMSO in brain heart infusion. Six strains of *Staphylococcus* and *E. coli* and four strains of *Pseudomonas* cultured in bouillon for 18–20 hr were grown in 10% DMSO-modified Mueller Hinton medium. The growth of three strains of *Pseudomonas* was inhibited, but that of other strains increased. The growth of all strains increased in the control culture medium without DMSO. Again, 5% DMSO-modified Mueller Hinton medium could inhibit only one strain of *Pseudomonas.*

An interesting example of the bacteriostatic effect of DMSO *in vivo* has been supplied by Levesque *(25).* Open infected wounds in horses were treated with DMSO. After 2 or 3 days treatment the wounds closed, and remarkable healing was noted. Underneath the rather loose scab formation that formed between each application, serous exudation instead of frank pus was found. A type of film seemed to form over the surface of the wound, protecting it from secondary invaders, yet allowing formation of the granulation tissue to proceed, but not to the

extent that proud flesh was developed.

Pottz *et al. (26)* reported an extensive series of tests of the bacteriostatic and bactericidal effects of DMSO on a selected group of medically important microorganisms. Tubes of brain heart infusion broth with concentrations of DMSO ranging from 5 to 80% were inoculated with 18-hour broth cultures of the test organisms, incubated for 24 hr at 37.5°C, examined macroscopically, subcultured to brain heart blood agar plates, reincubated for 24 hr at 37.5°C, and examined for growth. Results of this series of tests are shown in Table 1. To

TABLE 1

Bacteriostatic and Bactericidal Effects of DMSO on a Group of Selected Microorganisms

Test microorganism	Concentration DMSO used, %[a]									
	0	1	4	5	10	20	30	40	50	60
Escherichia coli	3	3	3	3	3	1	0	0	0	0
Aerobacter cloacae	3	3	3	3	3	1	0	0	0	0
Pseudomonas aeruginosa	3	3	3	3	2	0	0	0	0	0
Proteus vulgaris	3	3	3	3	3	1	0	0	0	0
Staphylococcus aureus	3	3	3	3	3	2	1	0	0	0
Streptococcus pyogenes	3	3	3	3	3	1	0	0	0	0
Streptococcus faecalis	3	3	3	3	3	2	1	1	0	0
Salmonella schottmulleri	3	3	3	3	2	1	0	0	0	0
Diplococcus pneumoniae	3	3	2	0	0	0	0	0	0	0
Candida albicans	3	3	3	3	1	1	0	0	0	0

[a] 0, No growth; 1, light growth; 2, moderate growth; 3, normal or unrestricted growth.

further delineate the bacteriostatic and bactericidal effects of DMSO, a series of cultures of these microorganisms was exposed to varying concentrations of DMSO, and colony counts were made by the pour plate method. Stock cultures of the test microorganisms were grown for 24 hr at 37.5°C in trypticase soy broth inoculated from this broth. A second series of trypticase soy broth was incubated for 4 hours; dilutions of 1:1000, 1:10,000, 1:100,000, 1:1,000,000, and 1:10,000,000 were made from trypticase broth containing concentrations of 5, 10, 15, 20, 25, and 30% DMSO and incubated for 24 hr. One ml from each of these dilutions was transferred to sterile petri plates; 20 ml of melted trypticase soy agar were added, mixed, and allowed to solidify. These plates were incubated for 24 hr at 37.5°C. Each plate was then

examined and the colonies were counted and recorded. Results of these colony count determinations are shown in Table 2. Bacteriostatic

TABLE 2

Bacteriostatic and Bactericidal Effect of DMSO by Plate Count Method

Test microorganism	Plate count on exposure to varying concentrations of DMSO[a]							
	Control	5%	10%	15%	20%	25%	30%	40%
Pseudomonas aeruginosa	640	640+	380	1	0	0	0	0
Salmonella paratyphi	1,070	1,070+	1,070+	27	13	10	8	0
Streptococcus (beta-A)	750	640	640	0	0	0	0	0
Staphylococcus aureus	28	32	24	20	5	4	4	0
Candida albicans	4	4	3	0	0	0	0	0
Streptococcus anginosus F	86	2,700	2,700	800	70	50	0	0
Streptococcus faecalis	7,500	7,500	7,500	850	240	5	4	0
Escherichia coli	37,550	37,500	37,500	9,000	90	0	0	0

[a] All counts in millions as determined by serial dilution plate counts.

concentrations shown here are in general agreement with those reported by Jacob, Kligman, Rampey, and others.

There appears to be close agreement as to the bacteriostatic concentration of DMSO among the several authors cited in the preceding paragraphs. There also appears to be some hesitancy to attribute bactericidal characteristics to DMSO. However, results shown from Tables 1 and 2 should remove any doubt on this point. Various writers mention the "sterilizing" effect of DMSO without applying the synonym "bactericidal." Investigations made in our laboratory indicate that the bactericidal concentrations required for *S. aureus* were 30% and for the remainder of the microorganisms as reported by Kligman and Rampey.

Krizek and Davis *(4),* working with *E. coli, P. aeruginosa,* and *S. aureus,* obtained results which indicate that DMSO is both bacteriostatic and bactericidal. Tube dilutions of DMSO were made in nutrient broth, producing concentrations from 1 to 100%. To these tubes were

added standard inocula of the test organisms. Saline dilution replaced DMSO in the control tubes. After 18 hr incubation at 37°C, each dilution was serially diluted and back-plated for accurate bacterial counting. The *Staphylococcus* series showed an increase in the inoculum in all control tubes; tubes containing less than 10% DMSO showed no reduction of organisms; tubes containing 10–20% DMSO demonstrated a definite decrease. As the concentration increased toward 30%, there was little growth (count actually less than the inoculum itself); DMSO in concentration greater than 36% did not permit growth or survival. The series using *E. coli* reacted in a similar fashion and showed no survival at concentrations of 33% or higher. The *Pseudomonas* series showed an abrupt drop in bacterial count as 20% concentration was approached, and complete inhibition at 25% concentration of DMSO.

These workers, in a later paper *(27),* reported increasing evidence that *Pseudomonas* burn sepsis yielded to typical therapy with DMSO. In a group of 40 rats subjected to a sublethal, 20% full-thickness scald burn, good control of *Pseudomonas*-induced infections was secured by a combination of 90% DMSO and 10% sulfamylon. In a similar series, neither continuous nor intermittent therapy with silver nitrate solution reduced bacterial levels, and the mortality rate exceeded 95%. Combined DMSO and sulfamylon reduced the mortality rate to less than 20%.

Jacob *et al (28)* in a series of thermal third-degree burns, observed that after 10 days of treatment with DMSO alone there was an absence of the characteristic surrounding postburn inflammation or infection seen under the eschars.

Huu and Albert *(29)* reported a companion series on the effect of DMSO on wound healing and tensile strength measurements in rabbits. While they failed to comment on the bacteriostatic properties of DMSO, it is of interest to note that they reported no infections in their susceptible procedure.

Clark *et al. (30)* reported several strains of *Mycoplasma* as possible pathogenic organisms in human rheumatoid arthritis, lupus erythematosus, and multiple sclerosis. He listed various methods of media selection, recovery, and culture technique for these fastidious organisms. The possible significance of these microorganisms under the above conditions interests the microbiologist. In our laboratory it has been found that DMSO in a concentration of 10% or less inhibits the growth of *Mycoplasma* spp. *in vitro.*

Barker and co-workers *(31),* in their work on the inhibition of hyaluronic acid degradation, noted the advantages of using DMSO for decreasing the process of degradation and its bacteriostatic qualities in the collection and preservation of cornea for storage at postmortem.

The mixed infections of eczematous otitis and the very often subsequently developing furuncular otitis are common and painful diseases. The skin within the external auditory meatus is considerably swollen, highly sensitive to pressure, and frequently even macerated to a greater or lesser degree. The results of therapy can be readily seen in this condition. Asen *(32)* reported a series in which DMSO was applied directly into the auditory meatus and to the adjacent outer parts by using a dripping-wet cotton-tipped applicator. Patients were treated once a day; in serious cases, twice daily. Most of the patients reported a distinct regression of pain after a few hours. However, this improvement lasted only for a short period of time after treatment with DMSO. A distinctly longer-lasting effect was achieved when antibiotics were applied, e.g., tetracycline and erythromycin dissolved in DMSO. In a number of these cases, strikingly dramatic results were obtained after only one or two applications. Some of the cases had previously been treated repeatedly with ointments containing cortisone and antibiotics without relief. Excellent results with complete remission of symptoms were achieved in 28 cases, 7 of which had been pretreated; 52 cases were significantly improved, 17 of which had been pretreated; and 22 cases remained unimproved. In the majority of cases, all symptoms had disappeared after two to four applications.

Asen used DMSO–antibiotic solution in cases of furuncles and infected or impetiginous eczema of the nose. The mixture was applied either to the skin or introduced into the vestibulum. Results proved to be most impressive to the investigator and to the patient. Generally, the feeling of tension and pain diminished within 1/2 hr. In most cases the treatment was terminated after two to four applications. Excellent results were obtained in 16 cases, improvement in 15, and no change in 3.

In the area of the mouth and pharynx, acute and subacute tonsillitis and pharyngitis were treated in 119 patients. Apart from the occasional subjective relief, the external application did not produce significant improvement. The preparation was therefore applied directly to the diseased sites and adjacent areas. In cases of acute inflammatory processes, a solution of antibiotics in DMSO proved to be superior to

DMSO alone. In cases of less acute forms frequently accompanied by changing complaints and a variable degree of pain, DMSO and mixed preparations proved to give equal results.

In a classic case Sanders *(33)* conducted a detailed and conclusive study to show the *in vivo* characteristics of DMSO as a bactericidal agent against *S. aureus*. The case reported was interesting from two standpoints, namely, no medication other than DMSO was used and that the clinical condition at the time treatment was initiated presented a potential problem of absorption of toxins. The patient studied had contracted osteomyelitis more than 30 years prior to the DMSO treatment. Primary diagnosis was made at Massachusetts General Hospital, and the diagnosis was confirmed by an almost pure culture of *S. aureus*. The patient had occasion to visit other clinics and a number of hospitals over the 30 years as the condition deteriorated. In all instances *S. aureus* persisted and reports indicated that the hemolysis varied from culture to culture. Frequently the cultures showed mixed bacterial flora. Irrigation of the open sinuses was started and continued for about 6 months. During the first month irrigation was carried out with a solution composed of 50% DMSO, 10% glycerol, and 40% water. The dosage was then increased to 90% DMSO and 10% glycerol in the amount of 3 oz given intermittently over the next 5 months. Cultures became negative for *S. aureus* and "bone pain" vanished; foul-smelling exudation which at times required almost hourly changing of dressings disappeared. Plastic surgery was performed to close the sinuses and the patient appears to be well on the road to recovery.

Yeats *(34)* employed 70% DMSO as a vehicle to test the effect of three drugs on tuberculoid markings and other lesions of patients already receiving oral treatment with dapsone for leprosy. The three groups of patients with leprosy were treated with the drugs in 70% DMSO as a skin wash. Improvement in all three groups was rapid and marked. This was a preliminary study, and while no controls using DMSO alone were tested, it was considered probable that the improvement in each group was attributable to the DMSO rather than to the drugs in solution.

It can be concluded that DMSO is both bacteriostatic and bactericidal. The present question appears to be whether this compound should be considered "only weakly antibacterial" as termed by Kligman *(11)* or a good bacteriostatic and bactericidal agent. Compared to many of the antibiotics in general use, DMSO must be considered weakly antibacterial. However, DMSO may have a broader spectrum than most

antibiotics when applied topically as a concentrated solution. Its possible therapeutic use as a bacteriostatic agent must be considered. This characteristic takes on added significance when DMSO is combined with antibiotics and acts as a potentiator. This bacteriostatic capacity of DMSO also must be considered from the standpoint of the effect on diagnostic cultures and on antibiotic susceptibility testing, and so forth. These points will be discussed at length later in this chapter.

The exact nature of this bacteriostatic or bactericidal effect on microorganisms is not fully understood. Jacob *et al. (28)* suggested the possibility that the antibacterial effect involves the production of methylmethane thiosulfonate from DMSO. This possibility has been studied in detail by Tsuchiya *et al. (6)* and at this time appears to be a plausible explanation for the ability of DMSO to limit the growth of or kill certain microorganisms.

Tsuchiya and co-workers mixed DMSO with an excess of hydrogen chloride and isolated an antibacterial spot using paper bioautography. They were able to establish that this active substance was methylmethane thiosulfonate.

Sulfoxides, being basic in nature, form salts with strong acids. Furthermore, it is known that sulfoxides are reduced by hydrogen halides to sulfides via diacid salts:

$$\underset{\text{I}}{R_2SO} \xrightarrow{HX} \underset{\text{II}}{R_2SO \cdot HX} \xrightarrow[-H_2O]{HX} \underset{\text{III}}{R_2SX} + X^- \longrightarrow \underset{\text{IV}}{X_2} + R_2S$$

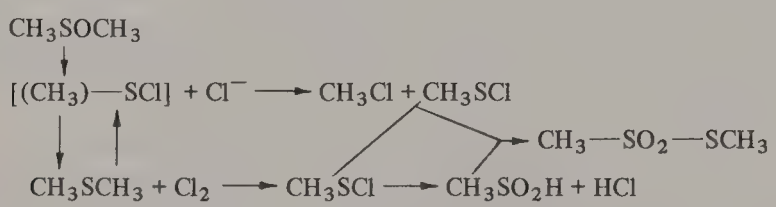
The reaction between DMSO and hydrogen chloride, however, is complicated and not fully understood.

When DMSO was caused to react with equimolecular hydrogen chloride under cooling, a semicrystalline mush, presumably the monoacid salt (II), was formed and no antibacterial activity against *B. subtilis* was observed. When DMSO was caused to react further with an excess of hydrogen chloride, however, a clear solution was obtained, and when this solution was allowed to stand, the temperature of the solution spontaneously rose to about 60°C and a considerable amount of gas was evolved. The low-boiling product collected was found to be composed mainly of hydrogen chloride and a water-soluble component which was presumably DMSO (IV). The resulting solution was neutralized thoroughly with powdered sodium bicarbonate or pyridine and, as described in the experimental section, repeatedly treated with organic

solvents to produce a crude product almost free from chlorine. This product was purified by vacuum distillation to active methylmethane thiosulfonate ($CH_3SO_2SCH_3$). The purified product gave analyses for methylmethane thiosulfonate, and the boiling point and infrared absorption bands corresponded well with those reported for the compound prepared by a different method.

Methylmethane thiosulfonate has been found to have antibacterial and antifungal activities, suggesting that the methane thiosulfonates have an antimicrobial effect. A series of antimicrobial tests of a variety of methane thiosulfonates has proved this to be true.

The interrelationships of the products in the reaction of DMSO with hydrogen chloride may be represented by the accompanying diagram. All the interrelationships except the formation of methanesulfenyl chloride (CH_3SCl) have been established.



The formation of methylsulfur trichloride (CH_3SCl_3) from methanesulfenyl chloride was established. The formation of methanesulfinic acid (CH_3SCl_3) by the hydrolysis of methylsulfur trichloride was found by Douglas and Farah *(35)*. The reaction of sulfenyl chlorides and sulfinic acid was applied to the preparation of thiosulfonate esters (RSO_2SR) by Sterling *(36)*. The formation of thiosulfonate esters from sulfenyl chlorides and sulfinic acid was also concluded by Douglas in the chlorination of alkyl disulfides.

Any mechanism that would account for the formation of methanesulfenyl chloride must involve the rupture of the sulfur–carbon bond. This kind of decomposition is rather unusual and the major known product of the decomposition is dimethyl sulfide. Several instances of this kind of decomposition have been found in the literature related to the chlorination of sulfides. An example is the formation of 2-nitro-4-methyl-benzenesulfenyl chloride from methyl-2-nitro-4-methylphenyl sulfide and chlorine. Methanesulfenyl chloride probably was formed by the nucleophilic attack of chloride ions on the carbon in the chlorosulfonium ions.

Experimentally, the product methyl methanethiosulfonate inhibited the growth of *Trichophyton rubrum, Trichophyton asteroides, B. subtilis,*

and *E. coli* in minimum concentration of 15.6, 31.2, and 125 μg/ml, respectively *(6)*.

Pottz and coworkers *(26)* reported that the mode of action of DMSO in killing microorganisms is unknown. However, it was noted from smears made from the broth culture sediments that the majority of the microorganisms had been dissolved. This was especially true of the *Diplococcus pneumoniae* cultures. A 4% concentration of DMSO added to young, vigorous broth cultures of *D. pneumoniae* serves as an excellent solubility test. Streptococci are not soluble at this low concentration.

III. EFFECT OF DMSO ON THE CHEMOTHERAPEUTIC SUSCEPTIBILITY OF MICROORGANISMS

The established ability of DMSO to act as a penetrant carrier has brought up the interesting question of its effect on antibiotic susceptibility testing. The eventual use of DMSO to transport antibiotics to hard-to-reach areas of the body, such as bone marrow, brain, and so forth, underlines the importance of this question. Also, the presence of DMSO in blood, urine, and other specimens might influence routine culture work and later antibiotic susceptibility testing. The added fact that DMSO acts as a bacteriostatic or bactericidal agent makes this question all the more interesting.

In conditions such as scleroderma, extensive burns, fungus infections, arthritis, and so forth, the accepted methods of treatment require the application of copious amounts of DMSO. The penetrant characteristic of the drug naturally assures a good blood level, urine level, spinal fluid level, and so forth, which will be reflected in specimens received in the laboratory for routine diagnostic procedures. The possibility exists that the concentration of DMSO in the clinical specimens used in biochemical, blood bank, and microbiological testing might be high enough to affect laboratory results. Unquestionably, a much broader and detailed investigation will need to be made in these areas.

One of the early promises of DMSO was its ability to act as a penetrant carrier of chemotherapeutic agents. A number of studies have been made to assess the effect of DMSO as a penetrant carrier and potentiator of antibiotics and other chemotherapeutics. Results reported to date by various researchers are both encouraging and discouraging. The methods used and the wide range of compounds tested have varied

so greatly that it is difficult to correctly evaluate the data that has accumulated.

Jacob *et al. (1)* in some of their earlier work involving 100 experiments using the intact urinary bladder of dogs, demonstrated that DMSO enhanced the absorption of sulfadiazine, insulin, sodium salicylate, heparin, and so forth. These workers were convinced that DMSO served as a penetrant carrier of these compounds and had a reversible effect on the biological membrane. Initial studies with plants revealed that DMSO exerted a profound effect on the biological membrane, altering its natural selectivity. Membranes treated with DMSO were rendered porous to compounds generally considered to be nondialyzable. Penetration of normally dialyzable ions and compounds was increased.

Jacob and co-workers *(2)* devised a procedure to determine whether or not DMSO would facilitate the transport of certain substances across the mucous membrane of the dog bladder. They believed better results were obtained if the bladder environment were maintained at an alkaline pH. Where sulfadiazine and salicylate were instilled, serum sulfadiazine and salicylate levels were markedly increased as compared with control animals. A six- to eightfold increase of serum sulfadiazine was noted in the DMSO-treated animals beginning at the initial 30-min period.

Kutscher *(37),* in a brief report, noted that 0.007 Decadron in a 70% solution of DMSO was found to have useful suppressant action on certain chronic lesions of the oral mucous membranes without indication of untoward effects.

Pope and Oliver *(38)* stated that the results of their investigations have shown that DMSO preadministered orally or parenterally does not greatly alter either the rate of absorption or the penetrability of the majority of drugs tested to date, although the cutaneous absorption of some drugs is increased when they are incorporated into concentrated solutions of DMSO.

These workers further noted that the mechanism by which DMSO increases membrane permeability is unknown. However, they suggested possible explanation including: (1) its solvency toward lipoidal and nonlipoidal material, (2) its ability to chelate metals, (3) its semipolar nature and small molecular size, (4) its hygroscopic nature, and (5) its heat of dissolution. They stated further that if DMSO is able to form calcium or other metallic chelates in the membrane, its ability to influence cell membrane permeability may be explained in part. Thus,

the nature of the solute, the site of administration, as well as the concentration of both solvent and solute all appear to contribute to the role of DMSO as a penetrant carrier.

Caccialanza and Finzi *(39),* in a brief report, summarized their studies with the conclusion that pharmacological and clinical research has indicated the DMSO has no useful local or general therapeutic action when used topically. Furthermore, DMSO as a vehicle for various drugs acting either on the skin or on visceral systems neither proved suitable for effecting transcutaneous penetration of very high concentrations of drugs nor increased their therapeutic effect to an extent greater than that obtained in the usual experiments without DMSO.

Kligman *(10)* used the fluorescent antibiotic dimethylchlorotetracycline hydrochloride (Declomycin) in a 20% DMSO solution to measure bladder penetration. The curve had much the same shape as the water controls, however, a sharp break was noted at about 70% DMSO concentration. Penetration was complete in 20 min at 90%, 55 min at 70%, and 120 min at 50%. In water alone, the time exceeded 3 hr. Occasion presents itself again to note that it is only with rather concentrated solutions that penetration enhancement becomes really striking.

Attention is called to the superiority of rate measurement for predicting the usefulness of DMSO to potentiate penetration. This is the measurement par excellence that reflects the actual quantities entering the skin per unit of time. It should again be emphasized that the effectiveness of DMSO increases sharply above 70%. Concentrated solutions, perhaps 80–90%, may be required if DMSO is to be clinically useful in accelerating the rate of diffusion of topical medication such as corticosteroids through the skin.

Kamiya *et al. (24)* demonstrated the effect of 5% DMSO on the restoration of sensitivity to antibiotic-resistant bacteria. They reported the sensitivity of bacteria to antibiotics was measured in 5% DMSO-modified Mueller Hinton medium and in control medium. Ten strains of *Pseudomonas, Staphylococcus,* and *E. coli* were isolated from patients. Sensitivity was determined on the basis of the growth-inhibitory circle diameters on this medium. Results were as follows: (1) *Pseudomonas.* As shown in Table 3, colistin-resistant strains 6, 8, and 9, tetracycline-resistant strains 6 and 10, and kanamycin-resistant strains 2 and 9 restored the sensitivity to these antibiotics in the medium containing DMSO. Sulfisoxazole had no effect on *Pseudomonas,* however, some strains (5 and 9) showed sensitivity to it. (2) *E. coli.* As

TABLE 3

Restoration of Sensitivity in *Pseudomonas* on Modified Mueller Hinton Medium Containing 5% DMSO

Chemotherapeutic agent	*Pseudomonas* strain number[a]																			
	1		2		3		4		5		6		7		8		9		10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Penicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	R	R
Kanamycin	S	S	S[b]	R	S	S	S	S	S	S	R	R	S	S	S	S	S[b]	R	R	R
Tetracycline	S	S	S	S	S	S	S	S	S	S	S[b]	R	S	S	S	S	S	S	S[b]	R
Chloramphenicol	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Erythromycin	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Colistin	S	S	S	S	S	S	S	S	S	S	S[b]	R	S	S	S[b]	R	S[b]	R	S	S
Sulfisoxazol	R	R	R	R	R	R	R	R	S[b]	R	R	R	R	R	R	R	S[b]	R	R	R
Leucomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

[a]R, Resistant (no inhibition zone); S, sensitive (good inhibition zone); A, medium containing 5.0% DMSO; B, medium containing no DMSO.

[b]Restoration of sensitivity.

TABLE 4

Restoration of Sensitivity in *coli* on Modified Mueller Hinton Medium Containing 5.0% DMSO

Chemotherapeutic agent	*Escherichia coli* strain number[a]																			
	1		2		3		4		5		6		7		8		9		10	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Penicillin	S[b]	R	R	R	R	R	S[b]	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	S	S	S	S	S	S	S	S	S	S	R	R	R	R	S	S	S	S	R	R
Kanamycin	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	R	R
Tetracycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S[b]	R
Chloramphenicol	S	S	R	R	R	R	R	R	S	S	R	R	R	R	S	S	S	S	R	R
Erythromycin	S	S	R	R	R	R	S	S	S	S	S[b]	R	R	R	R	R	S[b]	R	R	R
Leucomycin	S	S	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R
Oleandomycine	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Colistin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R
Sulfisoxazol	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	S	S	R	R

[a]R, Resistant (no inhibition zone); S, Sensitive (good inhibition zone); A, medium containing 5.0% DMSO; B, medium containing no DMSO.

[b]Restoration of sensitivity.

shown in Table 4, erythromycin-resistant strains 6 and 9, tetracycline-resistant strain 9; and kanamycin-resistant strain 7 restored the sensitivity for these antibiotics in medium containing DMSO. Penicillin had no effect on *E. coli,* however, some strains (1 and 4) showed sensitivity to it. (3) *Staphylococcus*. Staphylococci did not become sensitive to antibiotics in the medium containing DMSO.

Kamiya and coworkers, in a companion series, noted the effect of 5% DMSO on the increase of sensitivity to antibiotics. As shown in Table 5, 10 strains each of *Pseudomonas, Staphylococcus,* and *E. coli* were

TABLE 5

Effect of 5% DMSO in Modified Mueller Hinton Medium on Diameter of Inhibition Zones[a]

	Pseudomonas		*Escherichia coli*		*Staphylococcus*	
Chemotherapeutic agent	No. strains tested	Inhibition increased	No. strains tested	Inhibition increased	No. strains tested	Inhibition increased
Penicillin	10	0	10	2	10	1
Streptomycin	10	4	10	2	10	6
Kanamycin	10	4	10	2	10	3
Tetracycline	10	4	10	3	10	1
Chloramphenicol	10	0	10	1	10	0
Erythromycin	10	1	10	5	10	3
Leucomycin	10	0	10	0	10	2
Oleandomycin	10	0	10	0	10	4
Colistin	10	2	10	0	10	0
Sulfisoxazol	10	2	10	1	10	1

[a]Results based on disc method of sensitivity testing on petri plates of Mueller Hinton medium. The control plates contained no DMSO, test plates contained 5% DMSO. An increase of more than 2 mm in diameter of inhibition zone was considered enhancement.

tested by the disc method on control medium and on medium containing DMSO. All 30 strains were sensitive to the test antibiotics used. The difference of more than 2 mm of growth-inhibitory zone diameters between DMSO and control medium was regarded as an increase of sensitivity for different bacteria.

Stringer and Engle *(14)* achieved gratifying results in common paronychia in clinical investigation of a formula containing, bromsaline (0.5%), iodochlorohydroxyquinoline (USP), and DMSO. This

formula was designed to act upon a broad spectrum of infections, for many of the common cases of paronychia culture pyogenic cocci, *C. albicans, E. coli,* or fungi; mixed infections are all too common. The purpose was to dissolve relatively insoluble drugs in a highly penetrating base, and to make repeated small applications to the diseased tissue. The technique of application is 1 drop per nail fold painted on two or three times a day.

Pottz *et al. (5)* in early experiments indicated the possibility that a combination of penicillin and dimethyl sulfoxide would reverse the resistance of *S. aureus* to penicillin. These early experiments were with *Staphylococcus* strains isolated in the course of routine laboratory work. The assumption was made that this reversal of resistance was most likely brought about by the neutralization of the penicillinase elaborated by the microorganism.

An attempt to duplicate these findings by large-scale testing was conducted. Sixteen strains of penicillin-resistant *S. aureus* were obtained from the National Communicable Disease Center, Atlanta, Georgia. As indicated in Table 6, each of these 16 strains was tested and found to be coagulase positive. In turn, each strain was tested against 10 units and 40 units of penicillin G potassium and proven to be highly resistant. Growth of each of the 16 strains was unaffected by DMSO in concentrations of 0.5, 2.5, 5.0, and 10.0%. Growth of these strains was in no way restricted by exposure to 40 units of penicillinase in the culture medium.

In order to determine the effect of combined penicillin and DMSO on this group of resistant staphylococci, they were exposed to varying concentrations and combinations of the two compounds. Table 7 shows the results obtained with each of these penicillin-resistant strains. Fourteen of the 16 strains survived and multiplied without restriction in media containing penicillin varying from 1 unit to 40 units and DMSO in concentrations of 0.5–5.0%. In the case of these 14 strains, it must be assumed that no neutralization of the penicillinase or any degree of synergism between the penicillin and DMSO took place. In the case of strains no. 1 and 15, there appears to be a reversal of resistance of the microorganisms to penicillin. Cultures of the two strains were highly resistant to penicillin for several generations. It was assumed that this reversal was most likely chance mutation and not attributable to the combined action of the penicillin and DMSO.

The possible neutralization of penicillinase by DMSO was examined further by the series of experiments shown in Table 8. Three strains of

TABLE 6

Effect of Penicillin and DMSO on a Group of Penicillin-Resistant *S. aureus*

Staphylococcus strain[a]	Test substance used[b]							
	Coagulase test	Penicillin (10 units)	Penicillin (40 units)	DMSO (0.5%)	DMSO (2.5%)	DMSO (5.0%)	DMSO (10.0%)	Penicillinase (40 units)
1	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+
5	+	+	+	+	+	$	+	+
6	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+

[a] All 16 strains of coagulase-positive and penicillin-resistant S. *aureus* were furnished for this series by Jay O. Cohen, Bacteriology Section, National Communicable Disease Center, Atlanta, Georgia.

[b] + Indicates good growth on broth culture and blood agar subcultures.

TABLE 7

Effects of Combined DMSO and Penicillin on Susceptibility of Proven Penicillin-Resistant Staphylococci

Staphylococcus strain	Concentration of penicillin and dimethyl sulfoxide used[a]						
	Pen.(1 unit), DMSO 0.5%	Pen.(10 units), DMSO (2.5%)	Pen.(20 units), DMSO (2.5%)	Pen.(40 units), DMSO (2.5%)	Pen.(10 units), DMSO (5.0%)	Pen.(20 units), DMSO (5.0%)	Pen.(40 units), DMSO (5.0%)
1	++	–	–	–	–	–	–
2	++	++	++	++	++	++	++
3	++	++	++	++	++	++	++
4	++	++	++	++	++	++	+
5	++	++	++	++	++	++	++
6	++	++	++	++	++	++	++
7	++	++	++	++	++	++	++
8	++	++	++	++	++	++	++
9	++	++	++	++	++	++	++
10	++	++	++	++	++	++	++
11	++	++	++	++	++	++	++
12	++	++	++	++	++	++	++
13	++	++	++	++	++	++	++
14	++	++	++	++	++	++	++
15	++	+	–	–	–	–	–
16	++	++	++	++	++	++	++

[a] ++ Indicates good growth; + indicates moderate growth; – indicates the absence of growth in broth and on blood agar subculture.

TABLE 8

Neutralization of Penicillinase by DMSO

Penicillin-sensitive *Staphylococcus* strain used	Test substance used					
	Penicillin (5 units)	Penicillinase (40 units)	DMSO (5.0%)	DMSO (10.0%)	Penicillinase (40 units), DMSO (5%), penicillin (5 units)	Penicillinase (40 units), DMSO 10%, penicillin (20 units)
GHS-137-65	–	+	+	+	+	+
GHS-210-65	–	+	+	+	+	+
GHS-212-65	–	+	+	+	+	+
GHS-019-65	–	+	+	+	+	+
GHS-119-65	–	+	+	+	+	+
GHS-152-65	–	+	+	+	+	+

[a] + Indicates good growth of test organism; – indicates no growth of test organism in broth or blood agar subcultures.

penicillin-sensitive staphylococci were first tested and found susceptible to 5 units of penicillin. Growth of these microorganisms was unrestricted in the presence of 40 units/ml of penicillinase. Five and 10% DMSO in broth cultures failed to limit their growth. Penicillinase in the amount of 40 units/ml was added to protect these penicillin-sensitive strains. Combination of 5.0% DMSO and 4 units of penicillin/ml and 10% DMSO and 20 units penicillin/ml were added. The penicillin-sensitive staphylococci were protected by the penicillinase and showed unrestricted growth even in the presence of 20 units/ml of penicillin. Subcultures from this treated series were again sensitive to 5 units/ml penicillin. This entire series was varied by first adding the penicillinase to broth cultures of the microorganisms and then adding the combined DMSO and penicillin; by adding the microorganisms to broth tubes already containing the penicillinase, penicillin, and DMSO; and by adding the penicillinase, DMSO, and penicillin in that order to broth cultures of the test microorganisms. In each case the growth of the microorganisms, protected by the penicillinase, was unrestricted. This entire series appears to indicate that there is no degree of neutralization of penicillinase by DMSO.

The recognized importance of chemotherapeutic susceptibility testing in clinical medicine raises the question of the possible effect of DMSO on this significant laboratory procedure. To investigate the possibility

that DMSO might interfere with or enhance reactions in susceptibility testing, Pottz *et al. (26)* made a study of this question using *Salmonella paratyphi, Proteus vulgaris, P. aeruginosa, S. faecalis,* beta hemolytic streptococci Group A, *D. pneumoniae, E. coli,* and *A. cloacae* as the test microorganisms. High and low concentration levels of penicillin, chloromycetin, novobiocin, tetracycline, and dihydrostreptomycin were added to beef heart infusion broth cultures of the test microorganisms and incubated; presence or absence of growth was checked by subculture to blood agar plates. In a second series low and high concentration discs (BBL) of each of the chemotherapeutic agents were placed on beef heart infusion blood agar plates containing concentrations of DMSO varying from 0.5 to 10% on which the test microorganisms had been streaked. These plates were incubated and examined for zones of inhibition. In a third series the low and high concentration discs were first soaked in the various concentrations of DMSO and then placed on the streaked plates of beef heart infusion blood agar, incubated, and examined for zones of inhibition. A summary of these three series of experiments is shown in Table 9. A study of this data shows conclusively that the presence of DMSO in a concentration of 10% does not interfere with the chemotherapeutic susceptibility testing of this group of microorganisms. There is no apparent evidence that the presence of DMSO, in the tested concentrations, in any way enhances the reactions found in routine chemotherapeutic susceptibility testing. These findings give assurance that the small amounts of DMSO that might be present in such specimens as urine, blood, body fluids, and so forth, or in blood bank blood used widely in media preparation will not interfere with clinical laboratory susceptibility testing of microorganisms isolated in infection or disease.

The above series was repeated using the 16 strains of penicillin-resistant *S. aureus,* and the results obtained were essentially the same. The presence of DMSO did not interfere or enhance reactions of the chemotherapeutics used in the susceptibility tests conducted.

The experiments of Kamiya *et al. (24)* were repeated in our laboratory and the findings were consistent with those reported by these workers. However, the increase of only 2 mm in the diameter of the zones of inhibition in these tests was considered to indicate no more than enhanced diffusion of the antibiotics and not an enhancement of the sensitivity of the test microorganisms to these drugs.

Preliminary studies reported by Pottz *et al. (40)* indicated the possibility that the developed resistance of *M. tuberculosis* to streptomy-

TABLE 9

Effect of DMSO on Routine Susceptibility Testing with Six Commonly Used Chemotherapeutic Agents

Test microorganism	Penicillin (10 units)		Erythromycin (15 μg)		Chloromycetin (30 μg)		Novobiocin (15 μg)		Tetracycline (30 μg)		Dihydrostreptomycin (10 μg)	
	1	2	1	2	1	2	1	2	1	2	1	2
Salmonella paratyphi	R	R	R	R	S	S	S	S	S	S	R	R
Proteus vulgaris	R	R	R	R	S	S	S	S	S	S	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R
Streptococcus faecalis	R	R	R	R	S	S	R	R	S	S	S	S
Beta hemolytic streptococci A	S	S	S	S	S	S	S	S	S	S	S	S
Diplococcus pneumoniae	S	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli	R	R	R	R	S	S	S	S	S	S	R	R
Aerobacter cloacae	R	R	R	R	S	S	R	R	S	S	S	S

S, Microorganism susceptible to agent tested; R, microorganism resistant to agent tested; 1, test chemotherapeutic alone; 2, test chemotherapeutic agent plus DMSO to final concentration of 10%.

cin and isonicotinic acid hydrazide (INH) can be reversed by the combination of these agents with DMSO. A small series of cultures of *M. tuberculosis* of proven high resistance to both streptomycin and INH were killed *in vitro* with a combination of 5% DMSO and less than 10 μg streptomycin, or 5% DMSO and 0.05 mg INH, or both. This study has since been repeated using a total of 24 resistant strains and the same results were obtained. It is difficult to evaluate the significance of these *in vitro* studies. A full report and evaluation will of necessity await completion of animal studies.

In summary, it is of importance to the microbiologist to note that the presence of a small amount of DMSO does not affect culture results, results of chemotherapeutic susceptibility testing, the preparation of culture media, and so forth.

IV. EFFECT OF DMSO ON FUNGI

Of the thousands of distinct species of fungi, approximately only 45 are potentially pathogenic for human beings. All of them are true fungi and, with few exceptions, belong to the group termed Fungi Imperfecti. The diseases or infections produced by the true fungi are generally classified as (1) superficial mycoses involving the skin, hair, nails, and the mucous membranes of the mouth, nose, and vagina, and (2) the deep-seated or systemic mycoses. Most of the fungi can be placed into one of these categories, however, some such as *Actinomyces bovis, Aspergillus fumigatus* and *A. niger, Blastomyces dermatitidis, C. albicans, Coccidioides immitis, Cryptococcus neoformans, Giotrichum candidum, Histoplasma capsulatum, Monosporium apiospermum, Nocardia asteroides,* and some species of *Penicillum* may produce both systemic and superficial infection. (In this connection it may be stated that fungal diseases are so prevalent that they deserve the attention of *all* physicians because of the frequency with which the deep, disseminated, or systemic mycoses require consideration in the differential diagnosis of not only various obscure chronic infections but sometimes acute infections as well.)

The dermatophytes are an unique group of botanically related fungi with an affinity for epidermal scales and hair. Whether or not they are keratinophilic, i.e., digest "keratin" per se, is still undetermined. There are a minimum of six presently known soil-inhabiting species and a group of species primarily parasitic in the stratum corneum and hair of

living animals. Twelve of the 18 known strictly parasitic species are primarily or exclusively parasites of man.

It appears to be natural that the fungi would be one of the first groups of organisms to attract the attention of the early researchers experimenting with DMSO. In fact, Jacob and co-workers *(1, 2)* were experimenting with DMSO as a solvent for hard-to-dissolve fungicides when they accidentally discovered its penetrant capacity. Since this early experience, an impressive body of information on the effects of DMSO on the fungi has accumulated.

It was early recognized from known characteristics of the drug that it had great possibilities in the field of dermatology in the control of the superficial mycoses.

Goldman *et al. (16)* stress that dermatologists are interested in any material proposed for topical therapy, either as a direct medication itself or as a vehicle for other medication. They conclude that DMSO is both.

Jacob *et al. (1, 2, 28, 41, 42)* were early to recognize the possibility that DMSO might display characteristics of a fungicidal agent. They reported that initial studies with plants revealed that the chemical exerted a profound effect on the biological membrane, altering its natural selectivity. Membranes treated with DMSO were rendered porous to compounds generally considered to be nondialyzable. Penetration of normally dialyzable ions and compounds was increased.

Sulzberger *(43)* concluded that because of the demonstrated rapid and deep penetration by materials incorporated in DMSO into the horny layer of the human skin and into follicles indicated that DMSO might increase the effectiveness of incorporated antibacterial agents in the treatment of pyodermas, dermatophytoses, and acnes.

Pearce *(8)* reported good results with iodine in DMSO in treating fungus conditions of the skin and nails. Iodine has always been considered one of the best of the fungicides, but before its incorporation with DMSO it was not possible to introduce it to the tissues in depth.

Tsuchiya and co-workers *(6),* in their monumental work tracing the formation of methane thiosulfonate from DMSO, showed that this reaction product was antifungal. In their experiments with methane thiosulfonate, they demonstrated its activity as a fungicide against *T. rubrum* and *T. asteroides.* Rampey *(22, 23)* reported that DMSO had fungicidal activity against *Candida albicans* and *Cryptococcus neoformans.* He concluded that a concentration of 5–10% DMSO controlled these two pathogenic fungi.

Pottz *et al. (5)* also found DMSO in a concentration of 20% or higher to be fungicidal against *Candida albicans* and *Cryptococcus neoformans.* This was determined from both broth cultures to which the DMSO was added and by the incorporation of the drug into Sabouraud's agar plates on which the organisms were then streaked.

Stringer and Engle *(14),* noting the well-known difficulty of distributing antiseptics and antifungal agents into the entire diseased layer of keratin in nail and nail-fold infections, concluded that this could be partly overcome by use of penetrating vehicles. They reported good results in common paronychia using the following formula: bromsalans, 0.5%; iodochlorohydroxyquinoline (USP), 0.5%; DMSO, to 100%. This formula was designed to act upon a broad spectrum of infections; for many of the common cases of paronychia culture pyogenic cocci, *C. albicans, E. coli,* or fungi; mixed infections are also common. The purpose was to treat diseased tissues with relatively small applications. The technique of application was 1 drop per nail fold painted on two or three times a day over a 3- to 4-week period.

Pope and Oliver *(38)* reported the successful use of DMSO as a solvent for antifungal drugs in the treatment of ringworm disease and suggested that a wider and more detailed study should be conducted to assess its value in the treatment of these and other fungal diseases.

Kaminsky *(44)* discussed the possible applications of DMSO in the field of dermatology. He reported successful use of the drug as a carrier of fungicides and antibiotics in topical application. His report lists formulas for combination with bactericidal and fungicidal agents for topical treatment.

Belsky and Goldstein *(12),* however, did not find that DMSO enhance penetration in their experiments with a group of nonfilamentous marine Phycomycetes. They used *Thranstochytrium roscum, T. aurcum, Schizochytrium aggrogatum,* and *Dermocysitidium* spp. as test fungi. Paper sensitivity discs were impregnated with the various antifungal agents and placed aseptically on the surface of inoculated media in duplicate plates. Another set of duplicate plates prepared with 0.5% DMSO did not enhance effectiveness of any of the substances tested. It would be of interest to repeat these studies using a gradual increase in DMSO to 10% concentration.

Kligman *(10, 11)* recognized the commercial use of DMSO as a solvent for fungicides. He also conducted an interesting series of experiments which showed that DMSO alone possesses certain antifungal characteristics. The minimal inhibitory concentration of DMSO in

Sabouraud's broth to the nearest 10% was determined for three dermatophytic fungi: *Trichophyton mentagrophytes, Microsporum gypseum,* and *M. canis.* A loopful of dilute spore suspension was added to each tube which was then incubated for 1 week at room temperature. DMSO in a 10% concentration was inhibitory to all three test organisms. By subculture the fungicidal concentration was 30% for *Microsporum* species, while *T. mentagrophytes* survived the highest tested concentration of 50%. On the basis of these results, Kligman concluded that DMSO may be considered a weak antifungal agent.

Since ringworm infections are exclusively confined to the horny layer of the skin, which is rapidly penetrated by DMSO, the use of this agent as a vehicle for topical fungicides in its treatment should hold considerable promise. This possibility was explored in two preliminary, uncontrolled series of tinea pedia (athlete's foot). Male subjects were selected who presented typical signs of athlete's foot in the fourth interspaces which was verified by potassium hydroxide examination. In the first study, 11 subjects were treated twice daily by swabbing the interspace with 90% DMSO for 2 weeks. Clinical improvement was moderate in eight of these cases, but filaments were demonstrable in potassium hydroxide mounts of scrapings in six of the eight cases. By itself, DMSO was not sufficiently therapeutic to be considered superior to many other moderately active fungicides. There were no adverse effects, although some of the subjects experienced a transient burning sensation.

In Kligman's second series, 2% thiabendazole in 90% DMSO was applied bilaterally once daily for 14 days to the fourth interspace of 16 men with potassium hydroxide-verified athlete's foot. The clinical results were rated good to excellent in all patients, and fungus elements were not microscopically demonstrable in 13 of these 16 subjects after 14 days. The experimental design did not show whether or not similar efficacy would have been demonstrated by thiabendazole in another vehicle. Whether attributable to the thiabendazole, the DMSO, or the combination, this clinical result was decidedly encouraging.

Stone *et al. (45)* reported on a small series of patients treated for tinea nigra palmaris with combined DMSO and thiabendazole. The palm is generally resistant to penetration of therapeutic agents, and each of the patients in this series had previously failed to clear following application of several topical antifungal preparations. All patients in the series failed to respond to 90% DMSO applied twice daily, or to 4% thiabendazole ointment applied three times daily. These workers

obtained excellent results, however, using a 2% thiabendazole solution dissolved in 90% DMSO and applied once daily for 14 days. They concluded, on the basis of this small series, that the above combination applied topically once daily is an effective agent for treatment of tinea nigra palmaris. The amount of thiabendazole applied topically was so small that consideration need not be given to systemic effects of the absorbed material.

Zaias and Taplin *(46)* state that the direct demonstration of fungi in skin, nail, or hair scrapings is facilitated by dissolving the alkaline clearing agent, potassium hydroxide (20%), in a solvent of 60% water and 40% DMSO. With this reagent, hyphae can often be seen as well in 1 min without heating, as compared to 20 min or more with the classic plain aqueous alkaline solution. The DMSO-containing reagent is not recommended if more than 20 min will elapse between preparation of specimen and microscopic examination.

Goldman *et al. (16)* conducted a series of experiments to determine the fungicidal activity of DMSO. Concentrations of DMSO up to 1% incorporated in agar had no effect on *Microsporum, audouini, M. gypseum, M. canis, T. mentagrophytes, T. rubrum, C. albicans, C. neoformans, H. capsulatum, B. dermatidis,* and *S. schenckii.* One patient with a generalized *T. rubrum* infection had 90% DMSO applied for subtotal body inunction (approximately 90%) twice daily for 3 weeks. His serum, in 25% concentration added to agar plates, showed no antifungal activity against *T. rubrum* or *T. mentagrophytes*

While there has been a great mass of data concerning the medical uses of DMSO as an antifungal agent or as a transporter of fungicides, there have been limited studies reported on its uses and application in agriculture. An early study in this field was conducted by Keil *(47),* who found DMSO alone had little or no effect on the organisms studied at concentrations tested. However, DMSO proved to be a good solvent for many organic chemicals and inorganic salts and appeared to have the capacity to transport them into plants as well as animals. This property was of interest because DMSO could be used as a vehicle to carry toxicants to the site of infection. Much of Keil's work consisted of three types of tests: foliage spray, stem paint, and soil drench. By the first two methods he demonstrated enhanced control of certain fungal and bacterial diseases when DMSO was combined with various chemical compounds. Foliage sprays, however, were the most successful. Preliminary spray tests indicated that DMSO augmented the activity of certain antibiotics, chemical fungicides, and bactericides. Representative of

these are organic mercuries, quaternary ammonium compounds, iodine, hexachlorophene, Karathane, zinc sulfate, and oxytetracycline.

Working with bacterial spot disease of peaches caused by *Xanthomonas pruni,* for which there has been no effective control method, Keil found that control could be achieved by a combination of DMSO and oxytetracycline spray whether the disease was located in the leaves, twigs, or fruit. Data indicated enhanced control of peach bacterial leaf spot when DMSO at a concentration of 1% was included with sprays of oxytetracycline. The addition of DMSO to 28, 56, and 112 ppm of this antibiotic significantly increased disease control over comparable levels of antibiotic without DMSO. Additional studies showed that a 0.25% concentration of DMSO gave results as good as those obtained with 1% DMSO. Optimum results appeared to be obtained with 42 ppm oxytetracycline in 0.25% DMSO.

Greenhouse studies on peach bacterial leaf spot further indicated that residue resulting from the DMSO–oxytetracycline spray provided some protection of foliage against *X. pruni* infection for as long as 8–10 days. It was observed that terminal leaves that developed subsequent to spraying had significantly less bacterial spot infection than comparable leaves on the untreated plants. These results suggest absorption of the toxicant by newly developed terminal leaves. Preliminary soil drench tests conducted on peach trees under greenhouse conditions showed no enhancement of peach bacterial spot control when DMSO was combined with various chemical compounds. These tests, however, did indicate some effect by DMSO alone. This was a most interesting finding and indicates that further studies should be made along this line.

Exploratory field studies using an injection technique, were conducted to see whether or not material placed in one part of an apple tree translocates and controls disease in another part of the tree. In these studies, 100–150 ml of the test solution were fed to Rome Beauty trees through a hole bored into either the trunk or base of a large limb. Two or three applications were made at intervals of 2 weeks. Scab and powdery mildew ratings were recorded on the terminal leaves 6–10 ft from the injection site. Several chemical compounds combined with DMSO caused significant reduction of both diseases as compared with the untreated trees. DMSO alone had no effect on either disease. In general, there was approximately 50% reduction in scab disease with most of the effective treatments, but only about 20–30% reduction of powdery mildew. Orchard trees appeared to withstand 1–2% solutions

of DMSO alone without injury, but 10% solutions caused slight leaf tip burn.

Keil and associates *(48)* conducted a detailed companion study on the greenhouse and field control of bacterial leaf spot disease of the peach in which they compared the effects of oxytetracycline, zinc sulfate, iodine, and so forth, alone and in combination with DMSO concentrations of 0.25–2%. Iodine and zinc sulfate alone and in combination with DMSO were ineffective under the severe disease conditions of these tests. Oxytetracycline (28 ppm) combined with 1.0–2.0% DMSO greatly enhanced the control of bacterial leaf spot as reported in Keil's earlier work.

Keil concluded that the results obtained in this series of experiments were important for several reasons. A commercial product, DMSO, capable of dissolving many organic and inorganic compounds, was available. The apparent increased plant absorption and translocation of certain chemicals when dissolved in DMSO leads to speculations in regard to plant disease control. It seems that these offer an opportunity to control deep-seated and systemic infections impossible to control by conventional methods. Conceivably this could be brought about by absorption and translocation of the unchanged toxic moiety to the site of the infection. It is also possible that toxic decomposition products, formed after absorption could be translocated to the site of the infection. It is conceivable that a nontoxic molecule might, after absorption, alter plant metabolism and cause substances to be produced which fail to support growth of the causal organism. It is also possible that new compounds may be formed when certain chemicals are dissolved in DMSO.

Bean *(49)* reported a series of studies demonstrating the enhanced control methods, using DMSO with fungicides to combat *Helminthosporium* leaf spot and crown rot caused by *Helminthosporium* species in Kentucky bluegrass *(Poa pratensis)*. In the greenhouse, DMSO concentrations of 100, 50, 12, and 6% water solutions were phytotoxic to bluegrass, causing damage to the leaf tips. Concentrations of 3 and 0.5% did not damage foliage. A 3% concentration was used for field inoculation. Both Dithane M-45 (zinc ion and maneb) and Dyrene [2, 4-dichloro-6-(*o*-chloroanilino)-*s*-triazine] caused a significant reduction in the number of *Helminthosporium* lesions. Although there was no significant difference between check plots and the DMSO plots, the addition of 3% DMSO to Dyrene and Dithane M-45 caused approximately a 30% reduction in the number of lesions. The inhibitory effect of DMSO increased directly with increasing concentration, although at

1 and 0.5% spore germination was not affected. The presence of DMSO in the medium also reduced the growth of *Helminthosporium dictyoides* as well as *H. sativum* when colony diameters were measured. At concentrations below 1%, however, the growth rate of the two species was not affected by DMSO. Bean concluded that DMSO had little fungicidal activity by itself, but when added to the fungicides increased their effectiveness by approximately 30%. He was unable to explain these findings; however, since DMSO apparently is a good wetting agent, it may have been responsible for better coverage of the grass foliage. Another possibility, which requires testing, is that the fungicides may have become systemic in the plant because of DMSO.

Frank *et al. (50)* showed the effectiveness of nystatin in the control of fungal diseases of plants of high economic value. Nystatin is especially effective in the prevention of fungal spotting of orchids. In a similar investigation in our laboratory, Pottz found that nystatin in combination with DMSO provided good control of fungal diseases of roses when used as a foliage spray.

V. EFFECT OF DMSO ON VIRUSES

One of the surprising and encouraging characteristics of DMSO is its potential as an antiviral agent and as a transporter of certain antiviral drugs. The ability of DMSO to penetrate the biological membrane makes it ideal as a transporter of antiviral compounds directly into the cell where they are most effective against intracellular parasites. To date, there has been only limited investigation of the effects of DMSO against viruses.

Goldman *et al. (16)* reported the use of 90% DMSO and podophyllin in treatment of warts. They reported finding 5-iodo-2′-deoxyuridine (5-IDU) useful for the treatment of early lesions of severe herpes simplex. In herpes simplex, 5-IDU in DMSO was found to be more effective than DMSO alone. These workers also used *N*-methylisatin $=\beta$-thiosemicarbazone (1% in DMSO) as an antiviral chemotherapeutic agent in the treatment of warts and molluscum contagiosum. No significant results were observed in a small series of patients. Amspacher *(51)* obtained excellent results in a small group of herpes simplex patients using only DMSO in a 90% concentration. He reported the drying up and clearing of lesions and scabs after only two or three applications. There also appeared to be no, or only a few, recurrences in these patients after about 18 months. Past history of these patients

had indicated an average of three to four recurrences for each one.

Dake *(52)* obtained remarkable results using DMSO in the therapy of feline panleukopenia. Feline panleukopenia is a highly contagious viral disease of cats characterized by an explosive short course and a high mortality. The intestinal mucosa of the jejunum and terminal ileum becomes edematous, thickened, and inflamed. There is a reduction of cellular elements in the bone marrow and replacement of normal hematopoietic marrow by fat. There is a gradual or rapid decline in the leukocyte count, which may be reduced to 500/mm^3 or even lower. A total leukocyte count of 500–6000 showing neutropenia and lymphocytosis is usually considered diagnostic. Following the crisis, if the cat recovers, leukocytes are returned to the circulation at a rapid rate, with a neutrophilia usually accompanied by a shift to the left. Following natural exposure the incubation period is usually 5-days. In preacute cases death occurs within 12–24 hr after the first signs of illness. Kittens are often found dead 8–12 hr after having been seen apparently healthy and active. Seldom in acute cases does the course exceed 5–7 days; however, cats that survive longer than 9 days usually recover. Mortality in cats less than 6 months old approaches 90%, and in older cats 50–60% may die. Initially the temperature is 104–106°F the first 24 hr, almost normal for the next 24 hr, and then rises again. Some depression occurs during the first febrile period and is often profound at the second, with weight loss and dehydration following the same pattern. Dehydration becomes quite severe when vomiting or diarrhea occurs. Treatment is based on providing fluids, electrolytes, nutrients, preventing secondary infection, and combating severe dehydration. Conventional therapy consists of whole blood transfusions, fluids, vitamins, and antibiotics given on an intensive schedule and hospitalization of the cat.

Dake reported injecting 4 ml of 90% DMSO combined with vitamins, antibiotics, and fluids intraperitoneally in a series of cats with definite diagnosis of feline panleukopenia. One cat received daily injections for 2 days and two cats were injected on alternate days for a total of two injections. The others, six cats, were injected only once. All surviving cats stopped vomiting within 24 hr and were able to retain food and water within 1–3 days following administration of the DMSO. Nine cases were treated; six survived and three died. The overall mortality rate was 33%.

A double-blind test series was carried out by MacCallum and Juel-Jensen *(15)*, who investigated the control of herpes simplex skin

infections of man treated with combined DMSO and idoxuridine. Idoxuridine is soluble in watery solutions, and it therefore seemed that a solvent that would provide a higher concentration and/or allow greater powers of penetration of the drug through the skin to the affected cells was required. These workers chose DMSO as such a solvent. The results obtained with experiments in which primary herpes simplex virus intradermal infections in guinea pigs were treated with varying concentrations of idoxuridine in different dilutions of DMSO suggested that 5% idoxuridine in undiluted DMSO might be effective in the treatment of recurrent herpatic lesions (cold sores) in volunteers.

Sixteen patients participated in this series of experiments; each of five developed a lesion at a new site after an interval of time and were entered again as a separate cases. One patient developed a lesion at a new site while the old lesion was still healing and was reentered and treated with the same therapy used in the concurrent attack, making a total of 22 patient attacks. One patient did not complete the trial therapy. Lesions in 10 attacks were treated with active idoxuridine–DMSO solution, in 11 with only DMSO. In all, the clinical diagnosis was proven, the virus was isolated, and complement fixation and neutralization antibodies of herpes simplex virus were present. The average duration of the attacks until arrest of the lesions, leaving only a dry crust, was 1.2 days in the group receiving idoxuridine–DMSO and 2.45 days in those receiving only DMSO. The scabs were gone after 3.5 days in the former and after 5.45 days in the latter. The average normal duration of the attack, as estimated by the individual patient without treatment, was 9.8 and 9.55 in the two groups. There was a shortening of the average duration of attack of 6.3 days for the idoxuridine–DMSO-treated group, and 4.1 days for the DMSO-treated group, a difference of 2.6 days between idoxuridine in the solvent (100%) as compared to the solvent alone. DMSO caused a slight tingling or burning sensation when it was applied to the skin, and this inflammatory reaction probably increased the amount of antibody in the intracellular spaces and so aided healing.

No recurrence at the original site was observed during the succeeding 6 months in the group treated with the idoxuridine–DMSO combination, when an average of 1.7 recurrences per patient, based on past history, could have been expected. These workers concluded that idoxuridine in DMSO not only promoted healing but may also prevent recurrence at the same site if the lesion is treated early. The low recurrence rate in the group treated with DMSO may be attributable to

the early action of antibody because of local inflammation. Cases of this type could be a demonstration of one of the basic qualities of DMSO, its action as a catalyst, encouraging the production of natural antibody against the causative virus.

Pope and Oliver *(38)* noted significant clinical improvement in seven patients with severe large herpes simplex lesions treated with idoxuridine in 90% DMSO. These workers considered that additional studies of antiviral chemotherapeutic agents in DMSO were warranted. Furman *(53)* reported excellent results in two cases of herpes zoster which he treated with DMSO in 90% concentration. Asen *(32)* reported four cases of herpes simplex treated with DMSO; three improved and one remained unchanged. He also reported favorable results in two cases of long-lasting herpes zoster of the neck and ear that were treated with DMSO. The indurated and highly hyperalgesic skin became softer and free from pain soon after treatment.

Gordon *(54)* suggested that his experiments have given more than a hint that the continued use of DMSO may be valuable in treating corneal edema resulting from postoperative complications, as well as herpes uveitis.

Persky and Stewart *(55)* reported five cases of herpes of the genitalia which they treated with daily applications of DMSO. Two responded quickly and completely to DMSO, and the disease was arrested without recurrence. In the three other cases no apparent benefit occurred from use of the drug.

Amstey and Parkman *(56, 57)* conducted a detailed study of the effectiveness of DMSO in the enhancement of the infectivity of viral nucleic acid. DMSO was used as an agent to enhance cellular infection by polio ribonucleic acid using African green monkey kidney, rabbit kidney, and HeLa cell cultures. DMSO enhanced the RNA infection, with a nearly linear increase in polio RNA titer resulting when DMSO was increased from a concentration of 5 to 40%. The optimal DMSO concentration for enhancement was 40% for a 20-min absorption time at room temperature. These workers concluded that DMSO is a useful agent for increasing sensitivity in assaying infections polio RNA and presumably all infectious nucleic acids. Although there are many factors which may preclude the isolation of an infectious nucleic acid, DMSO may make it possible to assay the infective nucleic acids from some viruses of characteristically low infectivity titers.

Cochran *et al. (58)* found that water dilutions of DMSO, buffered to pH 8.5 with tris, partially removed tobacco mosaic virus (TMV) protein

when the DMSO concentration was higher than 70%. DMSO concentrations below 20% did not influence the infectivity of TMV or TMV RNA. With concentrations between 20 and 60%, the infectivity of TMV and TMV RNA varied inversely with the concentration of DMSO used. At 60%, DMSO injured the leaves of the host plant (Scotia bean). Systemically infected Turkish tobacco leaves were ground in equal weights of DMSO buffered with tris to pH 8.5. This suspension was placed above a layer of cellulose in a chromatographic column and then washed with 50% DMSO. The DMSO was removed from the eluate by dialysis against distilled water. Highly infective TMV was obtained after further purification steps. These workers concluded that use of DMSO in virus isolation techniques may result in improved viral and viral RNA activities.

Yehle and Doi *(9),* in isolating phages for *B. subtilis,* found that most of these phages were unstable during storage. These workers used a standard phage storage medium combined with various concentrations of spermine, glutathione, albumin, sucrose, and glycerol, with viable phage remaining after storage ranging from 1.3 to 34.5%. Using 10% DMSO with 0.5% glucose, they found that 100% recovery of viable phage was secured after 25-days storage at −20°C. Some of the advantages of the DMSO solution, as listed by Yehle and Dio, were (1) the phage was stabilized for a long period, (2) the phage could be thawed and frozen repeatedly with no loss of titer, (3) rapid dilution did not affect the phage titer, and (4) the preparation of the storage medium was extremely simple. The DMSO solution has been successfully tested with several unstable *Bacillus* phages and perhaps may be suitable for other phages sensitive to long periods of storage or to chemicals such as chloroform.

VI. EFFECT OF DMSO ON PARASITES

The various effects of DMSO on microorganisms and viruses reported are interesting, however, these observations were not altogether unexpected. The unexpected results against animal parasites have been most encouraging. To the present writing, there have been only scanty investigations in this field, however, those that have been reported suggest the need for further studies and the prospects of very intriguing findings involving use against various encysted helminthes.

One can visualize possible use of DMSO alone or in combination with antihelmintic compounds in combating such hard-to-reach conditions as amebic abscess of the liver, trichinosis of the striated muscles, schistosomasis, and so forth.

It is also of note that the addition of DMSO to staining procedures has made it possible to make diagnostic and permanent mounts of high quality easily. Mitchell *(18)* and Rampey *(59)* have both developed useful methods of parasite staining, combining DMSO with other compounds. These procedures will be reviewed later.

Creeping eruption (cutaneous larva migrans) has lent itself to experimental therapy with DMSO. Katz and Hood *(17)* conducted a double-blind study to evaluate a solution of topical thiabendazole for the treatment of creeping eruption. Thiabendazole in DMSO applied topically favorably altered the natural course of creeping eruption and was as effective as the drug administered orally. The vehicle, DMSO, had no effect on the course of the infestation when compared to placebo tablet therapy.

The primary disadvantage of thiabendazole is that the large oral dose required produces bothersome side effects in a large percent of the patients treated. Katz and Hood decided to determine whether or not topical thiabendazole favorably altered the course of the eruption without causing the unpleasant side effects.

Twenty-five patients with creeping eruption of varying severity were treated with either 2% thiabendazole in 90% DMSO, or with 90% DMSO alone. The patients were examined weekly by the same physician at each visit. The criteria for judging a lesion as inactive were cessation of pruritis and absence of erythema, elevation, vesiculation, and movement of the tract. Fifteen patients were treated with a single medication. Ten other patients had eruptions suitable for a bilateral simultaneous comparison of the two medications. In the 25 patients there were 35 treated areas. Good results were obtained with thiabendazole in 90% DMSO. Results were as good as with oral thiabendazole. From an average of over 300 lesions, there was a reduction to approximately 10 in 3 weeks; with oral thiabendazole the reduction was to approximately 20 lesions. The reduction with DMSO alone was very little different from that of the placebo tablets.

This important work has given rise to the question as to whether topical thiabendazole actually requires DMSO. Investigation indicated that topical thiabendazole is not metabolized on absorption into the skin. This may be important in regard to the reported antimycotic

properties of DMSO. There is still some question as to whether DMSO functions only as a rapid and superior carrier for thiabendazole or if it reacts with the compound. Katz and Hood could offer no data to substantiate that it does react with this or other antihelmintic drugs. It should also be noted that we do not know if there were significant epidermal changes in the area of the tunnel of the parasite to affect the worm. Also, there have been no *in vitro* studies of DMSO investigating its toxicity to the nematode.

Smith *(60),* after considerable experience with thiabendazole in DMSO in the successful treatment of tinea pedia and onchomycosis, considered the possibility of using this combination in treating creeping eruption. He felt that the possibility of putting the drug in contact with or into the invader would provide an easier and more dependable method of treatment. Smith's series was composed of 31 cases of cutaneous larva migrans in patients ranging in age from 20 to 54 years. Diagnosis was made in all cases. The lesions involved the trunk and extremities and ranged in number from 1 to over 100 parasites in the cases used. DMSO 90% concentration with 2% thiabendazole, DMSO 70% with 0.25% thiabendazole, and DMSO 90% without thiabendazole were the three preparations used. At the outset, an area about 2 cm in diameter at the active end of the tract was painted with an applicator soaked in one of the above solutions. When the activity of the parasite could not be located, the general area was painted, since no reaction, either local or general, was noted with the smaller areas treated. In many cases the larvae were so thick in an area that it was considered feasible to paint up to 8 cm in diameter. The plain DMSO solution was tried in a few patients, however, it was soon noted that satisfactory results were not forthcoming and treatment was discontinued. The patients were seen at 2-day intervals when possible, and treatment was stopped when there was no evidence of activity in the skin and pruritus had ceased. This was done to observe the lasting effect of the drug and to locate any new or not yet controlled parasites. A clinical cure, based on the complete disappearance of pruritus and cessation of activity of the parasite, was obtained in all but three cases. Since those responding did so within 96 hr, in the three not responding the medication was stopped after 1 week. It may be of interest to note that one of those not responding also failed to respond to oral thiabendazole.

Smith concluded that the rapid clearing of symptoms and signs of active parasites and the lack of relapse in any of the cases reported indicated that this was a method of treating creeping eruption worthy

of further study and wider usage to support or deny these findings. If further study and experience support these findings and those reported by Katz, DMSO–thiabendazole could become the treatment of choice for this troublesome problem.

Any discussion of the affects of DMSO in the field of parasitology would be incomplete without the evaluation of its effect against the insect vectors of some of the common parasites. Sulzberger *(43)* has suggested the possibility of applying a suitable insect repellent in DMSO so that its penetration into the horny layer and slow extrusion over many weeks could prolong the effectiveness of the repellents, rendering them much more useful in preventing insect-vectored diseases including malaria.

VII. MISCELLANEOUS FINDINGS IN THE FIELD OF MICROBIOLOGY AND ASSOCIATED AREAS

Various studies have been made to determine the effectiveness of DMSO in several special areas of microbiology and closely allied fields. While these findings may seem of a minor nature at present, they may open new areas of investigation of the effects of DMSO.

A. Use of DMSO in Staining Microorganisms

One of the earliest uses of DMSO in the clinical laboratory was in the routine staining of microorganisms. In this area it has proved to be a useful tool in the simplification of several complicated staining procedures. Pottz *et al. (7, 40, 61, 62)* recognized that as a penetrant carrier, DMSO, might serve to carry the primary stain into tubercle bacilli, thus simplifying the usually complicated procedure. Their stain for acid-fast microorganisms has been widely accepted and is coming into general use in this country and Europe as a replacement for the time-honored Ziehl–Neelsen and Kinyoun methods. The described technique has the advantages of ease of preparation of staining solutions, elimination of heating of smears and the accompanying mess, reduction of the staining procedure to two steps, combination of the acid decolorizer and the counterstain into one solution, lessening the chance of overstaining and excessive decolorization, retaining other bacteria present, and staining smears and tissue sections equally well.

The simplicity of the staining procedure assures excellent slides, whether they are prepared by a student or by an experienced technologist. The speculation that this unusual wetting agent might well serve to transport carbol fuchsin into the mycobacterial cell more rapidly and without the necessity for applying heat appears to be well founded.

This staining method gives excellent results with all mycobacterial species (including *Mycobacterium leprae).* It has also proved to be an excellent stain for bacterial endospores and ascospores of certain yeasts. It is interesting to note that the head portion of the human spermatozoon is also acid-fast when stained by this procedure. This acid-fastness of the sperm is attributed to the presence of a substance identical with or similar to the mycolic acid found in mycobacteria types. We have found this characteristic staining of the sperm very valuable in examination of vaginal smears taken in cases involving legal medicine.

Our experience with this new procedure has demonstrated that smears and sections of tissue may be stained in 2 min or less, and that decolorizing and counterstaining may be accomplished in a similar period of time. The actual staining with the DMSO method may be accomplished on glass rods over the sink or in Coplin jars. Complete instructions for the preparation of the staining solutions and the methods of staining by this procedure will be found in Section X.

Mitchell *(18)* and Rampey *(59)* have both successfully applied the above-described characteristics of DMSO to modify and simplify the tedious methods of preparation of permanent staining of intestinal parasites. Mitchell summarized the advantages of this modification over the classic Faust's modification of the Heidenhain iron–hematoxylin procedure in terms in agreement with those advanced by Rampey. Faust's modification has been found to be inadequate for demonstrating intestinal parasites because of unsatisfactory definition and differentiation of the nuclear structures and the cytoplasm. Although many modifications of Faust's stain have been reported, the procedures are time-consuming and relatively impractical for routine use. Attempts were made to improve this stain, with excellent results, by incorporating DMSO as a vehicle. This agent increased the penetrating power and decreased the time involved in mordanting and staining from a total of 40 to 6 min, with a subsequent increase in critical definition of the parasites. Further work has been done to develop a less time-consuming semiconcentration method, which was found to be much more effective than the time-honored zinc sulfate flotation method. Details of the preparation of solutions and methods recommended for this stain and Rampey's modified trichrome stain appear in Section X.

The incorporation of DMSO into other staining procedures has been reported by Pottz *et al. (40)*. These workers have investigated the possibility using DMSO to improve the Gram staining method, Albert's stain, Wright's stain, polychrome stain, Papanicolaou procedure, and so forth. While there is some saving of time in the iodine step of Gram's staining and in the Wright's stain, these results do not offer advantages great enough to be considered improvements over the original methods. However, the success of the above-described staining modifications clearly indicate that there is a possibility of fruitful investigation of the effect of DMSO in various staining procedures, tissue fixing, and so forth. Further studies along these lines are being conducted in this as well as other clinical laboratories.

B. Use of DMSO in Skin Testing

The possible clinical usefulness of the membrane-penetrating properties of DMSO in the field of routine and allergy skin testing has been investigated by several workers. Kligman *(10)* noted that the addition of 50% DMSO to solutions containing 1% old tuberculin (OT) enhanced positive patch test reactions in tuberculin-sensitive subjects. Furthermore, the dermatitis produced by 1% trypsin was prevented when the solution contained 50% DMSO. The probable explanation of this behavior is the formation of DMSO complexes with proteins, resulting in their denaturation.

In our laboratory, data has been collected which confirms that reported by Kligman with old tuberculin. However, it is of interest to note that positive skin reactions were obtained when intermediate-strength purified protein derivative of tuberculin in 90% DMSO was applied topically to the forearm of hypersensitive individuals. It was assumed that this protein fraction was carried through the outer layers of skin by concentrated DMSO but not deep enough to exclude localized skin reaction.

Smith and Hegre *(13)* investigated the effect of DMSO in allergy skin testing. To determine the optimal antigen concentrations, two patients with known positive skin reactivity to mountain cedar, a mixture of molds (containing *Alternaria, Hormodendrum, Helminthosporum, Spondylocladium, Fusarium, Aspergillus, Penicillum,* and *Phoma),* and ragweed were tested with various concentrations of antigen in DMSO. Dilutions of 1:40,000, 1:4,000, 1:400, and 1:40 of each antigen in 50% DMSO were used for testing. Starting with the

1:40,000 dilution; the antigens were applied directly to the upper arm with a cotton-tipped applicator. The controls, consisting of a buffer and DMSO with a buffer, were also applied in the same manner. The small drop was allowed to remain on the skin for 30 min. If there was no reaction, the next lower dilution was applied.

At concentrations of 1:40,000, 1:4,000, and 1:400, no objective or subjective reaction was noted. However, at the 1:40 concentration, one patient developed pruritus to mountain cedar and the mixture of molds. No erythema or objective change in the skin was observed. Pruritus developed in each of these areas between 3 and 4 min after application and lasted until the antigen was removed after 30 min. On the basis of these results, the 1:40 antigen concentration in 50% DMSO was selected for skin testing.

The clinical test patients were evaluated first with 1:20 scratch tests for inhalant and environmental antigens. Six of these patients also received 1:500 intradermal inhalant skin tests. Parallel skin tests to a 1:40 concentration of the antigen in 50% DMSO were applied in drop form in addition to the scratch tests on the back. Routine scratch testing gave positive results in six patients. Of the others who required intradermal skin tests, two had positive skin tests. The DMSO–antigen mixtures gave negative results except for one patient who developed pruritus to one antigen. No evidence of erythema, inflammation, or skin reactivity was found. Although 8 of the 10 patients had positive skin tests by conventional techniques, they had no reaction.

This study by Smith and Hegre was designed to evaluate the use of DMSO as a vehicle for allergy skin testing in lieu of scratch or intradermal testing. Initial screening suggested the possibility of a reaction because of the pruritus in response to two antigens in 1:40 dilution. However, in testing two patients, each with 31 skin tests, no evidence of reaction was found. This absence of reaction suggests two possible explanations, i.e., either the antigen went through the skin too fast to allow for skin reactivity, or it did not cross the skin. Previous data support the former explanation inasmuch as antibodies of bovine serum albumin were developed when a mixture of DMSO and bovine albumin was applied to the skin of rabbits.

Perlman and Wolf *(63)* used nonallergic males and employed immunological methods to demonstrate the passage of allergens with DMSO through the intact skin at a sensitized site containing the specific reagin, resulting in the typical Prausnitz–Kustner (P-K) reaction. A number of experiments were conducted with various sera and with a number of allergens varying in molecular size from penicillin

(molecular weight less than 350) to dog hair and house dust (molecular weight more than 300,000). Penicillin G potassium as the allergen proved most successful, since it could be applied with DMSO at an area remote from the sensitized site, thus eliminating the obscuring effect of DMSO which, in the concentration used, often caused redness and urtication. It was observed that with the specific reagnic sera penicillin flared up the sensitized site by a remote challenge either by scratch or intradermal test or by mucosal (sublingual) application. As little as 10 units of penicillin G potassium injected intradermally at a remote site could elicit full reaction; this was also noted with challenge by a scratch or sublingual application.

The sensitized sites were made in most experiments on the flexor surface of the arm and forearm by injecting 0.10 ml of the sera intracutaneously; both the place and the direction of the deposit of the serum were marked. The area for the remote challenge with the mixture of allergen and DMSO was the flank below the axilla. This mixture in the amount of 0.1 ml was applied in the center of a 25-cm^2 area and gently spread to the periphery. Then, with as much uniformity of pressure as possible, the entire area was rubbed 10 times with the head of the plunger of a tuberculin syringe. No abrasions or urticarial streaking were noted. Thus, when urtication from DMSO occurred it was quite uniform. Often there was a spread of this urtication beyond the 25-cm^2 area. The sensitized sites were observed for any reaction for several hours before negative results were recorded. However, 1 hr was arbitrarily accepted for a negative result.

These workers concluded that (1) employment of the P-K reaction offered a method for demonstrating the penetrant-carrying capacity of DMSO through the intact human skin; (2) an allergen of small molecular weight such as penicillin G potassium mixed with DMSO 90% was readily carried through intact skin; (3) allergens of high molecular weight (3000 and above) could not be shown to penetrate skin within the prescribed 1-hr time even when the challenges were made directly to the intact skin over the sensitized sites; and (4) the time required for a remote P-K reaction after challenge with penicillin in DMSO on intact skin was 40 min. This was in contrast to the more rapid rate of reaction after challenge with aqueous extracts of allergens by scratch, intradermal, or mucosal routes, which were usually complete within 20 min.

Kligman *(64)* compared sodium laurel sulfate (SLS), DMSO, stripping, and phenol as provocative agents in skin testing. He rates them in

this order: cumulative reaction rate of SLS, 85/125; DMSO (70%), 80/125; stripping (10 times), 65/125; phenol (5%), 61/125. DMSO provocative tests tend to have sharper borders than SLS tests and on this account may be easier for the uninitiated to read.

The usefulness of DMSO as a penetrant carrier in skin testing and allergy testing appears to be questionable at this time. It is evident, however, that additional work will be done along this line by interested dermatologists.

C. Use of DMSO in Mucus Dispersion

One of the more promising possibilities for DMSO, closely associated with the field of microbiology, is its use in mucus dispersion. Mucin is chemically an inert substance, insoluble in and not readily attacked by physiological fluids. It occurs almost universally in the animal kingdom. It is secreted by all epithelial surfaces where the normal function seems to be to provide a cohesive coat overlaying the epithelium and exerting a protective effect on the underlying cells. Mucin production can be stimulated by chemical and physical irritants, and it is greatly increased in inflammatory diseases. In chronic pulmonary conditions, the presence of abnormal amounts of material of high viscosity within the bronchial tree significantly reduces ventilation and causes retention of cellular debris and pathogenic microorganisms.

A method that will reduce the viscosity of these secretions *in vitro* is essential to the physiological mechanisms involved in the mobilization and removal of such secretions. The life expectancy of the patients with cystic fibrosis has been considerably increased as a result of antibiotic therapy and other treatment; consequently, the problem of liquefying mucin acquires new importance. The concept of localized pockets of bacteria-laden mucin adhering to the mucosal wall should be considered as a contributory factor in the development of ulcerations, particularly in view of the suggestion that the determining factor in the production of necrosis may be the length of contact between pathogenic bacteria and underlying cells.

Waldron-Edward and Skoryna *(65),* in a preliminary study, used DMSO as a mucin-dispersing agent. A solution containing 80% DMSO was successfully used to separate a clear supernatant containing dissolved mucin (protein–fucose ratio, 3.4:1).

The possible use of DMSO combined with one of the following might offer the desired results: carbamide (urea), formamide, aceta-

mide, dimethyl formamide, butyl acetamide, ethyl carbamide. Trypsin, an enzyme that digests the peptide links in the protein component of the mucus, mixed with DMSO might be used without the usual side effects (fever, hoarseness, irritation of the mouth, nausea and vomiting).

D. Utilization of DMSO in Freeze-Preservation of Spermatozoa

Response of spermatozoa to freezing varies according to the species, i.e., human spermatozoa remain highly active after freezing and thawing while rabbit spermatozoa are easily damaged by hypertonic salt solutions at temperatures below zero, when the medium becomes concentrated because of separation as ice crystals. Rabbit sperm are also easily impaired by a sudden rise in concentration of glycerol in their suspending medium.

Sawada and Chang *(66)* found that DMSO provides greater protection to rabbit spermatozoa than glycerol during the cooling to −79°C and that fertilized eggs were obtained by insemination of rabbits with semen frozen in a medium containing DMSO. A high proportion of rabbit spermatozoa retained active motility after freezing to and thawing from −79°C in a medium containing DMSO, glycine, and fructose. Experimental results revealed that DMSO protects rabbit spermatozoa more effectively than glycerol during freezing and thawing. Fertilized eggs were obtained in four does after intravaginal inseminations of nine rabbits and in five does following intrauterine insemination of eight rabbits with frozen semen. The number of eggs fertilized was increased when intravaginal insemination was performed with semen samples stored at −79°C for 3 hr. The proportion of eggs fertilized, however, was very low (9–59%).

Yankow *(67),* in a related study, investigated the effect of DMSO solution on the development of frog eggs. *Rana pipiens* eggs which had undergone the first cleavage were placed in DMSO solutions and were found to develop normally when the concentration was 1% or less. After the first cleavage had occurred, 40 eggs were placed in 200 ml each of the DMSO solutions in spring water. The experiment was run twice for 20 days with the following average survival rate: 32 in spring water control, 34 in 0.1% DMSO, 37 in 0.2% DMSO, 36 in 0.5% DMSO, and 37 in 1.0% DMSO. In 2% DMSO solution 19 eggs did not develop beyond the gastrula stage. All of the remaining embryos lagged in their development and showed abnormalities. Between the gastrula

stage and hatching, 10 embryos became deformed and died. The remaining 21 embryos were abnormal in appearance and all died by the 14th day. In 4% solution no eggs developed beyond the four-cell stage.

Zimmerman *et al. (68)* appear to have made the first study of the possible use of DMSO in the freezing and storing of human semen. They used 50 healthy medical students in the series reported. In this series of experiments, glycerol 1 part to 9 parts semen; DMSO 1 part to 9 parts semen; and DMSO 1 part to 18 parts semen were used as a preservative. Each of the specimens was frozen to −196°C by the liquid nitrogen method. They report the following results following freezing: in 1:9 glycerol, the average count was 71.7 million, average motility was 26% (which was 38% prefreezing motility), the alive/dead ratio (A/D ratio) was 24% (range 0–80%); following freezing in 1:9 DMSO, the average count was 81 million, average motility was 17% (which was 25% of the prefreezing motility), A/D ratio was 26.8% (range 0–70%); and following freezing in 1:18 DMSO, the average count was 62 million, average motility was 18.5% (which was 27.2% of the prefreezing motility), A/D ratio was 27.3% (range 8–63%). It was concluded that a concentration of 1:9 glycerol gave a higher motility than 1:9 DMSO or 1:18 DMSO. The two concentrations of DMSO produced greater survival than the 1:9 glycerol concentration.

Sherman *(69)* recognized the need for improving freeze-thaw protection of spermatozoa, since about 30% loss still occurs in spite of the 7–10% glycerol currently used in methods for preservation of human spermatozoa. His study involved pooled and split-sample comparisons with spermatozoa from one to three ejaculates obtained from each of 16 individuals.

DMSO and glycerol, at a concentration of 10% by volume, were compared as to their ability to protect spermatozoa during freezing to −196°C in liquid nitrogen vapor (at an overall average of 16°C/min) and thawing to room temperature in a 22°C water bath (at an overall average rate of 60°C/min). Comparison of 10% glycerol with 10% DMSO revealed a comparable effect in that (1) there was a slight but statistically significant average drop of 7% in the motility which was attributable to pretreatment with each substance prior to exposure to freezing conditions, and (2) motility after thawing was the same, 44%, in the presence of either glycerol or DMSO.

In comparing 5, 10, 15, and 20% DMSO, an interaction between protective and toxic levels was observed. Greater freeze-thaw survival was shown at the 10% level, least at 20%, while 5 and 15% were equal.

Toxicity of DMSO increased with increased concentration and was observed within 1 contact hour during 22°C storage both before freezing to −196°C and thawing to 22°C. A concentration of 10% reflected the most favorable balance between protection and toxicity among the concentrations evaluated.

Sherman cautioned that, in toxicity or freeze-thaw protection of human spermatozoa, the varied results reported with DMSO may in part be attributable to the toxicity of traces of impurities remaining in some commercial and reagent grades of DMSO. Concentration of protective substances, methods of addition, time and temperature of pretreatment, constituents of medium, and the like are common variables in this type of research. It is also important to use split-sample comparisons of the same specimen to avoid inconsistencies resulting from biological variation in spermatozoa from different individuals.

Sherman concluded on the basis of his findings that DMSO is not recommended as a substitute for glycerol in the preservation of human spermatozoa by freezing with the methods described.

In preliminary studies in our laboratories we have attempted to freeze human spermatozoa in normal saline–glycerol solutions containing varying concentrations of DMSO. In our experience, based on a limited number of semen specimens, we have not been able to recover a high percentage of motile spermatozoa on thawing from temperatures varying from −20 to −48°C. Concentrations of DMSO varied from 10 to 25%. Semen specimens showing a spermatozoa count of 60–100 million, with normal morphology and 90% motility, were used in our series. In view of the results reported by Sawada and Chang and others, it appears likely that the concentrations of DMSO were too great. Additional studies are being conducted using lower concentrations.

It may be of additional interest to note that our observations unequivocally show that DMSO in high concentrations destroys human spermatozoa. A semen specimen showing a high count and 90% motility can be placed on a wet-mount preparation under the microscope, the motility checked, and a drop of DMSO allowed to flow under the cover slip. In this experiment, using 50–90% DMSO, it will be observed that the spermatozoa are killed in 5–10 sec and are usually completely dissolved in a matter of 3–5 min.

Our observations, along with those of Sawada, Chang, and Yankow point strongly to the need of more detailed and thorough study of the effects of DMSO in the area of sterility and fertility.

VIII. EFFECT OF DMSO ON THE ANTIGEN-ANTIBODY REACTION

It has been observed in this laboratory that high concentrations of DMSO block the normal antigen–antibody reaction. When 1 drop of 100% DMSO was added to a saline suspension of *Salmonella typhi* cells, which gave a strong agglutination with the specific typing serum, the reaction was blocked. Tests that were strongly positive became negative in the presence of higher concentrations of DMSO. This same phenomenon was noted in connection with a febrile agglutination series, heterophile agglutinations, latex fixation, and other similar determinations. Furthermore, when 1 drop of 90% DMSO was added to a blood film on a view box, A-positive bloods (B, AB, and O positive as well) typed out to be O negative.

This possible interference of DMSO with the antigen–antibody reaction proved to be interesting because of the implications that could be drawn when considering the wide use of this basic principle in blood banking, serology, and medicine in general. In two series of over 200 each, A-positive, O-positive, B-positive, and AB-positive donor bloods which were tested with concentrations of DMSO from 0.5 to 100%, there was no interference with typing when concentrations from 0.5 to 65% had been added. Concentrations of DMSO between 70 and 100% produced increasing interference as the concentration was increased. Generally speaking, there was a slight increase in the time required for clumping and the particles were somewhat smaller in size, requiring more careful reading of the results. The time required for positive clumping did not in any case approach 2 min. Serial dilutions with the above diagnostic serological tests (heterophile, febrile agglutinations, and so forth) showed that concentrations below 50% DMSO had no effect on the results obtained. It was our conclusion, based on the work performed to date, that no interference with the antigen–antibody reaction occurred in diagnostic tests because of the presence of a small amount of DMSO in specimens submitted to the clinical laboratory.

IX. USE OF DMSO IN THE EXTRACTION OF BACTERIAL PRODUCTS

Adams *(70)* reports DMSO as a useful agent for extracting lipopolysaccharides from the bacterial cell and cell walls. In working with *Serratia marcescens, Salmonella typhimurium,* and *E. coli,* it was noted

that the DMSO method extracted approximately 10 times as much material as did the standard phenol extraction method. However, it was apparent that DMSO was also extracting a large proportion of the protein of the cell wall. These extracts contained the same sugar components, showing that the extraction procedures were not selective for certain polysaccharides. Experimentation showed that the optimum extraction with DMSO was 2 hr at 60°C. After separation of the protein, the DMSO extraction showed a reduction to approximately 1.6%, a value roughly comparable to the phenol-extracted lipopolysaccharide (1.21%). It was Adams' conclusion that the DMSO extraction procedure was four to five times as effective as the 45% phenol extraction method. Further experimentation, considering the whole bacterial cell and cell wall only, indicates that with the whole bacterial cell as starting material there is very little choice between the two methods. However, with cell wall preparations, there was a much higher yield of lipopolysaccharide with DMSO than with phenol.

X. APPENDIX–STAINING TECHNIQUES

A. Pottz-Rampey-Benjamin Acid-Fast Staining Method

The solutions required for staining direct smears of sputum, concentrated sputum, urine, gastric washing smears, or sections of tissue are identical. The following formulas are listed for preparation of the solutions.

1. Carbol Fuchsin-DMSO Stain

Fuchsin, basic, pure crystals, certified	4 g
Phenol, crystals, C.P.	12 g
Glycerol, C.P.	25 ml
Ethyl alcohol, 95%	25 ml
DMSO, reagent grade	25 ml
Distilled water to yield	160 ml

Dissolve the fuchsin in the ethyl alcohol, add the phenol which has first been liquefied in a water bath, mix well with a glass stirring rod, add the glycerol and DMSO, and make up to volume with distilled water. Allow the well-mixed stain to stand for at least 30 min, and then

pass through a coarse filter paper or a Seits serum-clarifying filter disc to remove any acid-fast organisms or debris that may be present. The stain prepared according to the above directions keeps indefinitely at room temperature if stored in an amber glass or plastic container.

2. Combination Decolorizer and Counterstain

Malachite green, 96% dye content, 2% aqueous solution	220 ml
Acetic acid, glacial 99.5%	30 ml
Glycerol, C.P.	50 ml

Mix in the order listed. No filtration is required and the solution keeps indefinitely at room temperature in a well-stoppered bottle.

3. Procedure for Staining Smears

a. Prepare smears in usual manner.

b. Fix smears by means of flaming or for 30 sec in absolute alcohol.

c. Over a sink or in Coplin jars, stain with carbol fuchsin–DMSO solution for 2 min (3 min for thick smears and tissue sections).

d. Wash with tap water to remove excess carbol fuchsin.

e. Counterstain and decolorize in glacial acetic acid–malachite green solution for 2 min. The slide should be returned to this solution for an additional 1 or 2 min if an even background of green or blue-green is not obtained in 2 min.

f. Wash in tap water and allow to air dry.

g. Examine under oil immersion. The beaded tubercle bacilli are brilliant red against a green or blue-green background.

Modification. The slides may be retained in the carbol fuchsin–DMSO or in the acid-counterstain for a period from 2 min to overnight without altering the results obtained.

4. Procedure for Staining Sections of Tissue

a. Cut paraffin sections approximately 4–6μ in thickness.

b. Remove paraffin in usual manner (pass through two changes of xylol, 2 min each; two changes of absolute alcohol, 1 min each, then two changes of 95% alcohol, 1 min each; and finally into distilled water).

c. Stain in carbol fuchsin–DMSO solution for 2 min.

d. Wash in distilled water to remove excess stain.

e. Decolorize and counterstain in the glacial acetic acid–malachite green solution for 2 or 3 min.

f. Wash well in distilled water.

g. Clear by passing through the alcohols and toluol or xylol in the usual way.

h. Mount in clarite or permount.

The stain and staining procedures described have yielded consistently reliable results with all types of tuberculous and leprosy material. The 2-min. time limit (3 min for thick smears and tissue sections) is recommended inasmuch as it sets an arbitrary minimal time limit for the completed stain; however, we have had excellent results when staining thin smears for only 1 min. Our experience with the use of the stain for smears, concentrates, and so forth, is that if the stain is applied for only 1 min or for an appreciable time in excess of 2 min the efficiency of the stain is not affected. Staffs in clinical laboratories with a large volume of smears and sections of tissues for acid-fast bacteria may find it to their advantage to stain for 30 min or even overnight. When slides are stained for periods in excess of 30 min, they should be thoroughly washed in water and permitted to remain in acid–decolorizer–counterstain solution for at least 5 min.

Tissue sections stained by means of the procedure described herein should be well cleared after the acid–decolorizer–counterstain solution bath if they are to be retained for a permanent collection; however, if the sections are only for routine examination, time may be saved by omitting the clearing steps and examining the water-washed slides without a cover slip.

B. Mitchell's Modification of the Heidenhain Iron-Hematoxylin Stain

1. Reagents

a. Schaudinn's fixative

Stock solution (stable indefinitely).

Mercuric chloride, saturated, aqueous	2 parts
Ethanol, 95%	1 part

The working solution is stable for 24 hr and is made by adding 5 ml of glacial acetic acid to 50 ml of stock solution.

b. DMSO.

c. Alcohol iodine. To 50 ml of 70% ethanol, add sufficient Gram's iodine (approximately 40 drops) to produce a port wine color.

d. Ferric ammonium sulfate–DMSO (mordant). Dissolve 2.0 g of ferric ammonium sulfate in 50 ml of distilled water. Add 5.0 ml of

DMSO, transfer to a Coplin jar, and place in a 55°C water bath. Stable for 24 hr.

e. Aqueous ferric ammonium sulfate, 1% (decolorizing solution).

f. Hematoxylin stain (stable indefinitely). Stock solution (10% alcoholic). To 100 ml of absolute alcohol (ethanol denatured) add 10.0 g of hematoxylin (slight heating may be required to dissolve completely). Allow to age 3 weeks before using.

The working solution (1% aqueous). To 50 ml of distilled water, add 5.0 ml of stock hematoxylin, and then add 5.0 ml of DMSO.

2. Procedure

a. *Concentration.* In a screw-capped centrifuge tube place 15 ml of saline solution and approximately 2 g of stool specimen. Shake well for 2 min (Kahn shaker) and filter through four thicknesses of gauze. Centrifuge for 5 min at moderate speed; discard the supernatant fluid and make thin slides from the sediment.

b. *Fixation and staining.* While still wet, place smears in hot (50–55°C) Schaudinn's fixative for 10 min. Transfer directly to the alcohol–iodine solution for 5 min., and then to 70% alcohol for 5 min. Rinse in running tap water. Examine while wet under 10× objective. Repeat the decolorization and examining process until the proper degree of staining is obtained. Wash in running tap water for 3 min. Place for 1 min in 70% alcohol containing 1 mg/ml of eosin Y. Pass through ascending concentrations of alcohols and xylol. Mount with a cover glass and examine.

3. Results

Karyosomes, nuclear chromatin, cell walls, cytoplasmic granules, and chromatoidal bodies are stained black. The glycogen vacuoles are unstained. Leukocytic nuclei stain similarly to cysts and must be differentiated. All bacteria, yeast, and cellular debris are stained black.

C. Rampey's Modification of the Trichrome Stain for Intestinal Protozoa in Fresh Fecal Smears

1. Procedure

a. Fix fresh fecal smears in 100% DMSO at room temperature for 2 min. Drain off excess.

b. Pass through 70% ethyl alcohol for 1 min. Drain.

c. Stain in Coplin jar of trichrome for 3 min. Drain.

d. Destain in 90% acidified ethyl alcohol, 1 drop of glacial acetic acid in 10 ml of ethyl alcohol, for 10 to 30 sec. Drain.

e. Pass slides through a series consisting of 95% ethyl alcohol, 100% ethyl alcohol, and xylol, 1 min. in each. If necessary, return to xylol until refraction at smear–xylol interface ends.

f. Mount with permount, clarite, or other mounting media.

2. Results

Cysts and trophoxoites of intestinal protozoa are well stained blue-green in color. Karysome material of *Endamoeba histolytica, E. hartmanii, Endamoeba coli,* and so forth, is well defined and stains a deeper blue-green to purple in contrast to the cytoplasm. Karysomal material of *Iodamoeba butschlii, Endolimax nana,* and so forth, tend to stain red. Chromotoid bodies stain red.

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Chapter 8

DMSO in Experimental Immunology

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I. INTRODUCTION

Oral vaccination requires large doses of antigen because much of the antigen is rendered ineffective by passage through the gastrointestinal tract. To avoid this loss, immunologists searched for substances which,

when added to oral antigen, would improve its absorption. Besredka has tried bile *(1)*. Möse *(2)* and Grossgebauer *et al. (12)* have used aristocholic acid unsuccessfully.

Since dimethyl sulfoxide (DMSO) increases the permeability of skin and mucosa to a number of substances, the immunological activity of mixtures of antigen and DMSO was studied in animal models.

II. MUCOSAL INOCULATION WITH MIXTURES OF *S. typhimurium* ANTIGEN AND DMSO IN MICE

Animals can be protected against virulent oral infection with *Salmonella typhimurium* by mucosal immunization with inactivated bacteria *(3–6)*. Schmudde *(7)* investigated the possibility that addition of DMSO to vaccine would improve absorption of the antigen via the mucosa, and result in reduced mortality.

The vaccine employed was a dry antigen from heat-inactivated *S. typhimurium* (Behring-Werke, Marburg). A portion of this vaccine was suspended in physiological saline solution and the remainder in a 30% aqueous DMSO solution. The solutions were adjusted quantitatively to give 10^9 bacteria in 1 ml liquid. White inbred mice were immunized orally, nasally, and rectally with the two vaccines. Oral and rectal inoculation was carried out with a tube. Each of these inoculations was 0.25 ml, and for nasal inoculation 1 drop of vaccine was instilled into each nostril. The mice were immunized daily for 10 consecutive days. Ten days after the last inoculation, all the animals, including the noninoculated controls, were infected orally with virulent *S. typhimurium* bacteria. The dosage was adjusted so that 50% of the control animals died. The prophylactic effect of the inoculation was measured by the mortality rate of 20 days after infection.

Results of six individual experiments carried out on 1822 mice produced a mortality rate among the treated mice of 39.5% after oral, 37.5% after nasal, and 40.5% after rectal inoculation. (Combined graphs of the mortality curves are shown in Fig. 1 for oral inoculation, in Fig. 2 for nasal inoculation, and in Fig. 3 for rectal inoculation.) This rate is significantly lower than the 60.9% mortality rate established with a control group. These data confirmed that effective protection against a virulent oral intestinal infection can be obtained by these

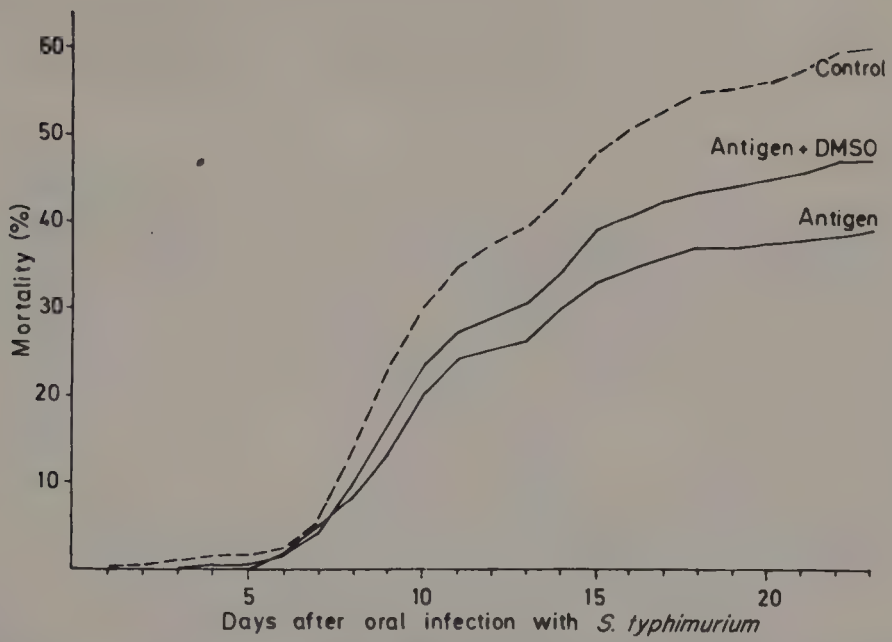

Fig. 1. Mortality of mice immunized orally with 0.25 ml of inactivated *S. typhimurium* in saline and 30% DMSO solution daily for 10 consecutive days and inoculated with virulent *S. typhimurium* (6 experiments, 781 mice).

three routes of immunization. If we compare the mortality curve of simple vaccine with that of DMSO–vaccine in Figs. 1–3, we see that in each case addition of DMSO impaired rather than improved the result of inoculation. This was equally true for all three experiments. The idea, based on theoretical concepts, that addition of DMSO would improve the results of inoculation was not substantiated.

A number of possible explanations for failure of DMSO to enhance the immunological activity of *S. typhimurium* were considered: (1) Is the immune reaction of animals impaired by DMSO during inoculation? From reports of other authors it is conceivable that the DMSO dosage was too high and possibly immunosuppressive. (2) Does DMSO damage the defense mechanisms of the intestinal mucosa so that animals subsequently become more sensitive to oral infection with *S. typhimurium* bacteria? It is possible that DMSO increased permeability for the pathogens, thus increasing the mortality rate. (3) Is the antigen itself altered by addition of DMSO so that its immunizing effect is reduced? DMSO, which is active chemically, could possibly have altered the antigen. It was considered appropriate to investigate these three possibilities in consecutive experiments.

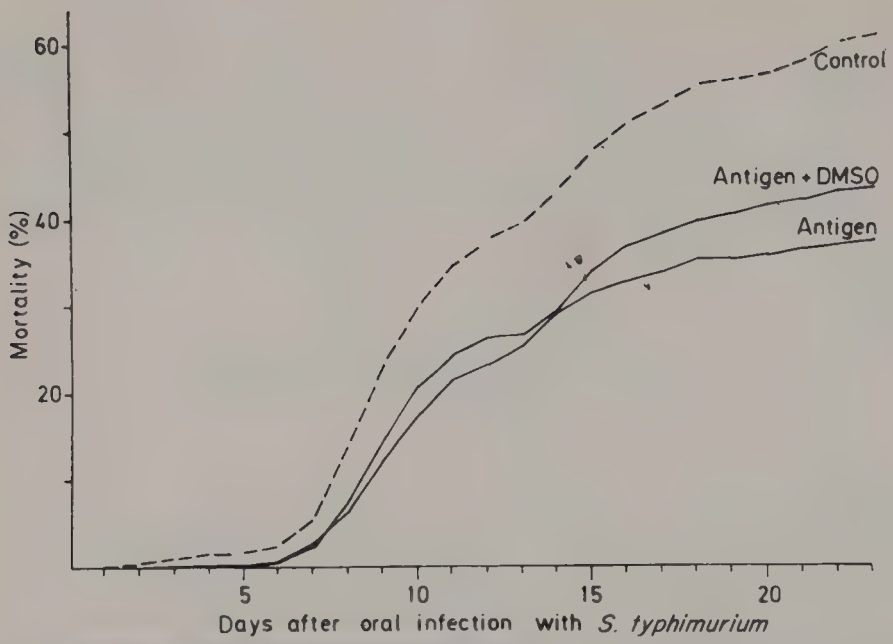

Fig. 2. Mortality of mice immunized nasally with 0.25 ml of inactivated *S. typhimurium* in saline and 30% DMSO solution for 10 consecutive days and inoculated with virulent *S. typhimurium* (6 experiments, 824 mice).

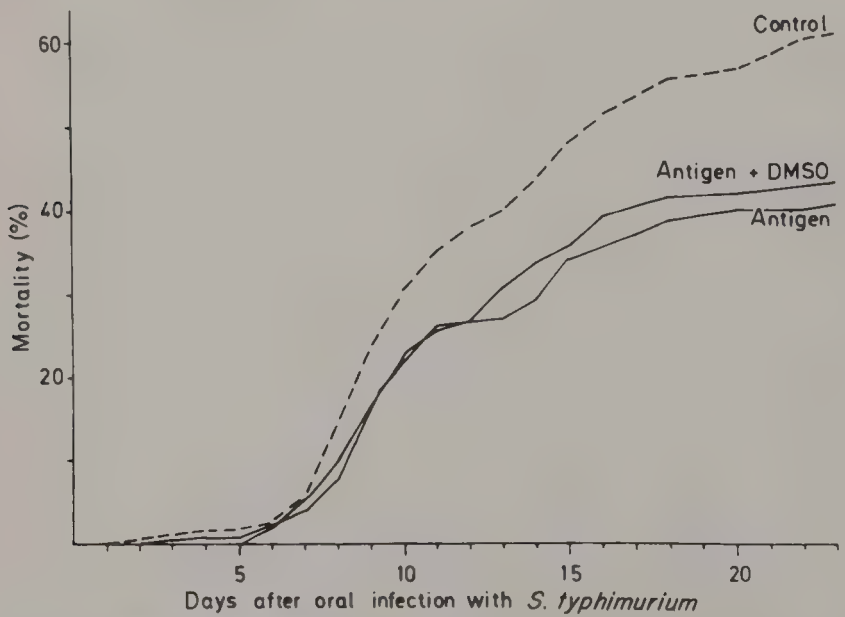

Fig. 3. Mortality of mice immunized rectally with 0.25 ml of inactivated *S. typhimurium* in saline and 30% DMSO solution daily for 10 consecutive days and inoculated with virulent *S. typhimurium* (6 experiments, 785 mice).

III. MIGRATION OF LEUKOCYTES AFTER ADMINISTRATION OF DMSO

In an attempt to answer the first question, we investigated the migration of leukocytes after oral administration of 15% DMSO to mice. The experimental animals received 0.25 ml of the DMSO solution, introduced via a stomach tube, daily for 10 consecutive days. The total leukocyte and differential count in the circulating blood was determined on the 1st, 5th, 10th, 13th, and 16th days of the experiment.

In our experience leukocyte counts in mice are significantly influenced by the techniques used in blood sampling. The usual method of sampling from the tail vein, in which bruising is unavoidable, is inaccurate. In earlier experiments it was found that a particularly good method is to take the sample from the femoral vein *(8)*. After shaving the fur and disinfecting with alcohol, the femoral vein was punctured with a cannula in the midportion of the inside thigh. In this way the blood flows well and spontaneously and can readily be sampled with a leukocyte pipet.

After preliminary experiments of this type, we carried out a more detailed experiment on 10 mice. Data obtained from these 10 mice treated orally with 15% DMSO showed a significant increase in leukocyte count over a prolonged period. During treatment the percentage of polymorphonuclear leukocytes decreased from a mean value of 24.4 to 16.3% and that of lymphocytes increased from 67.5 to 74.1%. It was also noted that the number of polymorphonuclear leukocytes did not actually decrease, but was essentially unaffected by DMSO therapy. A rise in the lymphocyte count from 5010 to 9700 was noted, however. (At its peak, this value was more than double the initial value.)

From these data it is concluded that the leukopoetic system is not disturbed by DMSO treatment; there is no leukopenia. On the contrary, there is an actual rise in the lymphocyte count which constitutes the major portion of the white blood cell count in the mouse. From these results it is believed that the answer to the first question is that DMSO given orally does *not* impair the leukopoetic system in the mouse, but actually stimulates it.

IV. INFLUENCE OF ORALLY ADMINISTERED DMSO ON A SUBSEQUENT INFECTION WITH *S. typhimurium* BACTERIA

In this experiment mice were fed different concentrations of DMSO without antigen by the same method used for oral inoculation with DMSO–antigen mixtures. They were then tested for susceptibility to virulent oral infection with *S. typhimurium* bacteria. If the mortality of the animals treated with DMSO proved to be higher than that of the controls, this would indicate that DMSO had a direct detrimental effect on the intestinal mucosa.

The experimental mice were fed via a stomach tube with 0.25 ml of 45, 30, and 22.5% DMSO solution daily for 10 consecutive days. On the 10th day after the final administration of DMSO, the experimental animals were infected orally with a dosage of virulent *S. typhimurium* bacteria that would cause 50% mortality.

A combined graph of the mortality curves shows the results of the three individual experiments carried out on a total of 526 mice (Fig. 4). These data demonstrate that pretreatment with DMSO does not increase the mortality of the animals in any case. An unexpected result was that fewer mice died after receiving concentrations of 30 to 45% DMSO when compared with the untreated control group. The differences in the final mortality, 44.0 and 43.4% on the one hand, and 60.2% for the control animals on the other, are at the limits of chance variation. Thus, a slight dose-dependent protection against *S. typhimurium* infection was achieved with pretreatment with DMSO. One consideration is that the leukocytic stimulation reaction demonstrated in the previous series of experiments could be responsible for this mild prophylactic effect.

These results give an unequivocal answer to our second question. Pretreatment with DMSO does not impair the defense mechanism of the intestinal mucosa. On the contrary, a slight prophylactic action is observed with higher DMSO concentrations. Consequently, one possible conclusion from these results is that addition of DMSO damages the antigen.

V. ORAL INOCULATION WITH DMSO-ANTIGEN FROM *S. typhimurium* BACTERIA

A third experiment was designed to determine whether or not DMSO impairs the immunological action of the vaccine. *Salmonella typhimu-*

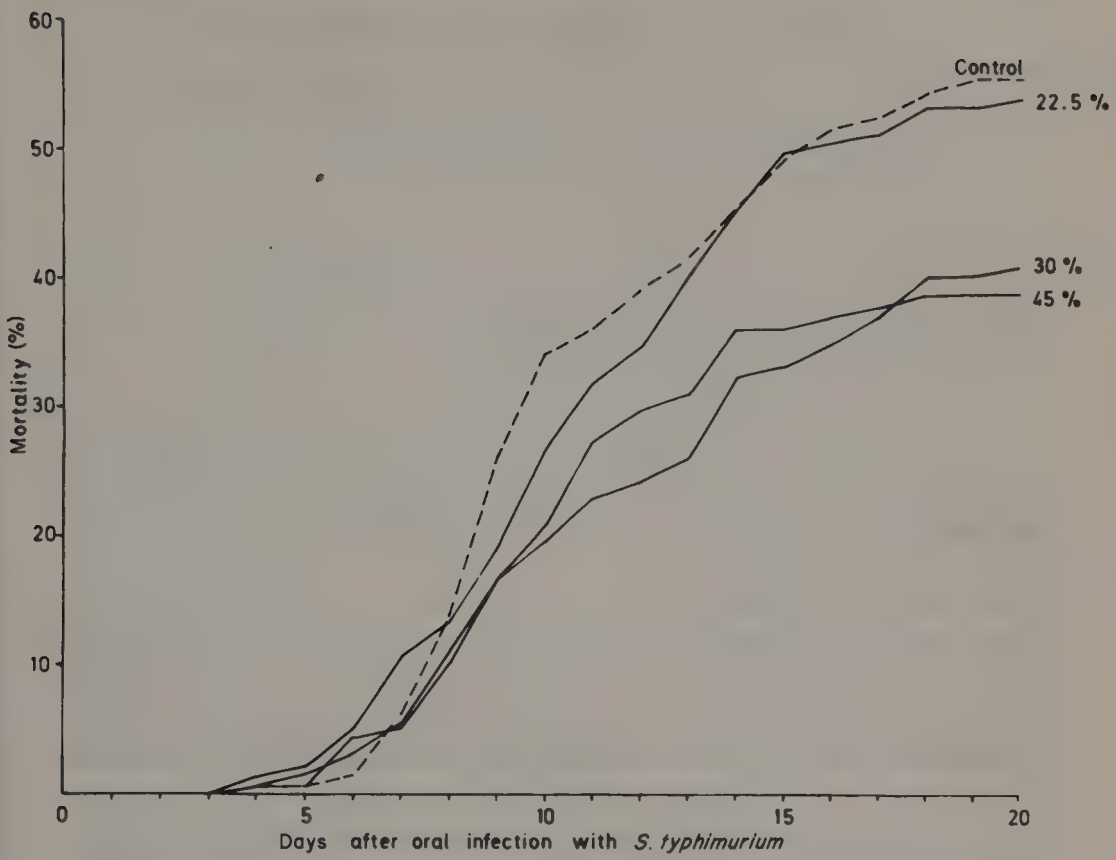

Fig. 4. Mortality of mice inoculated orally with *S. typhimurium* and pretreated with 10 consecutive daily doses (0.25 ml) of 22.5, 30, and 45% DMSO solution given via stomach tube (3 experiments, 562 mice).

rium was grown on agar plates. After washing once in physiological solution and adjusting the suspension to 10^9 organisms per milliliter, 100% DMSO, in a ratio of 1:1, was incorporated. A maximum temperature of 50°C was produced. After agitating for 24 hr with a magnetic agitator at room temperature, the suspension was still not sterile. Sterility was obtained only after the suspension had been kept for an additional 24 hr at 37°C. To reduce the DMSO concentration, this liquid vaccine was freeze-dried—a procedure that reduced the DMSO content to 3–5%. The dry substance was resuspended with an equal volume of physiological saline before use.

Using this vaccine, Kordes *(9)* immunized and infected white mice orally, rectally, and nasally as in the first experiment. The final mortality was reduced only slightly from 45.5 to 41.4%. These differences are within the region of chance scatter. Therefore, oral inoculation with DMSO–antigen provided the experimental animals with no protection. This is in contrast to other chemically inactivated vaccines

(e.g., formaldehyde, acetone), which in our experience have immunogenic activity when given by mouth.

In answer to the third question, then, it can be stated that treatment of the antigen with DMSO almost completely destroyed the immunogenic properties of the vaccine.

VI. INFLUENCE OF DMSO ON THE AGGLUTINABILITY OF *S. typhi* AND *S. typhimurium* BACTERIA

We next attempted to determine whether or not DMSO treatment would impair the agglutinability of *Salmonella*. This investigation was carried out using two species of bacteria, two DMSO dilutions, and two different periods of contact.

Salmonella typhi and *S. typhimurium* strains from our own collection were used. The live bacterial suspensions were titrated against standard sera by the Gruber reaction to give the initial value. The test was repeated after 24 and 48 hr on three different preparations: (1) live bacteria to which DMSO had been added; (2) bacteria that had been inactivated at 100°C for 5 min; and (3) heat-inactivated bacteria to which DMSO had been added. DMSO was used in dilutions of 45 and 22.5%, and the serum dilutions employed were from 1:50 to 1:25, 600, each dilution being double the previous one. Agglutination was determined in the standard way, visually with an agglutinoscope.

First, it should be pointed out that the two DMSO dilutions gave exactly the same results, and little difference between the agglutination values after 24 and after 48 hr was noted. The first unexpected result was that DMSO added to live bacteria did not impair agglutinability at all. The average values were actually somewhat higher. Inactivation at 100°C for 5 min reduced the agglutination end point by about three stages of dilution in both strains investigated. When DMSO was added to the inactivated bacteria, the agglutination values were reduced by six to seven stages of dilution as compared with the initial value.

Therefore, it was only the agglutinability of heat-inactivated bacteria that was reduced to any extent. Thus far, the author has not been able to explain why DMSO significantly alters the antigenic properties of bacteria after the action of heat. Further experiments should be carried out to explain this phenomenon.

VII. THE ACTION OF ALTERNATE ORAL ADMINISTRATION OF *S. typhimurium* ANTIGEN AND DMSO ON *S. typhimurium* INFECTION

In the previous experiments it was demonstrated that addition of DMSO impairs neither the immune reaction of experimental animals nor their natural defense mechanisms against an intestinal infection. It was shown, however, that vaccine lost most of its immunogenic activity and agglutinability when DMSO was added. Consequently, we carried out experiments to see if separate administration of DMSO and antigen would improve the prophylactic action. If the DMSO were given orally first and then the vaccine introduced orally a few hours later into the pretreated intestine, it was conceivable that absorption of the antigen would be improved.

This investigation was carried out in four phases: (1) one with controls in which mice were immunized orally with *S. typhimurium* antigen only; (2) one in which animals were fed 45% DMSO only, daily for 10 consecutive days; (3) one with a negative control, in which mice were infected without being treated; and (4) an experiment in which mice were fed 45% DMSO in the morning and immunized orally with *S. typhimurium* antigen in the afternoon. All experimental animals were infected orally with virulent *S. typhimurium* bacteria 10 days after the final oral treatment.

Figure 5 is a combined graph of the mortality curves produced from experiments on 938 mice. The mean final mortality, 71.7%, was higher than in the first group of experiments, which showed 60.9% mortality. The other values were correspondingly higher. Oral inoculation reduced the final mortality from 71.7 to 44.7% and, as in the first group of experiments, this difference is outside the limits of chance. Oral therapy with 45% DMSO alone slightly reduced mortality as compared with the controls, although again the reduced mortality was within the limits of chance. However, mortality shows that the specific prophylactic action and the nonspecific DMSO action are not additive. The final mortalities of 45.1 and 44.7% are almost identical. Comparison of the curves in Fig. 5 shows that there is a slight increase in the mean survival time for animals treated alternately with vaccine and DMSO up to approximately 24 days.

This group of experiments demonstrates that oral pretreatment of

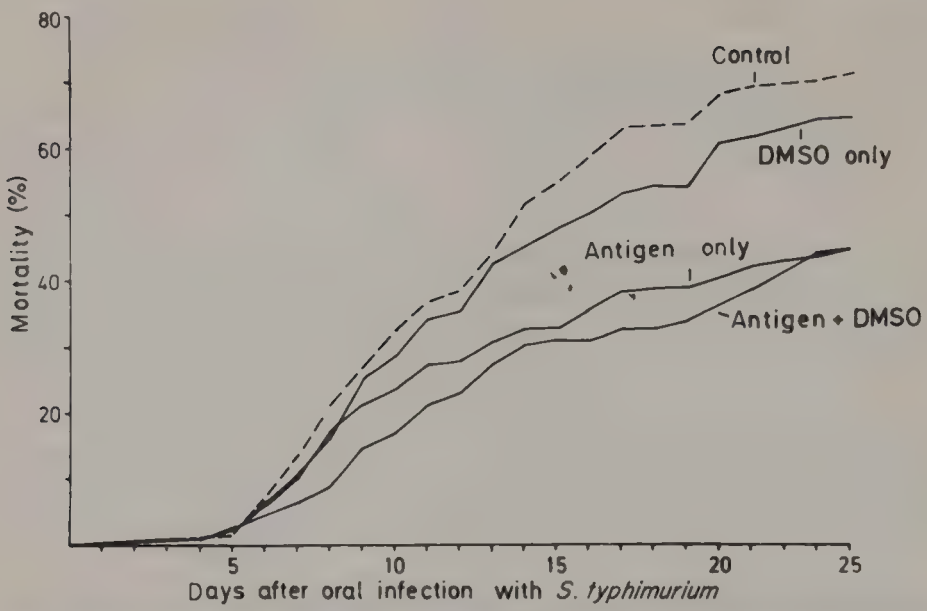

Fig. 5. Mortality of mice inoculated orally with *S. typhimurium* and immunized orally with *S. typhimurium* antigen with and without feeding of 45% DMSO for 10 consecutive days (5 experiments, 938 mice).

experimental animals with DMSO neither potentiates nor diminishes the activity of a separate oral inoculation.

VIII. LOCAL VACCINATION WITH MIXTURES OF SALK POLIOMYELITIS VACCINE AND DMSO

Neumann-Schönwetter immunized mice orally, nasally, and rectally with a commercial Salk vaccine prepared from inactivated poliomyelitis virus *(10)*. As in the first group, the vaccine was administered alone and mixed with DMSO. The prophylactic effect was measured by the mortality following parapoliomyelitis infection by the oral route.

This experimental procedure was based on investigations by Raettig. A parapoliomyelitis strain was introduced into the mouse by alternating intracerebral and oral infection, so that the animals became ill and died with paralysis. Since the Columbia-SK-parapoliomyelitis virus could not be grown on a tissue culture, the vaccine was produced from a brain emulsion taken from diseased animals *(11)*. The virus in the emulsion was inactivated by heat. Oral administration of this inactivated vaccine decreased the mortality from 51.2% for noninoculated animals to 22.6% for orally inoculated animals (1096 mice). Oral vaccination with poliomyelitis virus gave nonspecific protection against

oral infection with parapoliomyelitis virus to almost the same extent. Neumann-Schönwetter tested the action of DMSO on this model.

The vaccine used was a trivalent, formaldehyde-inactivated poliomyelitis vaccine (Behring-Werke, Marburg). This vaccine was diluted with equal parts physiological saline solution in one instance, and with 60% DMSO in the other, giving a 30% DMSO vaccine. The mice were vaccinated orally, nasally, or rectally with these two vaccines. Oral vaccination (0.2 ml), and rectal vaccination (0.1 ml), were given via a tube. For nasal vaccination the animals were given 1 drop in each nostril from a pipet. Each animal was vaccinated 5 times on 5 consecutive days. On the fifth day after the final vaccination, the mice were infected orally with virulent Col.-SK-virus in a dose adjusted to give a mean mortality rate of 50%. The prophylactic effect was measured by the paralysis rate on the sixth day after infection. Since all animals were killed immediately after the symptoms of paralysis had appeared (experience showed that these symptoms were always quickly followed by death), the curves for paralysis rate and for mortality are identical.

Neumann-Schönwetter carried out eight experiments on a total of 1046 mice *(10)*. His results show that the final mortality, which was 51.2% for the controls, was reduced by more than half of all three types of vaccine. This reduction is statistically significant, and the nonspecific results of the vaccination confirm earlier results *(11)*. Adding DMSO to the vaccine did not improve the result of the vaccination in any case, as the mortality curves in Fig. 6–8 show. Thus, the results from this model confirm those of the first group of experiments. The curves in Fig. 6 show that DMSO neither impaired nor improved the nonspecific action of the vaccine. However, nasal and rectal vaccination led to a higher mortality when DMSO was added to the vaccine (Figs. 7 and 8).

Thus, the results from the viral model confirm those from the bacterial model (first group of experiments). DMSO did not improve the results of vaccination. Under the experimental conditions investigated to date, an adjuvant effect cannot be ascribed to DMSO.

IX. ANTIBODY FORMATION FOLLOWING ORAL POLIOMYELITIS VACCINATION WITH AND WITHOUT ADDITION OF DMSO

Using the procedure described above, we carried out experiments to see whether or not DMSO damaged specific groups on the virus antigen. For this investigation Neumann-Schönwetter conducted a

neutralization test *in vitro* with serum from vaccinated animals and then tested the serum-inactivated poliomyelitis virus for residual infectivity *(10)*.

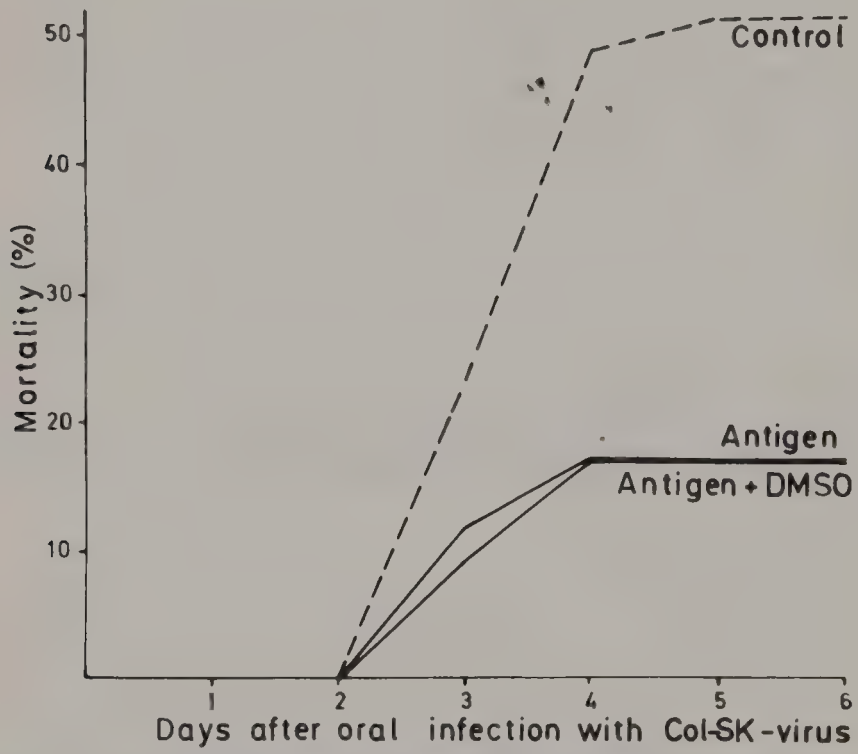

Fig. 6. Mortality of mice immunized orally with Salk vaccine and inoculated orally with Col.-SK-virus (8 experiments, 422 mice).

Five mice from the previous experiment were selected from each of the groups of orally vaccinated animals and from the control group 5 days after the final vaccination. Blood was sampled from these 15 animals by cardiac puncture, and serum was obtained in the usual way. The sera of each group were pooled and then diluted 1:10, 1:100, and 1:1000. A virulent poliomyelitis virus, strain MEF1, in a dilution of 10^{-2}, was added to the concentrated serum sample and to its three dilutions, and incubated at 37°C for 1 hr; 0.03 ml of the mixture so treated was injected intracerebrally into four young mice in each case. The prophylactic value of the serum was deduced from the fatalities following this infection.

Since four mice were employed in each of four experiments, a total of 16 animals was available for each portion of the experiment and at each dilution. The biological neutralization test with serum of mice orally vaccinated with Salk vaccine showed a high antibody content. Only one of the 16 animals died after intracerebral infection, while 13

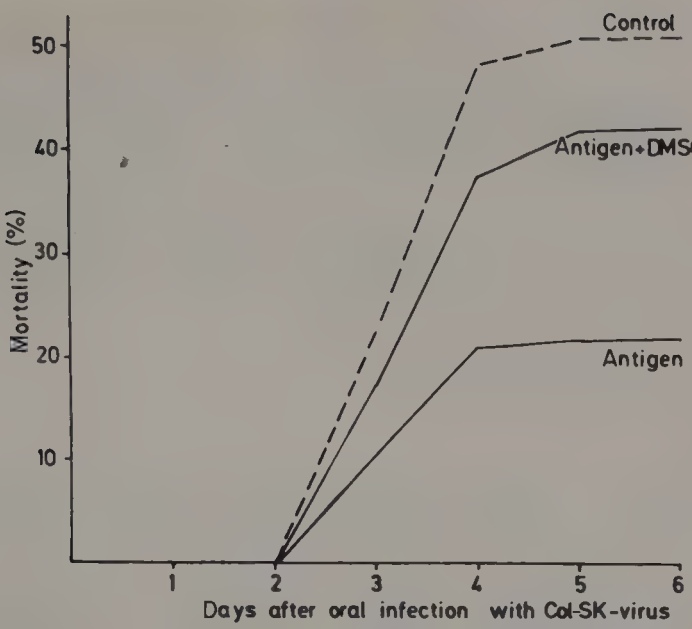

Fig. 7. Mortality of mice immunized nasally with Salk vaccine and inoculated orally with Col.-SK-virus (8 experiments, 473 mice).

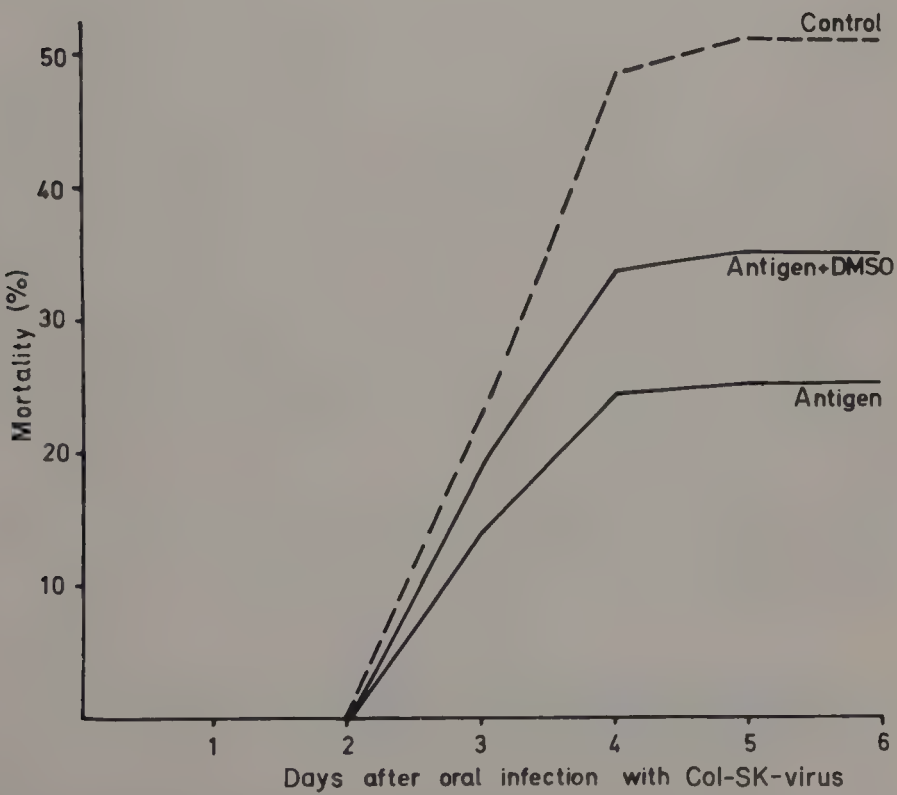

Fig. 8. Mortality of mice immunized rectally with Salk vaccine and inoculated orally with Col.-SK-virus (8 experiments, 475 mice).

of the nonvaccinated controls died. The antibody content of mice vaccinated orally with the Salk–DMSO mixture was slightly higher than that of the controls; 11 of the 16 animals died. By taking all the serum dilutions together, a mortality rate of 18.8% was obtained using sera after Salk vaccination, 67.2% using sera from untreated animals, and 50% using sera after treatment with the Salk–DMSO mixture.

From the results of these neutralization tests, just as for the bacterial model, it was concluded that the specific action of this viral antigen was also impaired by the action of DMSO.

X. SUMMARY

In experiments to date, addition of DMSO to vaccines did not improve the effect of the vaccine, either by oral, nasal, or rectal vaccination in both bacterial and viral models. This did not happen because DMSO increased the susceptibility of the experimental animals to oral infection or because it impaired their immune reaction. The DMSO concentrations used did not increase the incidence of leukopenia, nor was there a higher mortality rate after pretreatment with DMSO. On the contrary, we observed lymphocytosis and an increase in the activity of nonspecific defense mechanisms against infection.

The nonspecific prophylactic action of DMSO and the specific action of the vaccine were not additive. This was apparent because DMSO had a disruptive effect on the antigen. Three experiments supplied evidence for this assertion: (1) Immunization with DMSO-inactivated vaccine was unsuccessful (its immunogenic activity had been destroyed); (2) addition of DMSO to heat-inactivated *Salmonella* considerably reduced agglutinability; and (3) a mixture of DMSO and formaldehyde-inactivated poliomyelitis virus failed to induce formation of neutralizing antibodies in mice. This statement is confirmed by the experiment that demonstrated that separate oral administration of DMSO and inactivated bacterial vaccine did not result in damage to the antigen.

It is to be hoped that these results will stimulate other authors to investigate further the uses of DMSO in immunology.

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Chapter 9

Interaction of DMSO and Alcohol

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I. INTRODUCTION

Pharmacological and clinical testing of new drugs frequently reveals undesirable side effects. The characteristics of these effects are then described, and they can be expected to occur when a given drug is employed. However, little is known about the effects produced by simultaneous or consecutive administration of two or more substances.

According to current medical opinion, an intensified effect occurs when synergistic agents are administered, but a reduced action is

expected when antagonistic agents are combined. However, this generalization can be applied only with reservation since when two synergistic substances such as alcohol and glutethimide are combined, even though some activity may be increased, there may also be a decrease in another activity. The fact that the effect, as measured by a specific characteristic, produced by combining two substances with the same kind of pharmacological action is greater or smaller depends on the dosage used in the combination *(1)*.

Shortly after the clinical testing of dimethyl sulfoxide (DMSO) had begun, symptoms were occasionally observed in ambulant patients who had taken alcoholic drinks after treatment with DMSO. John *(2)* reported that the odor of alcohol diminished after the administration of DMSO. However, Jentschura *(3)* reported that the pronounced taste of "bad eggs" occasionally caused by DMSO could be eliminated with brandy. Boost's report *(4)* appears even more relevant; he commented on alcohol intolerance as follows: "Two patients who had received a cutaneous application of 5–7 ml DMSO, one for an injury and the other for a rheumatic process, both subsequently (although the exact interval between DMSO application and taking alcohol is not known) drank two Martini cocktails, and immediately fell down extremely drunk."

On the basis of these observations, Laudahn *(5)* discussed the possibility of a change in the blood-brain barrier produced by DMSO. With the combined action of alcohol and DMSO, this barrier could become more permeable to alcohol, and a rapid flow of alcohol to the brain could account for the intolerance.

Experiments on mice and on humans were set up to investigate the validity of this hypothesis. The effect of both substances was investigated not only by simultaneous but also by separate administration of the same dosage, since Boost reported that intolerance apparently occurred when the alcohol was taken soon after DMSO administration.

II. INVESTIGATION OF THE INTERACTION OF ALCOHOL AND DMSO IN MICE

Young adult white mice of both sexes weighing approximately 20 g were employed in this experiment. Lethality and survival time were selected as the end points for testing the combined action. The animals

were given no food for 12–15 hr prior to the experiment (although water was freely available until shortly before the experiment began). Then, by the method developed by Mallach *et al. (6,7),* the test animals received the median lethal doses of both substances orally and fractions of these dosages in all possible combinations. The mean lethal doses of alcohol and DMSO were equalized to 1.000 and the fractions designated 0.8, 0.6, 0.4, 0.2, 0.1, and 0 (the actual LD_{50} values of which were 24 g/kg DMSO and 9.0 g/kg alcohol).

Different values are given in the literature for the LD_{50} for DMSO by oral administration. Brown et al. *(8)* list it as 20 ml/kg (18.8 g/kg), Rosenbaum and co-workers *(9)* give it as between 15.0 and 21.5 g/kg, and Guenzel *(10)* as 16.5–21.4 g/kg. However, by Kaerber's method *(11),* under the conditions chosen for the combination experiments (duration of experiment 24 hr, environmental temperature 24°C), the value was found to be 24.2 g/kg by Mallach and Etzler *(12).*

Within the chosen limits the toxicity of alcohol depends on the concentration. Absolute alcohol, for example, is more toxic than 50% alcohol *(13).* Since simultaneous administration of DMSO and alcohol has a diluent effect, the dosage for absolute alcohol was used instead of 8.4 g/kg (LD_{50} for 50% alcohol).

Under these experimental conditions 980 animals (each combination was administered to 20 animals) received combined doses of alcohol and DMSO simultaneously, as well as 60 min before or 60 min after the administration of alcohol. In this experimental model the alcohol dosage remained constant while the DMSO dosage was reduced by stages, and vice versa.

Figure 1 shows the effect obtained with constant alcohol dosage and variable DMSO doses for simultaneous and separate administration of both substances. With simultaneous administration of high doses of both substances the effect is intensified. The effect of interposing the time interval on administration is clear. When alcohol is given 60 min after administration of DMSO, the toxicity is approximately 30% higher as compared with the toxicity observed after simultaneous administration of both substances (to 0.8). The mortality is 100% when alcohol is given 60 min before administration of DMSO. The following hypothesis was formulated on the basis of these results: In an unchanged and/or metabolized form, the substance administered initially either inhibits or activates the metabolism of the substance that follows, and this produces a toxicity greater than that produced by the simultaneous interaction of both substances *(14).*

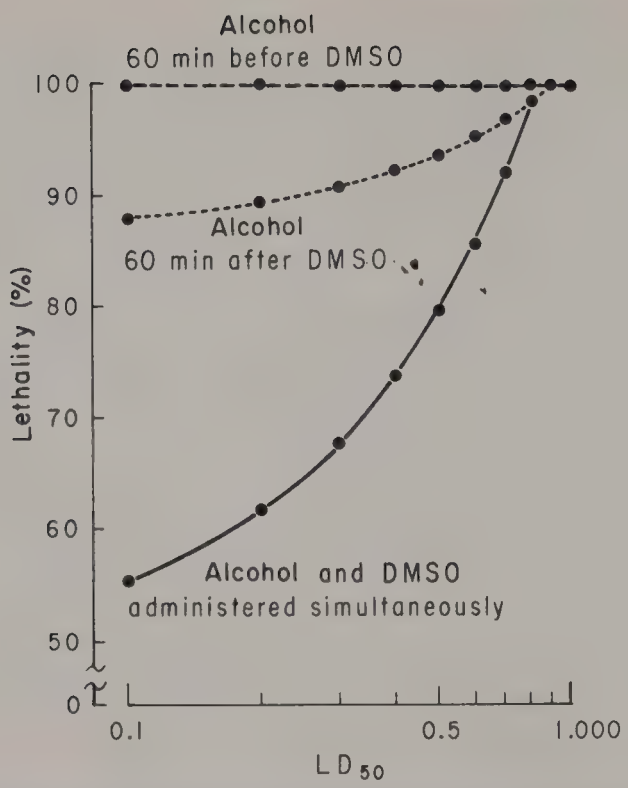

Fig. 1. Mortality curves resulting from the administration of alcohol and DMSO in varying doses to animals, simultaneously and with alcohol 1 hr before or after DMSO.

After demonstration of the mutual influence of alcohol and DMSO, particularly with interposition of an interval in administration, on the lethality of white mice, these effects were tested statistically. This was done using variance analysis as developed by Fisher *(15,16)*. At present no simpler method is known for determining and testing interactions.

In this analysis 2940 white mice, divided into three groups of 980 animals (20 animals per division) received alcohol and DMSO simultaneously, DMSO 60 min before the alcohol, and DMSO 60 min after the alcohol. Administration was oral via a stomach tube. For statistical reasons the end point considered was the survival time instead of lethality.

From this investigation the individual effects of alcohol and DMSO and of the interval in administration on the survival times of the experimental animals are significant with a probability of error of 1%. In addition to these individual effects there is a further factor, namely, the interaction between alcohol and DMSO, with a probability of error of 0.5%. Thus, the individual effects of alcohol and DMSO are not

simple additive phenomena; interaction between the two is an additional factor to be considered. The individual effects, however, are stronger than the interaction.

Although there are differences in the individual effects, it is not possible to confirm that alcohol had a greater influence on the survival time than DMSO did. Hence, all individual effects must be regarded as being of equal magnitude *(17)*.

III. INVESTIGATION OF THE INTERACTION OF ALCOHOL AND DMSO IN MAN

A. Experimental Method

This investigation was carried out on 46 young men who were instructed to appear on the morning of the experiment in a fasting state and also to have consumed no alcoholic drink the previous evening so that their bodies would contain no residual alcohol when the experiment was begun. The testing was carried out using Alcotest test tubes (Fa. Draeger, Luebeck).

Three groups were set up. In the first group of 15 volunteers 0.75 g absolute alcohol per kilogram body weight was given and immediately followed by pure 50 mg/kg DMSO. In the second group of 15 volunteers DMSO was given 1 hr before the alcohol. The third group of 16 volunteers was the control group and was given only alcohol.

Immediately before the beginning of the experiment, the test subjects were weighed to determine the dosage to be given. Alcohol was imbibed in the form of 55 vol.% carbohydrate-free spirits (vodka) within a 10-min period. DMSO was applied to the skin of the back. The duration of the experiment was 6 hr. Blood alcohol curves were determined from eight blood samples (fasting sample, 60, 90, 120, 180, 240, 300, and 360 min after the start of drinking). Test subjects in the second and third groups emptied their bladders before the beginning of the experiment. During the experiment the volume of each urine sample was measured and the time noted. The test subjects of the first two groups were given a light snack, usually two filled rolls, 180 min after the start of drinking. No liquid was given.

The blood samples were taken from a small vein. The alcohol content of these samples and of the urine samples was determined 3 times by

the Widmark procedure and 2 times by the ADH procedure. A graph of the blood alcohol curve was drawn for each test subject, and the time from the start of drinking until the peak was reached was noted. The height of the peak, the fall of the blood alcohol level per hour (β_{60}), and the Widmark constants c_0 and r were determined *(18)*. Table 1 gives means ($\bar{x}$) and standard deviations (s) for these characteristics, and also age, height, and weight of the test subjects. During the experiment the extent and intensity of the skin reaction and the breath odor of alcohol and/or dimethyl sulfide (DMS) were tested in each subject. The degree of intensity of skin reaction and the alcohol and DMS odor were evaluated on the basis of a scheme rated from 0 (none) to 4.5 (very strong).

B. Blood Alcohol Curves

Results from blood alcohol curves were determined for each subject. At the end of the experimental period, the level of alcohol fell more rapidly when it was given with DMSO than when it was administered alone. Average curves for the three groups are similar. The β_{60} values in Table 1 reveal that the fall in blood alcohol level is greater when alcohol and DMSO are given together than when alcohol is administered alone. The hourly decrease in the first group (DMSO immediately after alcohol) is 0.154‰, in the second group (DMSO before alcohol) 0.140‰, in the control group 0.120‰. The differences between the first and third and second and third groups are significant, with probabilities of error of 0.1 and 0.5%. Therefore, under the given experimental conditions, the blood alcohol level fell more rapidly under the influence of DMSO (although the effect is only slight).

C. The Alcohol and DMS Odor

According to Kolb *(19)*, one of the two DMSO metabolites, DMS, is eliminated in the breath. Gerhards *(20)* states that the second metabolite, dimethyl sulfone ($DMSO_2$), is eliminated in the urine. It is assumed that the characteristic odor following DMSO treatment is attributable to DMS. Therefore, 60, 180, and 360 min after the start of drinking we smelled the breath samples of the test subjects and, using a scale of 0–4.5, distinguished qualitatively between alcohol and DMS and assessed these quantitatively according to intensity. An odor of alcohol

TABLE 1

Statistical Comparison of the Values Necessary for the Assessment of Blood Alcohol Curves

Proved Sign	Group I (N = 15)		Group II (N = 15)		Group III (N = 16)		LP		
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	I with II	I with III	II with III
Age, years	22.7	2.1	23.8	1.4	23.6	2.8	0.10	0.40	0.90
Height, cm	180.5	7.8	178.8	5.8	179.7	6.3	0.60	0.80	0.80
Weight, kg	73.7	10.1	73.8	9.4	74.4	11.7	0.98	0.90	0.90
Blood alcohol maximum ‰	0.881	0.102	0.930	0.076	0.893	0.130	0.20	0.80	0.40
Minutes after first drink	83	34	73	19	79	20	0.80	0.70	0.50
c_0, ‰	1.115	0.121	1.103	0.095	1.009	0.095	0.80	0.02	0.02
r	0.680	0.073	0.685	0.056	0.753	0.075	0.975	0.02	0.01
β_{60}, ‰	0.154	0.021	0.140	0.021	0.120	0.014	0.10	0.001	0.005

that became less and less intense could be detected between the first and third hour of the experiment in some test subjects who had received DMSO directly after the alcohol, but the odor could not be detected at all in the second group of subjects who had received DMSO 1 hr before the alcohol.

By comparing the intensities detected by the olfactory sense with the DMS concentrations measured by gas chromatography in samples of exhaled air taken at the same time, and using coordinates in which the odor was correlated with time on a decimal basis and concentration on a logarithmic basis, close positive correlations were obtained in the first and also in the second group. This result corresponded to the physiological observation that the intensity of sense impression is a logarithmic function of the quality of the stimulus. The statistical evaluation of the gas chromatograms revealed that DMS concentration in exhaled air was more pronounced in the test subjects from the second group.

With administration of the two substances, one directly after the other (group 1), DMS concentration in the breath 60 min after administration of alcohol was only 1.26 μg/liter, while the second group, which had received DMSO 60 min before the alcohol showed a significantly higher average value of 7.11 μg/liter. There was also a marked difference between the values obtained between the two groups 180 and 360 min after administration of alcohol. All differences were statistically significant with a probability of error of 0.1%. Figure 2 illustrates the relation of the DMS concentration in the breath to the alcohol concentration in the blood. If it is assumed that the DMS content of breath and that of blood are in equilibrium, these greatly

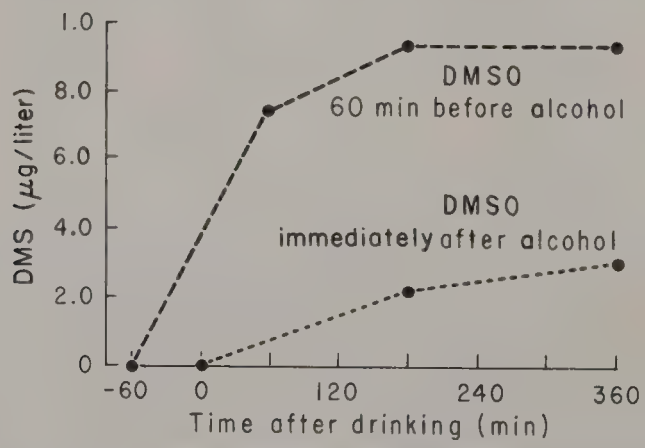

Fig. 2. Graph of DMS production resulting from simultaneous or subsequent action of alcohol [after Heck *et al.* (24)].

differing DMS curves permit the conclusion that DMS production is either inhibited or that further metabolism is promoted under the action of alcohol. According to Rammler and Zaffaroni *(21)*, methyl sulfate can be produced *in vitro* from DMSO, and according to Schueppel *(22)* alcohol accelerates the demethylization of drugs *in vivo*; theoretically it could therefore be inferred that alcohol causes demethylation of DMSO.

This raises the question as to what extent the enzyme systems controlling DMSO metabolism are identical to or coordinated with those of alcohol metabolism. Finally, it should be mentioned that the odor of alcohol on the breath is disguised by DMS, although chemically it is still detectable.

D. Diuretic Action of DMSO and Alcohol

According to Jacob *(23)*, DMSO has a diuretic action in dogs following intravenous administration. This mechanism may be based on an osmotic effect. Nevertheless, it seemed of interest to learn whether or not DMSO and alcohol in combination exert any influence on diuresis. After comparing urine volumes with the amount of alcohol excreted in the urine for groups II and III, our investigations *(24)* indicate that this is not the case.

E. Motor Nerve Conduction Rate under the Influence of DMSO and Alcohol

During the 1965 DMSO Symposium in Berlin, Jacob reported that in experiments on the cat the analgesic action of DMSO involved a reversible block of stimulus conduction. Jacob postulated this was attributable to the osmotic effect of 25% DMSO in Ringer solution. Measurements of the motor nerve conduction rate (NCR) in man had not previously been carried out.

Our electromyographic investigations *(25)* led to an interesting result. When the test subjects received 0.75 g alcohol/kg, the NCR decreased by 6.3 m/sec to the range 0.0–1.0‰. When 50 mg DMSO/kg was applied to the skin of the back 60 min before administration of alcohol, there was a greater decrease in the NCR of 11.6 m/sec (Fig. 3). This difference is statistically significant. It is not clear how DMSO and alcohol, alone or in combination, affect the NCR. Since conduction rate

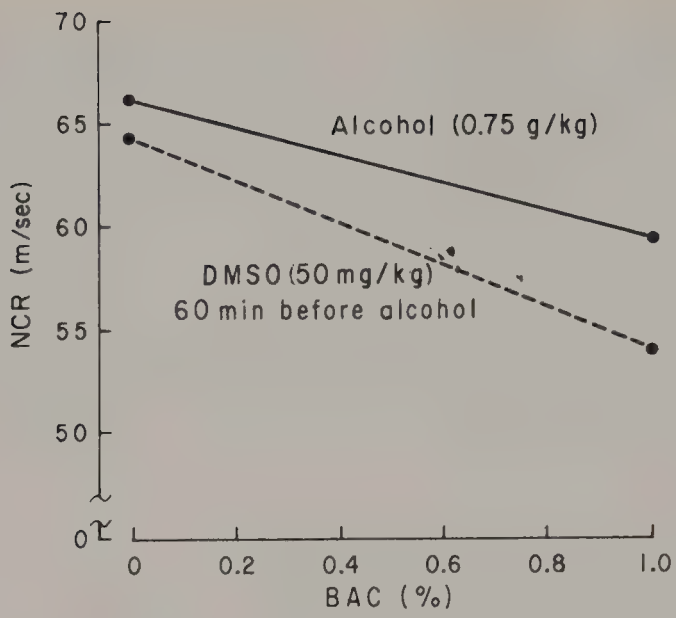

Fig. 3. Influence of DMSO and alcohol on the NCR.

is a function of many factors, including fiber thickness, tissue temperature, and blood carbon dioxide content, appropriate additional investigations should be performed.

F. Influence of DMSO and Alcohol on Mental Functions

Attention focus and concentration, reliability and speed of reactions, adaptability and stress capacity were also experimentally tested, i.e., mental capacities which, according to K. Mayer (26–28), result in end points that can be compared statistically. Dosage and administration of DMSO and alcohol were as previously described.

For example, the quantitative and qualitative performance of a test subject was assessed in a test of power of concentration, a prolonged calculation lasting 30 min. The deterioration in performance, measured by the percentage of error, after administration of DMSO and alcohol simultaneously or in doses separated by an interval of 1 hr, was significantly greater than that which occurred under the influence of alcohol alone. This deterioration of performance was particularly prominent 2 hr after administration of both substances (Fig. 4). Similar impairment was seen in the reactions to simple optical stimuli.

Independent of the objective deterioration in performance, the test

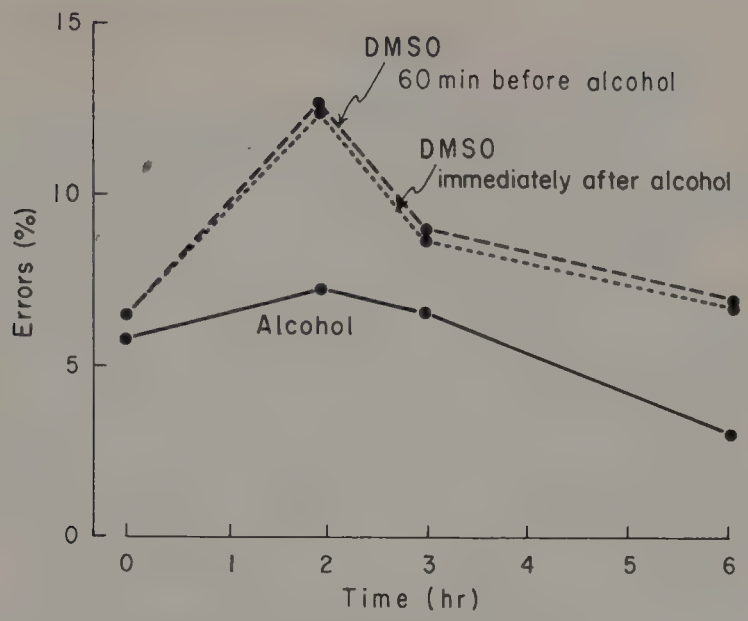

Fig. 4. Average percentage error in the power concentration test as influenced by alcohol and by combined alcohol and DMSO action.

subjects believed that their functional capacity was impaired more under the combined action of DMSO and alcohol than under the influence of alcohol alone. However, no alcohol intolerance such as had been described by Boost *(4)* was observed in these experiments *(24)*.

IV. SUMMARY

In mice simultaneous administration of alcohol and DMSO via the oral route increases mortality at a rate greater than that obtained when DMSO is administered prior to alcohol.

Results from studies of blood alcohol levels taken from human volunteers indicate a more rapid decrease in blood alcohol level under the additional influence of DMSO administered immediately after or 60 min before.

DMS concentration in the breath was significantly higher in subjects who received DMSO 60 min before alcohol than in those to whom DMSO was given immediately after consumption of alcohol.

Results of urine volume comparisons between subjects receiving DMSO 60 min before alcohol and those receiving only alcohol showed

no significant difference, indicating the absence of any diuretic effect of DMSO in humans.

Nerve conduction rate was affected both by 0.75 g/kg alcohol administered orally and by 50 mg/kg, DMSO, however, DMSO decreased the rate nearly twice as much as alcohol alone.

Deterioration in mental performance was significantly greater after the administration of DMSO and alcohol simultaneously or after an interval of 1 hr than after the administration of alcohol alone.

Knowledge of the interactions between DMSO and alcohol is limited, although more information was gained from experiments on man and animals than from investigations of interactions between other drugs and alcohol. Because of its physical and chemical properties DMSO is an excellent model substance. There should be more intensive psychopharmacological, physiological, and biochemical research into the effects of DMSO on the action of alcohol, and vice versa.

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Chapter 10

DMSO and Plants

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I. INTRODUCTION

During the past 6 years a number of interesting and, in several cases, potentially useful effects of DMSO on lower and higher plants have been reported. Many of these reports are in the form of abstracts, preliminary announcements, or brief papers lacking sufficient data for critical evaluation. Relatively few of the findings have been corroborated by independent investigators. Hence, a critical review of this subject seems premature, and an attempt will be made in this chapter to recount the findings largely as reported.

II. TOXICITY

Several studies have sought to establish levels of toxicity for higher plants and microorganisms, usually in order to evaluate the solvent when DMSO was used as a carrier, but in at least one case with the objective of possible use as a herbicide.

Aqueous solutions of 40% or greater sprayed to runoff on cacao seedlings caused marginal necrosis, spotting, and yellow blotching; lower concentrations had no toxic effects *(1)*. Cotton seedlings sprayed to runoff daily with 25% DMSO showed tip burn around the edges of the primary leaves at the end of 2 weeks *(2)*. A 10% DMSO spray caused severe burning of leaves followed by necrosis in *Datura stramonium (3)*. A 5% spray produced no toxic effects in this species, but in *Datura tatula* caused yellowing and margin necrosis of leaves, streaking, and abscission of small buds. However, some flowering and fruit set occurred even in these cases. Aqueous solutions of 6% or greater when sprayed to runoff on Merion bluegrass *(Poa pratensis)* caused damage to leaf tips; no damage was caused by concentrations of 3% or lower *(4)*. Spraying 2% DMSO on Bartlett pear trees at flowering time caused some browning of petals and slight blackening of the leaf margins *(5)*. No injury was observed in trees sprayed with DMSO containing 50 ppm streptomycin. DMSO applied as a foliar spray at rates up to 4 gal/acre on natural old field vegetation produced no obvious symptoms of toxicity *(6)*. Small droplets of DMSO applied to bean leaves caused localized necrosis *(7)*.

Daily spraying of cotton seedlings with DMSO concentrations up to 25% over a 4-week period did not affect growth or produce any signs of

systemic disorders *(2)*. Corn plants were not injured by application to their bases of pure DMSO at a rate of 30 gal/acre.

Injection of 300 ml of 1 *M* DMSO into the trunks of dormant peach trees delayed leaf development in some trees but caused no serious injury *(8)*. Injection just prior to bud swell or at full-leaf stage caused severe leaf scorch over most of the foliar surface and dieback of one or more branches, usually those directly above the point of injection. Wilting commenced within 24 hr of injection and marginal leaf necrosis developed rapidly. These trees did not recover during the season of application but grew normally the next year. Injection of 0.5 *M* or less concentrated solutions just before leaf swell produced appreciably less damage, and the affected branches were rapidly hidden by new growth so that the trees appeared normal by midsummer.

Weekly applications of 100 ml of 1% aqueous DMSO to the soil of *Datura ferox* and *D. innoxia* potted in 1-gal containers caused burning and drying of the leaves; a 0.1% solution had no toxic effects *(9)*.

Sprouting of tubers of purple nutsedge *(Cyperus rotundus)* planted in soil treated with DMSO was greatly retarded *(10)*. The inhibitory effect varied with the DMSO dosage. Even after the delayed appearance of shoots, root development was still inhibited completely; after transplanting the tubers to untreated soil, roots were formed immediately and grew normally. After 60 days of contact with the original tubers, the treated soil was no longer toxic to a second planting of tubers.

Bean plants cultured in nutrient solutions containing 1.4 m*M* DMSO showed retardation of elongation *(11)*. At 2.8 m*M* the fresh and dry weights of shoots and roots also were significantly lower than the controls. A concentration of 1% DMSO was only slightly, if at all, toxic to cells of the yeast *Schizosaccharomyces* on prolonged contact *(12)*.

III. ENTRY, TRANSPORT, AND FATE OF DMSO IN PLANTS

Information on entry and movement of DMSO has been obtained both by studies using the ^{35}S-labeled compound and by observations of physiological effects. In one experiment a dilute aqueous solution of radioactive DMSO was applied to the external surface of the bark of a young pear tree *(Pyrus calleryana)* just above the ground line *(13)*. The attached leaves were monitored with a Geiger–Muller tube. All leaves on the tree became radioactive. In general the activity increased with time up to 9 days after application although irregular fluctuations were

observed in some leaves. Activity tended to be higher in leaves near the ends of the branches. No evidence was presented as to whether or not the DMSO had undergone any chemical reaction. In another investigation, all parts of seedlings of *Lupinus albus* became radioactive when the seeds were germinated in the presence of ^{35}S-labeled DMSO. The embryo showed the highest activity, the seed coats and cotyledons the least *(14)*.

DMSO injected into the stems of peach, apple, pear, and cherry seedlings moved rapidly to the leaves as shown by injury at the leaf margins *(15)*.

The foliar symptoms exhibited by bean plants cultured in nutrient solution containing DMSO suggested that transport took place via the xylem *(11)*.

DMSO penetrated carrot xylem parenchyma rapidly and did not act as a functional osmoticum *(16)*. However, a significant portion of the DMSO that entered from aqueous solution did not readily reequilibrate when the tissue was transferred to water *(17)*.

Peach trees receiving trunk injections of DMSO emitted from the leaves within 30 min a characteristic odor described as "intensely sulfurous" which persisted for several days *(8)*.

IV. EFFECTS OF DMSO ON ABSORPTION AND TRANSPORT

There are many indications that DMSO influences uptake and movement of a variety of substances. The evidence is direct and unequivocal in a few cases, indirect or circumstantial in most. There is as yet very little information as to the mechanisms by which such effects are produced.

A. Water

The diffusion coefficient for radial movement of water in roots of *Zea mays* seedlings was not affected by 1 *M* DMSO *(51)*. DMSO (0.44*M*) administered either as a pretreatment or together with mannitol did not significantly alter the hydraulic permeability of carrot xylem parenchyma *(16,17)*.

B. Salts, Nutrients, Metabolites, Dyes

An early statement, without supporting details, that treating plants with DMSO rendered the membranes more permeable both to dialyzable ions and compounds and to substances generally regarded as nondialyzable stimulated many of the subsequent investigations *(18)*.

The effect of DMSO on absorption of phosphate through the roots of strawberry plants *(Fragaria ananassa)* was investigated in two experiments *(13)*. In the first, two comparable bare-rooted plants were placed in Hoagland's nutrient solution containing ^{32}P. After 3 hours sufficient DMSO was added to one solution to make it 0.05%. Thereafter, at intervals of 1.5, 6.5, and 25.5 hr, measurements were made of the radioactive emission from selected leaf samples from the two plants. At each sampling the DMSO-treated plants showed nearly twice the activity of the control. In the second experiment a dilute aqueous solution of DMSO (50 ml of 200 ppm DMSO per plant) was applied to the soil of strawberry plants potted in 1-gal cans. Ninety-six hours later an aqueous solution of $Na_2H^{32}PO_4$ was similarly applied; after a further 4 hr, leaf samples were removed and the radioactive emission determined. The activity of the DMSO-treated plants was 21 times as great as that of controls which had received only water.

Inclusion of 2% DMSO in solutions of various iron salts and chelates applied to leaves of chlorotic citrus trees approximately doubled the subsequent chlorophyll formation *(19)*. DMSO in concentrations up to 10% has been reported to stimulate the uptake of zinc by excised barley roots but to depress severely the absorption of sodium and rubidium *(20)*. These effects were thought to be the result of an attack by DMSO on some metabolic process rather than an alteration of membrane permeability, since loss of zinc to a desorbing solution was not enhanced by the presence of DMSO.

Bean plants cultured in nutrient solutions containing 1.4 or 7 m*M* DMSO decreased the conductivity of the solution significantly more than control plants *(11)*. Electrolyte efflux from plant tissue placed in deionized water was not affected by brief exposure to pure DMSO or by prolonged contact with dilute solutions *(16,17)*.

Fifteen minutes of incubation of intact tobacco callus tissue in buffer containing 5–15% DMSO resulted in extraction of the pool of endogenous serine *(21)*. This facilitating effect of DMSO on entry of substra-

tes and release of reaction products was utilized in designing procedures for assay of tryptophan synthetase in intact cells.

Rapid transport through plant tissues of dyes dissolved in DMSO has been reported *(22)*.

Acquisition by certain yeasts of the ability to metabolize exogenous citric, tartaric, and malic acids after prolonged incubation in DMSO was attributed to increased penetration of these substrates *(12)*.

C. Herbicides and Growth Regulators

In experiments on entry and transport of ^{14}C-labeled 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) triethanolamine salt in big leaf maple seedlings, it was found that the use of 50 or 100% DMSO as solvent enhanced severalfold the penetration of herbicide applied to leaves; export from the treated leaf was not significantly affected, however *(23)*. Application of the solutions to the base of the stem produced rather erratic results. The presence of DMSO in solutions injected through a slit in the bark increased movement of the herbicide in the stem but decreased transport to the new shoot growth and nearly prevented its movement to the roots.

The toxicity of dalapon (2,2-dichloropropionic acid) and diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] to oats was not significantly altered by the addition of DMSO *(24)*. However, a formulation of diuron plus 1% colloidal X-77 in 100% DMSO was more effective than in 10% aqueous DMSO.

In late postemergence field applications, DMSO enhanced the activity of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) on dicots but not on monocot weeds *(2)*. Typical herbicidal symptoms were evoked by 25 μg of 2,4-dichlorophenoxyacetic acid (2,4-D) applied in a 50 μl droplet of DMSO solution to bean leaves, whereas 100–200 μg were required to elicit the same response when applied in water. When the herbicide was injected directly into the pith of the hypocotyl, the two solvents were equally potent.

Using leaf abscission as a physiological indicator, it was found that 2,4-D entered bean leaves much more rapidly from DMSO than from water or ethanol solutions *(7)*. DMSO also expedited entry of the poorly penetrating lithium salt of 2,4-D but appeared not to promote absorption of the octyl ester which alone penetrates very well; however, the dose of octyl 2,4-D in this experiment may have been too large to detect possible enhancement by DMSO. Application of DMSO alone

had no abscission-promoting effect but did result in localized necrosis. That the necrosis was not the cause of the expedited entry was shown by an experiment in which applications on areas prekilled by liquid nitrogen did not enhance the effectiveness of 2,4-D in aqueous solution and reduced the effectiveness in DMSO. Injections of 2,4-D solutions into the hypocotyl resulted in identical physiological effects when either DMSO or water was the solvent, a further indication that DMSO influences entry but not translocation or activity of the growth regulator.

Penetration of sodium cyanate and sodium pentachlorophenate into cucumber leaves and of indoleacetic acid into tomato plants was increased by DMSO *(25)*.

The growth-retarding effect of B995 (N-dimethylaminosuccinamic acid) repeatedly sprayed on *Datura tatula* was slightly enhanced, and of CCC (2-chloroethyltrimethylammonium chloride) was marked enhanced by the presence of 2% DMSO in the solutions *(3)*. In a similar experiment with *D. innoxia,* DMSO did not potentiate the growth-retarding effect of B995 *(26)*. The elongation-accelerating effect of gibberellic acid solution sprayed repeatedly on *D. tatula* seedlings was slightly, but significantly, greater when 2% DMSO was used as solvent *(27)*. The growth-retarding effect of phosphon (tributyl 2,4-dichlorobenzylphosphonium chloride) applied to the soil of potted *D. ferox* seedlings was at first potentiated by the presence of 0.1% DMSO; however, this effect of DMSO was later eliminated or even reversed *(9)*.

Inclusion of 30% DMSO in solutions of herbicides applied to bean leaves increased the phytotoxic effectiveness of 2,4-D, diquat dibromide (1,1′-ethylene-2,2′-dipyridylium dibromide) or paraquat dichloride (1,1′-dimethyl-4,4′-dipyridylium dichloride) but not of 2,3,6-trichlorobenzoic acid of dicamba (2-methoxy-3,6-dichlorobenzoic acid); experiments with picloram (4-amino-3,5,6-trichloropicolinic acid) gave inconsistent results *(28)*.

Response of velvet mesquite *(Prosopis juliflora* var. *velutina)* seedlings to foliar applications of dicamba, picloram, or 2,4,5-T triethylamine salt was enhanced by the use of 50% or 100% DMSO as solvent but strongly antagonized by 20% DMSO *(29)*. At the higher concentrations DMSO also altered the pattern of injury, producing marked effects on the roots and in the leaves above those to which the herbicide was applied; without DMSO these organs showed little injury. Microscopic studies of the injured tissue led the author to the conclusion that while DMSO may influence translocation of 2,4,5-T the two substances do not necessarily move together. In a subsequent study it was found that

DMSO did not enhance the action of picloram and several other herbicides on mesquite, but that in combination with diesel oil and a lypophilic surfactant it was an effective carrier for the butoxyethanol ester of 2,4,5-T *(30)*.

DMSO increased substantially the effectiveness of several herbicides against a number of weeds *(6)*. Among the herbicides were monosodium methanearsonate, disodium methanearsonate, amitrole (3-amino-1,2,4-triazole), 2,4-D diethylamine salt, and Tordon 101 (a mixture of picloram and 2,4-D); susceptible weed species included *Verbena* spp., Johnson grass, and alligator weed *(Alternanthes philoxeroides)*.

D. Insecticides

A mixture of DMSO and dimethylformamide has been proposed as a solvent for chlorinated insecticides *(31)*.

Lindane, dieldrin, DDT, parathion, malathion, endosulfan (hexachloro-hexahydro-methano-2,4,3-benzodioxanthiepin-3-oxide), dimethoate [O,O-dimethyl *S*-(N-methylcarbamoylmethyl) phosphorodithioate], Kelthane [1,1-bis (*p*-chlorophenyl)-2,2,2-trichloroethanol], and Guthion [O,O-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3(4*H*)-ylmethyl) phosphorodithioate] injected into the stems of peach seedlings were transported to the leaves *(15)*.

An insecticidal composition containing DMSO and ground stems of *Ryania speciosa* (as a source of the insecticidal alkaloid ryanodine) has been patented *(32)*.

E. Fungicides and Antibiotics

As early as 1959 spraying with nystatin dispersed in 0.5% DMSO was reported highly efficacious in protecting orchids against both natural and artificial fungal infections; however, in these trials there were no comparisons with formulations lacking DMSO *(33)*. A mixture of DMSO and dimethyl formamide has been proposed as a solvent for mercurial fungicides *(31)*.

The effect of DMSO added in 3% concentration to formulations of the fungicides Dithane M-45 [active ingredient is a mixture of zinc and manganese ethylenebis(dithiocarbamates)] and Dyrene [2,4-dichloro-6-(O-chloroanilino)-*s*-triazine] for control of *Helminthosporium* diseases of Kentucky bluegrass was studied under field conditions *(4)*. Seven weekly applications were made throughout the spring. The plots sprayed with fungicide combined with DMSO had about 30% fewer

lesions than those treated with the fungicide alone, a statistically insignificant difference. Growth during the last week of the experiment, as measured by fresh weight of clippings, was 60% greater in those plots receiving DMSO, apparently a significant difference.

The protection of cacao seedlings against *Phytophthora palmivora* by several fungicides was not clearly enhanced by addition of DMSO at 2 or 5% concentration *(1)*. Indeed, in an experiment in which the treated seedlings were subjected to considerable rain during the approximately 2-week period between application of the fungicide and inoculation with the fungal spores, there was a suggestion that DMSO may have diminished the control capacity of several of the fungicides.

The effectiveness of soil applications of Lanstan (1-chloro-2-nitropropane) and Quintozene (pentachloronitrobenzene) in controlling clubroot infection of cabbage was not augmented by incorporation of DMSO; on the contrary, high levels caused phytotoxicity and low levels produced symptoms of manganese deficiency *(34)*.

Addition of DMSO at 1–2% concentration augmented markedly the activity of sodium pentachlorophenate in repressing growth and sporulation of *Monilia fructicola* in agar culture *(35)*.

No synergism was found between DMSO and cycloheximide or acrizane chloride in inhibition of growth of the oak root rot fungus, *Armillaria mellea,* on potato dextrose agar *(36)*.

The antibiotics cytovirin, cycloheximide, and streptoviticin A were translocated to the leaves of fruit tree seedlings after solutions in DMSO were injected into the stems *(15)*.

Control of bacterial leaf spot *(Xanthomonas pruni)* on peach trees by spraying with oxytetracycline was significantly enhanced by inclusion of 0.25–2% DMSO *(32,37,38)*. The greater effectiveness was attributed to increased penetration and transport of the antibiotic; nevertheless, the fruit at harvest time had no detectable antibiotic residue or off-flavor.

Control of fire blight of pear by sprays of streptomycin was diminished by inclusion of 1–2% DMSO *(5)*.

V. EFFECTS OF DMSO ON OTHER PHYSIOLOGICAL PROCESSES

A. Growth

There have been several reports of stimulation of growth by DMSO administered in various ways to a number of species of plants.

In a greenhouse experiment in which flats of zinnia, marigold, bean, corn, and cucumber were watered with 5 ppm DMSO in addition to weekly subirrigation with water, the treated shoots were taller and heavier than the controls which presumably received only the subirrigation *(39)*. The effect of spraying four species of *Datura* repeatedly with 2% aqueous DMSO was studied in a series of experiments *(3,9,26,27,-40)*. In one, sprayed *D. tatula* plants grew slightly but significantly taller than unsprayed controls. Regrettably, this experiment did not include a control sprayed only with water. The dry weight of the stems was also increased by DMSO, while that of the roots was decreased; the weight of the plant as a whole was unaffected by the treatment.

In an otherwise similar experiment with *D. tatula* carried out at a different season, DMSO did not significantly influence the elongation but did cause a somewhat smaller gain in fresh and dry weights. Growth of *D. ferox, D. innoxia,* and *D. stramonium* was not affected by the DMSO applications.

Spraying sugar beets at 10-day intervals during the growing season with 10–15 ppm DMSO at a rate of 100 gal/acre resulted in a 6% increase in the gross sugar yield *(39)*. This was attributable in part to a higher sugar content in the treated beets (102% of the control value) and presumably in part to a greater production of beets.

Fruit yields from Milton prune trees sprayed or injected with DMSO at a concentration of 10 ml/gal were 10 and 20% greater, respectively, than those from the untreated controls *(39)*.

Several investigators have observed that DMSO stimulates growth of shoots from tubers and roots. Dipping cut tubers of Irish potato in 2% DMSO solution increased the number of sprouts per tuber piece and resulted in higher crop yield *(39,41)*. Dahlia tubers responded similarly *(39)*. Dipping sweet potato roots for 5–15 min in 8–12% DMSO more than doubled the number of shoots produced, while the average weight per plant was not significantly affected *(42)*. Although indolebutyric acid alone had no effect on the number of sprouts produced, the DMSO effect was in some cases greater after treatment with both compounds.

One experiment indicates that the growth-promoting effect of DMSO applied to soil may be indirect and complex in nature *(43)*. DMSO was added to pots of soil at a rate of 0.56 mg per gram of soil. During the next 80 days these pots were cropped twice with cabbage plants grown to the early seedling stage; it was assumed that the DMSO was probably degraded to other compounds during this interval. Two bean plants were then grown to the flowering stage in each pot. The dry

weights of the shoots were significantly greater (about 36%) than those of the controls without DMSO. The total amount of nitrogen per shoot was approximately the same in treated plants and in controls; hence, the concentration of nitrogen per gram of shoot was lower in the treated shoots. The concentrations of sulfur and manganese were severalfold higher in the shoots of treated plants; the phosphorus content was not affected. The treated soil also showed a manyfold increase in sulfate and an appreciably lowered pH.

Growth of *Lilium longiflorum* pollen tubes was not affected by 1% DMSO but was markedly inhibited when DMSO was added to give a final concentration of 5%; growth resumed at essentially the original rate when the concentration was subsequently reduced to 1% by dilution *(44)*.

The growth of cultures of carrot tissue was not significantly affected by incorporation of 0.01–10 m*M* DMSO in the medium *(45)*.

Vegetative growth of *Penicillum notatum* and *Aspergillus niger* in fortified corn steep broth was increased about 10-fold by addition of DMSO up to a concentration of 3% of the total medium weight *(39)*. Above 5% growth was depressed. In another experiment with *Aspergillus* cultured in sucrose medium, a 30% greater yield of citric acid was obtained in the presence of 0.5% DMSO. Growth of the fungus *Stemphylium solani* was not inhibited by DMSO concentrations up to 20%. *(46)*.

The growth of colonies of *Helminthosporium sativum* and *H. dictyoides* on potato–dextrose–agar medium was not influenced by DMSO at 1% or less but was reduced by 3% DMSO *(4)*. Growth of the oak root rot fungus, *Armillaria mellea,* on potato–dextrose–agar medium was partially inhibited by incorporation of DMSO at 1% concentration and completely inhibited at 10% concentration *(36)*. DMSO at 200–1000 μg/ml stimulated growth of *Ashbya gossypii* by 10–20%, while depressing the synthesis of riboflavine by about the same percentage *(47)*.

B. Flowering, Sporulation

Four weekly applications of 100 ml of 0.1% aqueous DMSO to the soil of *Datura ferox* seedlings potted in 1-gal containers had no influence on the time or extent of flowering *(9)*.

Sporulation of *Penicillium notatum* and *Aspergillus niger* was depressed by concentrations of DMSO below 3%, which markedly

stimulated vegetative development *(39)*. Conidia production by *Stemphylium solani* was stimulated by 1–10% DMSO *(46)*. The effect was not specific to DMSO but was elicited by ethanol, ethylene glycol, and propylene glycol. A suggested mechanism is the triggering of some developmental process by extraction of ergosterol from a bound state in the preconidiophores.

C. Germination of Seeds, Pollen Grains, and Spores

Soaking seeds of tomato, cucumber, bean, corn, pea, soybean, morning glory, jimson weed, yellow foxtail, and mallow in 10^{-4} to 1 *M* DMSO solutions for periods up to 24 hr had no consistent effects on germination *(2)*. All seedlings showed normal morphology and development during a 2-week observation period. DMSO concentrations of 2.5 *M* and higher tended to decrease the percent germination. Wheat and pea seeds immersed 15 min in a mixture of 1 part DMSO and 2 parts dimethylformamide by weight and then planted in soil showed somewhat greater percent emergence and equal or greater growth in comparison to water-treated controls *(31)*.

Immersion of imbibed rice seeds for 8 hr in DMSO solutions up to 10% concentration had no effect on germination but did decrease the rate of shoot elongation *(48)*. Higher concentrations both decreased the percentage germination and delayed the germination process. Five percent DMSO, which alone had no effect on germination and only slightly retarded elongation, markedly potentiated the inhibitory action of ethyl methanesulfonate on these processes.

The germination of pollen of *Lilium longiflorum* was not influenced by DMSO at 1% or lower concentration, was prevented entirely by 5% or higher, and was partially inhibited between 1 and 5% *(44)*. The high concentrations did not permanently damage the pollen since normal germination occurred after removal or dilution of the DMSO.

Conidia of *Helminthosporium sativum* germinated 100% in tap water containing 1% DMSO, 90% in 25% DMSO, 29% in 50% DMSO, and not at all in pure DMSO *(4)*. The germ tubes were progressively shorter with increasing DMSO concentration.

Germination of spores of the myxomycete *Lycogala epidendrum* was accelerated by treatment with DMSO solutions *(49)*. With DMSO concentrations up to 20% and exposure times up to 120 min, germination was maximal when the product of these two variables was about 600 and fell off with longer exposures at higher concentrations. The

effect was an activation or acceleration manifested as increased germination at 24 hr after treatment; by 48 hr the controls had germinated to the same extent as the treated spores.

Spores of *Bacillus pantothenticus* were activated to germinate at room temperature by pretreatment with DMSO *(50)*. The effect was not specific, as dimethylformamide was even more effective. Both these highly polar substances alter the tertiary structure of proteins by rupture of hydrogen bonds. It is of interest to note that DMSO and elevated temperature, which bring about the same physiological response in this case, also have similar effects in carotenoid biosynthesis of tomato plants *(vide infra)*.

D. Cyclosis

Protoplasmic streaming in root hair cells of *Zea mays* seedlings was not prevented by immersion of the radicles in 0.1 *M* DMSO *(51)*.

E. Abscission

The rapidity and extent of leaf abscission in young bean plants (*Phaseolus vulgaris* ev. 'Red Kidney') receiving foliar applications of 2,4-D solutions were increased markedly by use of DMSO as solvent *(7)*. DMSO alone did not cause abscission.

F. Suberization

Dipping cut Irish potato tubers in 2% DMSO enhanced suberization *(39;* see also *41)*.

G. Metabolism

A 30-min dip of detached mature green tomatoes in 10% DMSO inhibited the subsequent red coloration and resulted in predominantly yellowish fruits *(52)*. This behavior was attributable to diminished production of the acyclic carotenoids phytoene, phytofluene, ζ-carotene, and lycopene, while synthesis of β-carotene and other cyclic carotenoids was not significantly affected. DMSO thus has an effect similar to that produced by high ripening temperature in certain tomato varieties and may provide a useful tool for elucidating the pathways of carotenoid

biosynthesis. A parallel similarity in the influence of DMSO and high temperature on activation of spore germination was pointed out in Section V, C.

The effect of foliar or soil applications of DMSO on alkaloid production by several species of *Datura* has been investigated *(3,9,26, 27,40)*. The results of treatment were rather variable, with indications of significant increases in a few samples, of significant decreases in some others, but no effect in most. There was a suggestion that quite different results might occur at different seasons of the year *(27)*.

Repeated weekly spraying of *D. innoxia* with 2% DMSO was reported to lead to a significantly lower content of chlorophyll than in the control *(26)*. In sprayed *D. tatula* there were appreciably higher concentrations of water-, ether-, and petroleum ether-extractives but a lower content of ethanol-soluble substances *(27)*.

The sucrose content of sugar beets sprayed repeatedly during the growing season with 10–15 ppm DMSO was 102% of the control value *(39)*. The presence of DMSO at concentrations up to 10 m*M* in the medium did not significantly alter the content of glucose, fructose, or sucrose in carrot tissue cultures *(45)*.

Experiments with pollen of *Lilium longiflorum* showed that DMSO is not a general inhibitor of metabolism although it does inhibit initiation and elongation of pollen tubes *(44)*. Pollen accumulates considerable starch in the presence of a concentration of DMSO that prevents germination. Respiration measurements led to the conclusion that DMSO inhibits reactions that utilize high-energy phosphate but does not directly affect the process of oxidative phosphorylation. Respiration of isolated mitochondria from green tomato fruits was not affected by the presence of 5% DMSO *(53)*.

The rate of endogenous respiration of the yeast *Schizosaccharomyces acidodevoratus* was increased markedly by 1% DMSO *(12)*. Oxygen uptake was stimulated more than carbon dioxide production so that the respiratory quotient of the treated cells was 0.8 in contrast to the control value of 1.6. *Schizosaccharomyces pombe* behaved rather differently; the oxygen uptake was not appreciably affected but the carbon dioxide production was decreased greatly so that in this species also DMSO treatment lowered the R.Q. from 1.9 to 0.8. Prolonged incubation of *S. acidodevoratus* in DMSO also resulted in utilization of exogenous citric acid and, to a lesser extent, of tartaric acid, which did not occur in the controls. Similarly, prolonged incubation of *S. pombe* in DMSO permitted the oxidative decarboxylation of malic acid.

Addition of 0.5% DMSO to the culture medium resulted in a 15% increase in streptomycin production by *Streptomyces griseus (39).* Production of riboflavine by *Ashbya gossypii* was decreased slightly by DMSO at 200–1000 μg/ml although growth was somewhat stimulated *(47).*

H. Enzyme Activity

Little attention has yet been given to the effects of DMSO on enzymes from plant sources. Catalysis of the condensation of serine and indole by a partially purified tryptophan synthetase was unaffected by the presence of 10% DMSO but inhibited about 30% by 15% DMSO *(21).* However, the conversion of indoleglycerol phosphate and serine to tryptophan and glyceraldehyde-3-phosphate by the same preparation was inhibited about 80% in the presence of 10% DMSO.

I. Mutagenesis

Mutagenicity of DMSO in *Arabidopsis thaliana* was investigated by applying a small droplet of 5% aqueous solution to the growing point when the first flower buds were at the premeiotic stage and subsequently determining the frequency of *albina* mutants in the selfed progeny *(22).* DMSO alone was nonmutagenic, but when applied in mixture with ethyl methanesulfonate the mutation frequency was double that of the latter compound alone. The result was attributed to increased and/or more rapid uptake of the ethyl methanesulfonate.

No mutagenic effect of DMSO, as measured by frequency of chlorophyll mutants, was observed when imbibed rice seeds were immersed for 8 hr in 5% DMSO, nor did DMSO affect the mutagenicity of ethyl methanesulfonate in this case *(48).*

J. Freezing

Pretreatment with 2 *M* DMSO was highly effective in protecting cortical parenchyma cells from winter twigs of the mulberry tree *(Morus bombycis)* against injury from rapid cooling to temperatures down to −196°C followed by rapid rewarming *(54,55).* Cells of collard were protected against freezing more by solutions of DMSO than by

sucrose *(56)*. Cultured cells of *Linium usitatissimum* and *Haplopappus gracilis* survived a month of storage at −50°C in a medium containing 10% DMSO *(57)*. Epidermal cells of various species of *Campanula* were injured by 25% aqueous DMSO.

Young potato plants sprayed to runoff either with 0.5% DMSO or with water were placed at 20°F for 4 hr and then returned to the greenhouse. All plants were injured to some degree, but the DMSO-treated ones were larger, grew more vigorously, and produced the equivalent of 100 sacks per acre more tubers than the water-treated controls *(39)*.

In the presence of 5% DMSO, isolated mitochondria from tomato fruit withstood storage in liquid nitrogen for periods up to 4 weeks without loss of respiratory control or decrease in efficiency of oxidative phosphorylation *(53)*. However, DMSO did not prevent freeze damage when mitochondria were stored at −18°C.

K. Nodulation

Bean plants growing in pots of soil to which DMSO had been added at a rate of 0.56 mg per gram of soil did not produce root nodules; this result may be attributable to the markedly increased acidity and solubilization of manganese in the treated soil *(43)*.

VI. INSECTICIDAL, VIRUCIDAL, AND FUNGICIDAL EFFECTS OF DMSO

DMSO injected into the stems of fruit tree seedlings showed partial control of the two-spotted spider mite in some experiments, but the effect was not consistent *(15)*.

Injection of DMSO into the trunks of peach trees infected with peach mosaic or necrotic ringspot viruses suppressed the disease symptoms during the first year *(8)*. This action appeared to be virustatic rather than virucidal, since all the treated trees showed some symptoms of the disease the following year. However, the disease syndromes were atypically mild, suggesting that the viruses had been modified.

Injection of DMSO into the xylem of pear trees previously infected with the virus causing "stony pit" markedly reduced the percentage of deformed fruits in both the first and second seasons after treatment

(58). Injection of DMSO into healthy peach trees in full leaf prevented development of disease symptoms after the trees were inoculated with peach mosaic virus or with *Prunus* ringspot virus. Injection into Eureka lemon trees produced striking control of a latent virus. Bitter pit of Jonathan apples was partially controlled by spraying repeatedly with 100 ppm DMSO.

Fully infectious tobacco mosaic virus (TMV) could be extracted from leaves by aqueous DMSO at concentrations below 20%; the infectivity decreased at higher concentrations *(59)*. However, it has also been reported that addition of 1–5% DMSO to TMV suspensions decreased the infectivity *(58)*.

Effects of DMSO on the growth of cultures of some pathogenic fungi were described in Section VA.

VII. DMSO AS A NATURAL PRODUCT

Although there is as yet no direct evidence that DMSO is a natural constituent of plants, it is an interesting speculation that this might be the case *(60)*. Both the oxidized and reduced relatives, dimethyl sulfone ($DMSO_2$) and dimethyl sulfide (DMS), respectively, do occur in plants.

$DMSO_2$ has been identified in several species of *Equisetum (61,62)*. It can be produced by oxidation of DMSO by a microsomal system *(63)*.

DMS is thought to be formed in the alga *Polysiphonia* by enzymic reactions *(64,65a)*. It is known also to be readily produced, both by certain molds *(66)* and by nonbiological reactions, from *S*-methylmethionine sulfonium ion

$$(CH_3)_2{}^+SCH_2CH(NH_2)COOH$$

which is of widespread occurrence in plants *(67–73)*. However, there is no evidence that DMS is oxidized to DMSO in plant tissue.

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CHAPTER 11

DMSO in Veterinary Medicine

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I. INTRODUCTION

A body of conclusive evidence now exists that supports the claim that dimethyl sulfoxide (DMSO), in concentrations of 50–90%, crosses the barrier of animal skin rapidly and in high concentrations *(1–3)*. DMSO transports some molecules through the skin, into underlying structures, and even into the blood *(4,5)*.

DMSO also exerts influences which are analgesic *(6)*, antiinflammatory, and antiedematous *(7)*, and alters fibroblastic activity *(8)* in ways that may be beneficial to living tissues. There can be no question that some of the foregoing properties have the inherent ability to enhance consequent toxicities and undesirable side effects. There is little published evidence, however, of serious injury resulting from the use of

DMSO alone. With the exception of changes in animal lenses *(9–11)* resulting from overwhelming daily dosages in the range of 5–10 g/kg and administered over extended periods, necropsy evidence of pathology is essentially negative.

Teratogenic effects produced in laboratory animals required amounts and techniques beyond those involved in therapeutic practices in veterinary medicine *(12)*.

The only apparent contraindication to topical DMSO therapy, at usual clinical doses, is its use in combination with toxic or irritant solutes.

II. TOPICAL DMSO IN HORSES

The antiinflammatory effect of DMSO can be dramatically demonstrated in horses. When horses are hypersensitized with purified fractions of human gamma globulin they react violently to a small challenging dose of antigen, and a massive inflammatory reaction develops at the site of injection. The injected area becomes so inflamed and edematous that animals refuse to move their necks. The inflammation subsides in 8–10 days and necrosis which is slow to heal follows.

Teigland and Saurino *(13)* produced lesions in hypersensitized horses by challenge injections of antigen, and prevented pain, edema, and necrosis by topical application of DMSO to the injection site. The untreated control injections produced massive inflammatory reactions that developed central necrotic areas. Open wounds, many of them unresponsive to prior conventional treatment, were treated with various mixtures of DMSO. The authors reported that "local edema was quickly minimized, normal oozing of serum did not occur....Excessive granulation...was adequately controlled...(and) the animals never seemed to get as sore as with previously known treatments. In five of the wounds treated, synovial drainage was involved. These areas sealed and the wounds healed in unusual *(sic)* short periods of time." In more than 50 cases of bursitis and synovitis they report nearly 90% response which was rated from "some improvement" to "complete return to normal." In 65 cases of osteoarthritis and periostitis they observed that "Clinical improvement...was noted in 60%...." In 20 cases of metacarpal periostitis ("bucked shin"), "quick relief was attained in 90% of those treated." Admittedly, the evaluation of response to treatment of

pathology of bones and joints is difficult, but the foregoing results seem to be considerably better by comparison than those obtained with conventional procedures. Teigland and Saurino are cautious, however, to point out that "unless a period of rest was observed and counter irritation avoided following this treatment, the condition would re-occur."

They also treated 22 cases of tendinitis (bowed tendon) with response "superior to that produced with any previously used external treatment." Nevertheless, they concluded prudently that "it would appear that no cure for the bowed tendon has been found in this approach."

McGee *(14)* reports treating cases of severe open wounds in horses, characterized by guarded prognosis with conventional therapy, that healed rapidly and with minimum complications following the topical application of DMSO.

Keown *(15)* obtained results similar to those reported by other clinicians with the exception of a severe soft tissue reaction in a horse following prior treatment with a decongestant, counterirritant, and analgesic poultice. Marked swelling and sensitivity followed within 12 hr after the application of DMSO to an area that had been traumatized by a tight protective bandage.

Levesque *(16)* commented on the bacteriostatic effect of DMSO topically applied to open wounds of horses and further claimed a remarkable control of excessive granulation tissue.

Arnold *(17)* observed that DMSO appeared to be of benefit to acute traumatic lesions that were not infected.

An effort was made to find documentary evidence of adverse reactions in large animals following treatment with DMSO, but with little success. Rumors have circulated of occasional "blow-ups" (local reactions involving inflammation and swelling), and of one report of the death of a horse associated with circumstantial evidence of DMSO treatment. Ford *(18),* as official veterinarian of the Washington State Horse Racing Commission, has seen evidence of adverse reactions said by the owner to have resulted from the application of DMSO in conjunction with iodine therapy.

III. TOPICAL DMSO IN SMALL DOMESTIC ANIMALS

Knowles *(19),* found that topical application of DMSO reduced the treatment time required for mammary gland engorgement in the bitch

by about one-half (from 5 to 7 days to within 3 days). He reported that in postsurgical edema, local allergic reactions, infectious dermatitis, and tendinitis "DMSO reduced the cardinal signs of inflammation promptly." He used DMSO as a carrier for indicated drugs in the treatment of interdigital cysts (six cases) and severe infectious dermatitis (nine cases). He observed prompt improvement in all instances. Only the garlic odor and some skin scurfing are recorded in criticism of DMSO per se. "However, in one case it did serve as a vehicle for the introduction of lethal quantities of a material (Benzene Hexachloride) that otherwise is safe for topical application."

IV. INTRAPERITONEAL DMSO

This chapter thus far has considered only the avenue of topical application of DMSO, however, intraperitoneal use has also been documented *(12,20)*. It occurred to us that if intraperitoneal solutions of indicated drugs bathed the gastrointestinal tract it would afford advantages in the treatment of gastroenteritis and peracute cases of diarrhea in calves (calf scours). Calves were treated at the Washington State University Veterinary Clinic in the winter of 1964 with a variety of commonly used medications mixed with DMSO. There was no evidence of favorable response. All of the calves died, but the necropsy report specified that there were neither gross nor microscopic lesions attributable to DMSO. With this assurance Dake *(21)* treated a series of nine cats diagnosed with feline panleukopenia, a highly contagious viral disease characterized by an explosively short course, edematous enteritis, and high mortality, by intraperitoneal injections of 4 ml of a 90% DMSO solution. Six of the nine cats survived. This was approximately twice the expected rate of recovery. After DMSO was withdrawn from investigational use, the next 12 cats with feline panleukopenia presented at the clinic were treated by routine therapy and all died. Omission of DMSO from the therapeutic regimen was the only significant change, and neighboring veterinarians reported no variations in the syndrome during this period.

V. SUGGESTIONS FOR FURTHER CLINICAL STUDIES

Before contemplation of other clinical implementation, it should be emphasized that DMSO has many technical biological characteristics

likely to have extensive indication, such as cryoprotective and radioprotective properties for living cells *(22)*.

Beneficial responses in experiments with laboratory animals and in applied studies in human medicine make it reasonable to speculate that DMSO may be found to have many uses in veterinary medicine in addition to those already described, particularly in pathological conditions of which satisfactory management is not presently possible. The connotation of clinical observations of beneficial changes following the application of DMSO to lesions involving excessive granulation, abnormal fibroblastic activity, sclerotic deformity, and inflammatory complications *(23)* has tremendous significance for the veterinary practitioner.

The transport of pharmaceuticals such as antibiotics, corticosteroids, and so forth *(24)* through the skin via topical application appears to promise extensive usage. German workers have reported the absorption of penicillin dissolved in 90% DMSO from the skin of the udder of cows *(25)*. Udder edema, postparturient edemas, and the swellings of purpura hemorrhagica of horses are logical targets for research with an agent that might have corrective value in accelerating the rate of removal of fluids.

Topical cutaneous anesthesia is a concept that excites the practical mind. Some experimental results confirm this accomplishment *(26)*, but to date efforts with most anesthetics have been unsuccessful. Penetration of the DMSO-dissolved anesthetic is apparently limited to the epidermis.

Goldman *et al. (27)* reported tatooing of the skin of a chemist produced by contact with copper chloride in DMSO. Artificial pigmentation of light-colored or unpigmented skin might provide protection from excessive solar irradiation, discourage the progress of photosensitive skin neoplasia, and a means of indelible identification.

Persistent coughs of dogs associated with chronic tracheobronchitis have diminished and ceased following topical application of DMSO to skin of the throat region *(28)*. Any effective approach to control of chronic coughs would be a great boon to veterinary medicine.

If DMSO can alleviate Peyronie's disease in man *(29,24)*, it might mitigate the sequelae to hematoma of the penis in the bull.

Observations have been made in human medicine that caused physicians to suggest that DMSO therapy may contribute to the suppression of cancer cell growth *(30,31)*. Other workers have reported that DMSO exerts bactericidal action against oral flora infections *(32)*. Oral actinomycosis and actinobacillosis are disease problems of consid-

erable magnitude in veterinary medicine, and any progress in control would be welcomed.

"Crampy" or the Spastic Syndrome of cattle, now without effective treatment, appears to be an invitation for DMSO experimentation. These are but a few examples of the many problems that beg the proof of a medicament that offers hope of increased efficacy beyond our present concepts.

VI. CONCLUSIONS

Although critical controls are difficult in the conduct of clinical veterinary medicine, there appears to be a consensus of opinion that inflammatory, edematous, and fibrotic or sclerotic lesions of recent origin are likely to respond to DMSO per se and/or DMSO solutions. These pharmacodynamic properties suggest possible approaches to many enigmas that plague the profession.

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Chapter 12

Some Effects of DMSO on Connective Tissue

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I. INTRODUCTION

Collagen is the principal protein of connective tissue. It has three individual polypeptide chains wound together as the strands of a rope. The chain is held together by hydrogen bonds between carbonyl and imino groups in adjacent chains preponderantly. The molecular weight is about 300,000 and each molecule contains approximately 3000 amino acid residues. The molecules are lined end to end and have no side chains. The helices form bundles of molecules known as fibrils.

Collagen has an unusual molecular composition, which accounts for its structure. About one-third of the amino acid residues are glycine, which likewise has no side chain, and about one-fourth are proline and

hydroxyproline. Hydroxyproline is exclusively related to collagen and elastin.

When collagen fibrils are heated, they shrink and the triple helix unfolds and assumes a random coil configuration. The temperature necessary to produce such a change is referred to as the "shrinkage temperature" and is a measure of the strength of cross-linkages holding the structure together.

The fibrous protein network of connective tissue is embedded in the ground substance. A large part of which is mucopolysaccharide and consists of uronic acids and other sugar molecules. They are present as polymers and may be combined through covalent bonds with proteins other than collagen.

II. EFFECT OF DMSO *IN VITRO*

Dimethyl sulfoxide (DMSO) penetrates skin readily, and when applied topically a high percentage of it is absorbed *(1–3)*. Thus, for a short time at least, relatively high concentrations are attained in the skin at a treated area *(4)*.

Kolb *(5)* exposed leather to 100% DMSO and after 24 hr found that the structure of the material was so altered that a finger could easily be pushed through it. We observed that untanned rabbit skin responded in a manner similar to that described by Kolb for leather. Rabbit skin treated with 100% DMSO showed a loss of strength which could be attributed to a defect in the tertiary structure of the collagen, that means a loss of its cross linkages.

In vitro effects of DMSO on rabbit skin were studied, and changes in the content of neutral salt-soluble, acid-soluble, and insoluble collagen were measured *(6)*. After 24 hr the amount of neutral salt-soluble collagen was reduced, while the amounts of acid-soluble and insoluble collagen were unchanged. The loss of neutral salt-soluble collagen may be attributable to a solvent effect of DMSO.

The action of different DMSO dilutions on insoluble collagen from isolated, air-dried, rat tail tendons was tested by observing changes in resistance to tearing *(6)*. DMSO was effective in this experiment only when used in concentrations greater than 95%. The tails lost their tensile strength, becoming swollen and contracted, and at the same time highly elastic. Swelling occurred in the absence of water, following

dipping into 100% DMSO. These experiments suggested that in addition to a loosening of the cross-linkage between peptide molecules a rearrangement of the peptide chains may have occurred and, if so, would offer an explanation for the contraction and increased elasticity.

Russell and Winkelman *(7)* studied temperatures necessary for thermocontraction of the skin and found the temperature to be higher after *in vitro* treatment with DMSO. The maximum effect was obtained in 75% DMSO. This action was believed to be partially explained by the loss of bound water whereby the peptide chains come nearer to one another, thus facilitating the formation of secondary cross-linkages. Thermal shrinkage increases with dehydration *(8)* and has been observed with aliphatic alcohols and other dehydrating agents *(9)*. The exact mechanism of this phenomenon, however, remains hypothetical.

Elastin is highly elastic and is much more thermostable than collagen *(10)*. However, under the influence of DMSO, insoluble collagen assumes properties similar to those of elastin. When treated with high concentrations of DMSO, tendons, i.e., insoluble collagen, not only resemble elastin in mechanical properties but also in sensitivity to temperature. These changes, following DMSO treatment, could be the result of only one effect on the collagen, i.e., the result of a rearrangement of the fiber structure.

The thermal contraction of rat-tail tendons is influenced by a mucoproteinase *(11)*. This enzyme removes mucopolysaccharides from the fibers. Hyaluronic acids, the presence of atmospheric oxygen and a reducing agent, i.e., ascorbic acid or hydroquinone, are depolymerized. *In vitro* this depolymerization can be completely inhibited by DMSO in concentrations as low as 1% *(12)*, however, lower concentrations were not investigated. This observation suggests that DMSO treatment stabilizes mucopolysaccharides *in vitro* and might be related to the increased shrinkage temperature of collagen after DMSO treatment.

III. EFFECT OF DMSO *IN VIVO*

A. Effect on Extracellular Components of Connective Tissue

The above mentioned *in vitro* experiments were performed with relatively high concentrations of DMSO. Lower concentrations were

not active. Similar concentrations obviously would not result *in vivo* from topical treatment. Therefore, effects obtained after *in vivo* treatment have inevitably another mechanism of action. DMSO was applied topically to the backs of rats, and tail tendons were subsequently examined for swelling in a citrate–phosphate buffer of pH 2.2 *(13)*. Water was taken up much more quickly by the tendons of DMSO-treated animals than by those of untreated controls. Furthermore, within 30 min after the buffer treatment gelatinization, loss of tensile strength, increased elasticity, and contraction of the tails occurred as the consequence of the loss of cross-linkages. This same phenomenon was observed in the control animals, but only after 2 hr. The effect on tail tendons was observed even though the DMSO treatment was localized far away on the skin of the back.

Results of experiments in which tail tendons from treated and control rats were incubated with trypsin at pH 7.6 were not as clearly defined *(13)*. Rats were treated topically with 0.25 g DMSO for 10 days. Tail tendons were then incubated with trypsin, and free hydroxyproline was subsequently measured. Tendons from treated animals produced less hydroxyproline than those from untreated controls. Surprisingly, the hydrolysis of hydroxyproline was enhanced when the DMSO dose was increased to 0.25 g twice daily.

Rabbits treated for 36 days with 5 g 100% DMSO daily failed to show changes in the different fractions of collagen of skin except for a slight increase in the acid-soluble fraction *(6)*. There was, however, an increase in uronic acid, a constituent of the mucopolysaccharides. This change might be the result of inhibition of oxidative reductive depolymerization.

It was surprising that DMSO had no influence on the quantity of collagen in the skin of healthy animals. Scherbel *(14)*, however, has shown that DMSO treatment of scleroderma patients resulted in a loss of histologically altered collagen.

Skin from patients with telocobalt radiation subcutaneous indurations was examined both before and after DMSO treatment. These specimens were compared with those obtained from subjects with healthy skins *(15)*. It was found that all forms of collagen were initially increased in the irradiated area. After local treatment with DMSO for 2–3 months, the amount of neutral salt-soluble collagen had almost completely normalized, while the citrate-soluble and insoluble collagen fractions did return to normal.

Surgical wounds of animals were treated with DMSO and compared with those in untreated controls. A variety of tests, including histopa-

thology of scar collagen, hydroxyproline content of an implanted sponge, wound breaking strength, and so forth, revealed no remarkable effect of DMSO on the newly synthesized collagen and scar repair *(16,17)*.

Collagen synthesis in a cotton wool granuloma, the model of a sterile chronic experimental inflammation, was not significantly influenced over a 20-day period of DMSO therapy *(13)*.

The influence of DMSO on collagen synthesis, however, was demonstrated in a fibroblast culture *(18)* in which a 1% DMSO concentration caused a lag in generation time; however, treatment increased the concentration of hydroxyproline in the cells.

Collagen contains about 12% hydroxyproline, while little if any is present in other proteins. It is synthesized by oxidation of proline, after incorporation of proline into the peptide chain. For this reason, and because it is not reutilized by the body, the excretion of hydroxyproline in urine is considered a measure of collagen breakdown *(19,20)*. Several patients with various diseases were treated with DMSO. A reduction in excreted hydroxyproline *(13)* was found following treatment, thus suggesting an inhibition of collagen breakdown. Exceptions were found in diseases involving fibrosis or sclerosis, such as scleroderma, in which hydroxyproline excretion was increased. While patients with scleroderma initially excreted high levels of hydroxyproline, these values later fell during the course of treatment. The reduction was concurrent with improvement in the condition *(14,21)*. The mechanism of this change has not been elucidated; however, it is possible that DMSO inhibits collagen metabolism as well as its synthesis. A simultaneous effect on collagen synthesis and breakdown has been reported for several hormones and other antiinflammatory agents *(22–25)*.

Although there was a local decrease in the amount of collagen in the area of radiation induration following DMSO therapy, a total increase in urinary hydroxyproline was not observed. Apparently, the loss of collagen observed at these sites following DMSO treatment was not great enough to exceed the reduction of collagen degradation that occurred in healthy tissues. It is, therefore, important to recognize that a local increase in collagen breakdown might not necessarily be reflected in urinary hydroxyproline levels.

Scherbel *(14)* observed histochemically that skin from patients treated with DMSO showed a qualitative increase in mucopolysaccharides. These findings resembled *in vitro* studies previously discussed.

Synthesis of mucopolysaccharides, as measured by the content of uronic acid and amino sugars, in rat cotton wool granulomas was

studied following topical DMSO therapy *(6)*. Granulomas treated with DMSO formed fewer mucopolysaccharides than those from untreated controls. It appears that DMSO decreased the rate of both synthesis and degradation of mucopolysaccharides. According to the circumstances the effect upon synthesis, however, can be less significant, or the healthy rabbit skin would not have shown a mucopolysaccharide increase following treatment with DMSO.

B. Action on Cellular Elements of Connective Tissue

Changes in extracellular elements of connective tissue are usually related to modifications on an intracellular level.

Berliner *et al. (26)* and Stenchever *et al. (18)* investigated the growth of fibroblasts in tissue cultures and found that 1% concentrations of DMSO inhibited cell proliferation. Lower concentrations did not affect the cells.

Mast cells were increased in DMSO-treated tissues, even with small therapeutic dosages *(27–29)*. It is not certain whether the primary response was to protect the cells from degranulation, or whether there was an actual increase of tissue mast cells, since DMSO also protects mast cells from the degranulating action of histamine liberators such as C 48/80 *(29)*.

Willoughby *et al. (30)* have shown that cells other than those found in connective tissue are influenced by DMSO. They reported that capillary permeability was increased and found that India ink particles penetrated vessels more readily than untreated cells. Leukocytes were found to migrate more readily into tissue following DMSO therapy. This observation was supported by the work of Formanek and Kovac *(28)* who reported that DMSO increased the number of leukocytes in the subcutis during production of experimental acute inflammations.

In spite of these effects on the cellular constituents, Formanek and Kovac *(28)* did not find a consistent effect in investigations of the action of DMSO on experimental models of acute inflammations such as dextran, formalin, ovalbumin, and traumatic edema. Only a histologically detectable increase in acid mucopolysaccharides was observed in the necrotic areas.

Few studies have been made on the elastic fibers of connective tissues. Scherbel *(14)* reported that scleroderma patients treated with DMSO showed no remarkable histological change in elastic fibers.

IV. DISCUSSION

Rabbit skin and rat tail tendons treated *in vitro* with DMSO showed changes with suggested alterations in collagen cross-linkages and some disruption of tertiary structure. When DMSO concentrations exceeded 95%, the collagen fibers became elastic, contracted, and demonstrated gelatinization. The changes, however, failed to alter solubility of the treated collagen fibers.

DMSO treatment of healthy skin, cotton wool granulomas, surgical scars, or skin from patients with other diseases which do not show pathological fibrous proliferation failed to produce significant changes in collagen fractions. It was found, however, that hydroxyproline levels were diminished in the urine of patients treated with DMSO, suggesting that collagen metabolism had been inhibited by DMSO.

Patients with diseases involving fibrous proliferation, such as scleroderma, excrete elevated quantities of hydroxyproline following DMSO therapy. Collagen breakdown subsequently diminishes, even with continued DMSO therapy, and hydroxyproline urine levels return to normal. The increase in urine hydroxyproline occurs only when the rate of pathological collagen breakdown exceeds the rate at which normal collagen metabolism is inhibited by DMSO. Many questions arise as to the mechanism of this action: (1) Is pathological collagen acted upon by normal cellular constituents, such as metabolic enzymes, which are directly influenced by DMSO? (2) Is the DMSO effect one of a nonspecific solvent toward a somewhat altered collagen molecule? (3) To what extent do other factors affect this phenomenon, i.e., mast cell increase, facilitation of leukocyte migration from capillaries, and general increase in capillary permeability?

It appears that DMSO increases the stability of mucopolysaccharides to oxidative reductive depolymerization. Both *in vitro* and *in vivo* studies showed elevated mucopolysaccharide content of treated skin. However, in animals with cotton wool granulomas DMSO treatment appeared to inhibit mucopolysaccharide synthesis. This difference might be attributable to a simultaneous inhibition of both synthesis and a breakdown of mucopolysaccharides.

We believe that the actual effect of DMSO is not only an action on the cellular elements of connective tissue, but may to a large extent be the result of an influence on the metabolism of extracellular constituents such as fibers and ground substance.

V. THE INFLUENCE OF DMSO ON CONNECTIVE TISSUE–SUMMARY

I. Collagen	
A. Content of neutral salt-soluble collagen	
1. Skin of rabbit treated *in vitro* with DMSO	Reduced
2. Skin of rabbit after percutaneous treatment *in vivo* with DMSO	No effect
3. Skin of patients with radiation indurations after percutaneous treatment with DMSO *in vivo*	Reduced
B. Content of acid-soluble collagen	
1. Skin of rabbit treated with DMSO *in vitro*	No effect
2. Skin of rabbit after percutaneous treatment with DMSO *in vivo*	Increased
3. Skin of patients with radiation indurations after percutaneous treatment with DMSO *in vivo*	Reduced
C. Content of mature insoluble collagen	
1. Skin of rabbit after treatment with DMSO *in vitro*	No effect
2. Skin of rabbit after percutaneous treatment with DMSO *in vivo*	No effect
3. Skin of patients with radiation indurations after percutaneous treatment with DMSO *in vivo*	Reduced
4. Cotton wool granulomas after percutaneous treatment of rats with DMSO *in vivo,* as compared to granulomas of untreated control animals	No effect
5. Resistance of scars to tearing, as indication of collagen synthesis after DMSO treatment *in vivo*	No effect
6. Amount of collagen histologically detectable in scleroderma cases after percutaneous treatment with DMSO	Reduced
D. Hydroxyproline elimination	
1. Normal subjects and patients without sclerosis and fibrosis undergoing percutaneous treatment with DMSO	Reduced
2. Patients with radiation indurations of the skin undergoing percutaneous treatment with DMSO	Reduced
3. Patients with scleroderma undergoing percutaneous DMSO treatment	
a. Initially	Increased
b. With further treatment	No effect

E. Qualitative changes in mature structure collagen
  1. a. Strength of skin after use of 100% DMSO *in vitro* — Reduced
     b. Strength of rat tail tendons after use of DMSO (95% and higher) *in vitro* — Reduced
  2. Stability of skin collagens to thermocontraction after administration of DMSO *in vitro* — Increased
  3. Stability of rat tail tendons to swelling in acid medium after percutaneous administration of DMSO *in vivo* — Reduced
  4. Stability to proteolytic enzymes (trypsin)
     a. After percutaneous administration of DMSO *in vivo* in small daily amounts — Increased
     b. After percutaneous administration of DMSO *in vivo* with higher daily amounts — Reduced

II. Behavior of the mucopolysaccharides of the ground substance
  A. Stability of hyaluronic acids to oxidizing, reducing depolymerization — Increased
  B. Uronic acids content in rabbit skin after percutaneous administration of DMSO *in vivo* — Increased
  C. Quantity of histologically detectable mucopolysaccharides in skin of scleroderma patients after percutaneous administration of DMSO *in vivo*
     1. Initially — Increased
     2. Later — No effect
  D. Quantity of uronic acids and amino sugar in cotton wool granulomas after percutaneous treatment of rats with DMSO *in vivo,* as compared with granulomas of untreated control animals. — Reduced

III. Behavior of cellular elements of connective tissue
  A. *In vitro* influence of DMSO on the proliferation of fibroblasts in cultures
     1. In low to medium dosages — No effect
     2. In high doses — Reduced
  B. Mast cell content of tissue after percutaneous administration of DMSO *in vivo* — Increased
  C. Leukocyte migration into tissue after percutaneous administration of DMSO *in vivo* — Increased

IV. Vascular permeability of capillaries to India ink after

percutaneous administration of DMSO *in vivo*	Increased
V. Histological changes in elastic fibers of skin from scleroderma patients after percutaneous treatment with DMSO *in vivo*	No effect

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Chapter 13

DMSO in Dermatology

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I. INTRODUCTION

The chief limitations to topical therapy are the skin's protective properties, which prevent the passage of substances from the external environment into the entire thickness of the skin. The development or discovery of an innocuous vehicle that could traverse the skin's barrier zone and deposit known available chemotherapeutic or prophylactic agents within a dermatological lesion would make possible the treatment of numerous dermatoses without recourse to systemic therapy and

its inherent risks. The wide spectrum of biological activities *(1,2)* attributed to dimethyl sulfoxide (DMSO) encouraged various dermatological investigations to ascertain whether or not this organic substance possessed remedial properties in itself and whether or not it could serve as a vehicle for the transport of currently available dermatological agents into the skin. DMSO possesses two pharmacological features that are advantageous for dermatological use: (1) apparent lack of permanent histotoxicity, and, (2) an ability to enhance percutaneous penetration. Moreover, DMSO causes few or no serious side effects, the only untoward manifestations of the drug following topical application being the oyster- or garliclike taste on the breath and an initial local irritation of the skin. Skin irritations can include erythema, urticaria, a burning sensation, and chapping from repeated use for long periods of time.

This chapter devotes itself primarily to some of the many investigations that have been carried out to ascertain whether or not DMSO might have potential therapeutic value in clinical dermatology.

II. EFFECTS OF TOPICAL APPLICATION

In practically every clinical study on the topical application of DMSO, its affect on skin was most significant at concentrations from 70 to 90%. Sulzberger *et al. (3)* observed that a single drop of 100% DMSO applied to the skin of the backs of human volunteers produced within 5–15 min many tiny follicular papules surrounded by an erythematous halo. By 20–30 min the papules coalesced to form a single large wheal which persisted for about 1 hour. There were also differences in the response of different individuals to the topical application of DMSO. In some persons concentrations as low as 20% caused whealing and erythema, while in others no whealing or marked erythema was produced even with 90% concentrations.

Scherbel and co-workers *(4)* found that the neck, face, and axillae were more sensitive to topical application of DMSO than the back or the extremities. They noted that cutaneous reactions usually subsided in the majority of their patients after 7–10 days, at which time the concentration of DMSO could be increased even to full strength without recurrence of irritant effects.

Zuckner *et al. (5)* stated that an aqueous solution of 90% DMSO "always" caused a burning sensation of the skin which persisted for

10–30 min. This reaction was usually accompanied by "a rash, peeling, scaling, pruritus, redness, and occasionally vesiculation of the skin."

Following topical application of 90% DMSO to 500 patients, Steinberg *(6)* observed almost immediate erythema which was particularly intense in fair-skinned individuals. The erythema, usually transitory, disappeared within 1 hr. Subsequent to the erythema, the patients complained of a burning sensation at the treated site. This was also temporary and did not persist for more than 1/2 hr. Pruritus was also a frequent symptom, especially when the administered dose of topically applied DMSO had dried on the skin. With continued use of the drug, mild scaling of the skin at the site of application developed in many of the patients; this occurred, however, only upon long-term application.

Arno and co-workers *(7)* reported that burning was the predominant local side reaction in 60% of their patients with episiotomy scars topically treated with DMSO. The patients also complained of local itching and scaling of the treated sites, which was no different from that observed in the nontreated control patients.

In more than 1300 patients studied by Goldman *et al. (8),* using topical DMSO for the treatment of a variety of dermatoses, local irritation was considered to be of relatively minor importance. Ayres and Mihan *(9),* in their series of 174 patients, also felt that local irritation of the skin did not constitute a significant problem.

Kligman *(10)* pointed out that the initial local irritation produced by higher concentrations of DMSO diminished with continued application. In his study, using 21 volunteers, 90% DMSO was applied to the entire trunk from the chin to the pelvic girdle once daily for 26 weeks. During this 6-month period, one-fourth of the volunteers experienced transient erythema during the first 2 weeks of topical application, and three-fourths of all the subjects experienced a transient burning or stinging sensation. After 2 or 3 weeks two of the volunteers developed a mild, scaly, diffuse erythematous dermatitis which disappeared upon continued application.

The primary irritant or allergic contact like effects on skin exposed to topical DMSO were shown to be the manifestation of DMSO's histamine-liberating property *(11).* Biopsies of human skin exposed to 90% aqueous DMSO for 1 hr exhibited mast cell degranulation in a manner similar to that seen in skin exposed to 48/80 (a condensation product of *p*-methoxyphenethylmethylamine with formaldehyde, a known histamine liberator). The irritant potency of DMSO was determined and compared with several related organic solvents. The

ID_{50} (the dose that irritates 50% of those exposed) for DMSO was 38% as compared to 54% for dimethylacetamide and 45% for ethylene glycol monomethyl ether *(10)*.

In brief, the topical application of aqueous solutions of DMSO in concentrations of 70% and above produces a local erythematous reaction which may or may not include urticaria. Burning and pruritus are frequent features. Once the skin becomes hardened to this immediate irritant effect, superficial scaling and dryness of the site may result. In practically all of the studies employing topical application of DMSO in concentrations between 70 and 90%, the skin reactions were reversible upon withdrawal of the drug. Upon long-term, repeated applications of the drug, the reactions either disappeared or became less intense.

III. INTRACUTANEOUS EFFECTS

Intradermal injections of DMSO in concentrations as low as 0.01% regularly produced the histamine-mediated wheal and flare reaction. With concentrations above 50%, inflammatory papules were noted at 24 hr and in some instances the lesions were decidedly painful *(3,10)*.

Scratch tests with concentrations of DMSO from 15 to 90% showed little difference in the degree of healing produced by lower concentrations from that produced by higher ones. The wheal and flare reaction, however, was somewhat less at lower concentrations. In neither the scratch test nor the intracutaneous injection tests with DMSO was follicular whealing noted, and the response was specific only to topical application *(3)*.

IV. EFFECTS WITH OCCLUSION

Human skin subjected under short-term occlusive exposure to 90% DMSO for 1 hr invariably developed erythema and urticaria. In some cases a papulovesicular reaction was produced.

Daily applications of 90% DMSO under occlusive dressing to the skin of the back for 35 days caused the skin eventually to become "hardened." The erythematous urticarial dermatitis usually present in 24 hr increased in severity in about 12–15 days, but gradually regressed

thereafter until by the 35th day the skin became essentially refractory. The clinical appearance of the skin, however, was not quite normal, and it showed a pigmentary change and slight superficial desquamation *(10)*.

In summary, under occlusive wrap the irritant effects of DMSO are accentuated. Erythema and urticaria become a constant feature and in some instances an erythematous papulovesicular response occurs. Even this form of injury to the skin is reversible, leaving no permanent damage.

V. HISTOLOGY

Topical application of aqueous 80% DMSO to human skin for 1 hr caused epidermal changes *(3,11,12)*. Some of the upper malphigian cells exhibited a "washed-out appearance," with a single large vacuole and the nucleus displaced eccentrically against the cell wall (see Fig. 1). In the midepidermis, disorder and alteration of the prickle cells also resulted. In some places the horny layer showed an increase in thickness and a separation of the cells by almost blisterlike spaces, which gave the stratum corneum the appearance of lace or network. This alteration could simply be the result of a washing out of certain materials from the horny layer and their replacement by water, especially when one considers that the feel and histological appearance of the skin resemble that of skin subjected to prolonged exposure to water, or skin that has remained under occlusive dressings or wet compresses for a period of time *(3)*.

Skog and Wahlberg *(12)* demonstrated macroscopic and microscopic changes in human skin exposed in different ways and for various periods of time to different concentrations of DMSO. Aqueous solutions of 25, 50, and 80% DMSO were applied in limited amounts either as occlusive patch tests, or in excess as cup tests. Exposure times varied from 1, 2, and 6–24 hr. Four-millimeter punch biopsy specimens were taken from the treated skin sites and histologically examined using hematoxylin–eosin, van Gieson, and Wiegert's stains. The results showed that histological changes were mildest at skin sites treated with 25% DMSO for 1 or 2 hours. Slight edema occurred in both the epidermis and dermis, as well as a dermal perivascular cell infiltrate of mononuclear cells and occasionally an admixture of eosinophils. These

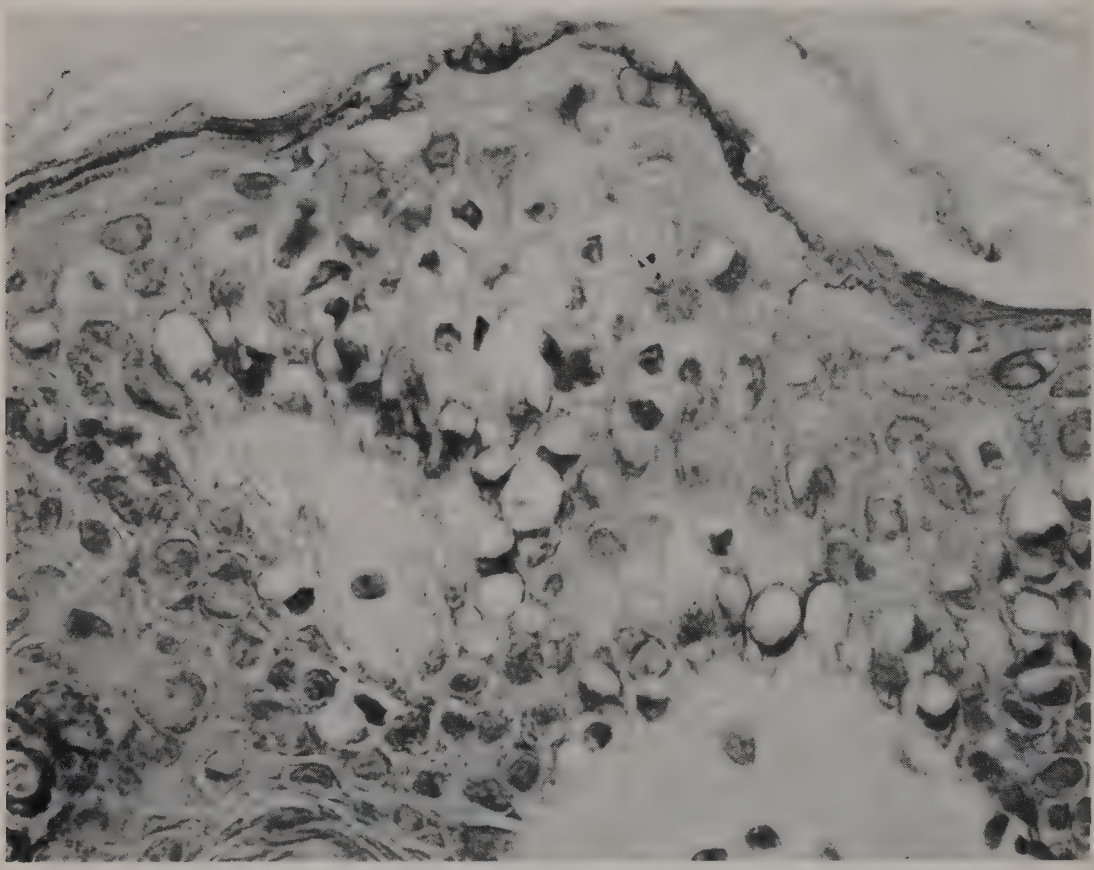

Fig. 1. Vacuolated epidermal cells with displaced nuclei and lacy appearance of the stratum corneum following topical application of 80% DMSO for 40 min. (Hematoxylin–eosin, ×470.)

changes were somewhat accentuated at the sites exposed to either 25% DMSO for 6 hours or to 50% DMSO for 1 hour. The effect on the epidermis was most striking, however, with patch tests of 25% DMSO for 24 hr, 6 and 24 hr with 50% DMSO, and at all the exposure times with 80% DMSO. Staining ability of the epidermis became significantly reduced. Cellular edema was prominent within the epidermis and was occasionally localized in areas of the basal cell layer. In some places cellular rupture, with the formation of microvesicles, had occurred. The latter change was particularly evident at 80% DMSO patch test sites, as well as at cup test sites. After 24-hour exposure the entire epidermis revealed necrosis and separation from the dermis. These histological changes may account in part for the effect DMSO has in enhancing percutaneous penetration with increasing concentrations.

The dermal changes varied only in degree, depending upon the concentration of DMSO and the total exposure time. A mild cellular

infiltrate, mostly of mononuclear cells, was seen perivascularly and, in some instances, about the appendages. At times a conspicuously large number of eosinophils were present.

Biopsy of skin test sites 24 hr after intracutaneous injection with 50% DMSO showed little epidermal change. The dermis revealed edema, intense lymphocytic infiltration, variable admixture of eosinophils and polymorphonuclear leukocytes, and vasodilation *(19)*.

Epidermal regeneration time of skin treated with 90% and higher DMSO concentration under short-term occlusive wrap was studied by Kligman *(10)*. By 48 hours the epidermal cells were regenerating and traces of a new granular cell layer were present. By 96 hours epidermal regeneration was complete, leaving the epidermis mildly acanthotic. When the skin was exposed to a concentration of 90% DMSO continuously for 14 days, the epidermis not only showed moderate thickening but parakeratotic scaling. The dermal reaction was minimal, consisting of vasodilation and a slight lymphocytic infiltrate.

The recent studies of Montes and co-workers *(13)* on the ultrastructural changes of the horny layer following local application of 90% DMSO to guinea pig skin may bear a significant relationship to those effects seen in the human epidermis. They noted under the electron microscope that the normal pattern of the horny layer underwent striking changes within 4 hours after a single topical application of DMSO. The basal portion of the horny layer showed cellular enlargement and was frequently separated from the underlying granular cell layer. The intermediate portion of the horny layer was less altered than either the superficial or basal portions and revealed greatly enlarged and distended empty spaces containing a granular fibrillar material.

The cytoplasmic content of the various cells in the superficial horny layer assumed a homogenous amorphous appearance and as time progressed became almost electron transparent. The fibers either disappeared (uppermost cells) or became clumped, producing a "homogenization" of their entire cytoplasmic content (lower cells). The plasma membranes, as well as the membrane coating granules remained fairly intact.

VI. PERCUTANEOUS ABSORPTION

In some instances DMSO was more effective than applications of water in producing increased permeability of the skin *(14)*. The manner in which DMSO penetrates the skin is still not clear. Yet, the ability of

DMSO to overcome the skin's barrier zone to gain entrance into the cutis and the bloodstream is borne out by the fact that the breath becomes malodorous and a garliclike taste is produced.

Studies on the topical application of radioactive DMSO-^{35}S to animal and human skin proved that DMSO does in fact penetrate the skin and appear in the blood. Kolb *et al. (15)* reported that in man the radioactivity could be detected in the bloodstream as early as 5 min after cutaneous application. The maximal plateau was reached between the fourth and sixth hours and remained almost unchanged for 1½–3 days.

Whole-body radioautograms of rats 1, 4, and 24 hr after cutaneous application of ^{35}S-labeled 90% DMSO revealed intense contamination of all the bones as early as 1 hour. There was no accumulation of radioactivity in the brain, spinal cord, vertebral discs, fatty tissues, or the adrenals of these animals 24 hr after topical application.

Elimination studies of DMSO-^{35}S in the urine of rats, rabbits, dogs, and man showed that better than 50% of the radioactivity appeared in the urine within 6–8 hr following cutaneous application. In man 10–15% of the radioactivity was eliminated in the urine within 24 hr and about 40% during the first week following daily cutaneous application for 20 days. In one case 50% of the applied dose of DMSO-^{35}S was recovered in the urine by the 10th day.

Experiments on the recovery of radioactivity in human breath following cutaneous application of labeled aqueous 90% DMSO revealed that during the first 6 hours only 1% of the radioactivity was recoverable, and 1–3% by the end of 24 hr. Gas chromatographic analysis of the expired air detected the presence of DMS during the first 6 hr, but not at 24 hr.

Stoughton and Fritsch *(14)* showed by quantitative measurements that DMSO enhanced the percutaneous absorption of the following agents: (1) hexopyrronium bromide (quaternary) and hexopyrronium-^{14}C chloride (tertiary), (2) naphazoline hydrochloride, (3) fluocinolone acetonide, and (4) hydrocortisone-^{14}C. That DMSO indeed enhanced the penetration of these substances *in vivo* was based on its ability to reduce the threshold concentration of these agents in producing their biological effects on the skin. For example, percutaneous penetration of hexopyrronium dissolved in 95% ethyl alcohol was increased about 25-fold in the presence of only 20% DMSO. A similar increase of absorption was noted with naphazoline in 50% DMSO under occlusion. With 10 and 25% DMSO a fivefold enhancement was shown for

fluocinolone acetonide. Although the data is useful, Kligman *(11)* pointed out that the measurements did not specifically indicate rates of penetration. He stated that a factor increase of 25 did not necessarily mean 25 times as much absorption per unit of time. Therapeutic efficacy of agents carried by DMSO might be dependent upon their penetration rate.

Various dyes, antiperspirants, testosterone, procaine hydrochloride, lidocaine hydrochloride, and triamcinolone acetonide in dilutions of DMSO (70% and above) have been shown to penetrate the horny layer of human skin rapidly *(3,11)*. Visual tracers, for example, such as methylene blue or iodine in 90% aqueous DMSO were topically applied to the skin of the backs of human volunteers. Microscopic examination of frozen sections of skin biopsies taken 20–45 min after dye application revealed staining throughout the stratum corneum, but none could be seen in the stratum malpighii. Perhaps the concentration was too low to be visible. Even after washing with 70% isopropyl alcohol, these dyes could not be removed from the horny layer *(3)*.

Methylene blue was also found to penetrate through the stratum corneum when applied under occlusive wrap for 1 hr to skin pretreated with 90% DMSO *(3,11)*. Following application of 1% aqueous methylene blue solution under occlusion for 2 hours to DMSO-pretreated skin test sites, the dye had penetrated to the glistening layer but none was seen in the layers below. The study suggested that although DMSO does not produce irreversible change in the horny layer's permeability, it can initially overcome the barrier zone to a sufficient degree to allow the percutaneous penetration of materials subsequently applied without actually serving as a carrier *(3,11)*.

Maibach and Feldman *(16)* showed that DMSO used as a vehicle for steroids increased the percutaneous penetration of hydrocortisone and testosterone in man about threefold. Stoughton *(17)* further demonstrated that hydrocortisone-^{14}C and fluocinolone acetonide, when incorporated in 40% DMSO, established a significant stratum corneum reservoir within a few minutes after topical application. The reservoir of glucocorticoid was not only resistant to removal by surface washing with soap and water and alcohol but persisted for over a 16-day period.

In an attempt to estimate the molecular size of substances that can traverse the skin's barrier zone in the presence of DMSO, the Prausnitz–Kuestner (P-K) technique on human skin was employed. Allergens of small molecular weight, such as penicillin G potassium, when mixed with 90% DMSO readily penetrated the stratum corneum following

topical application. Allergens of higher molecular weight, 3000 and above, such as house dust, dog hair, timothy pollen, and castor bean, did not penetrate the intact skin to give positive P-K reactions. When the stratum corneum was removed by cellophane tape stripping, positive P-K reactions occurred with either low or high molecular weight allergens *(3,18)*. The effects of DMSO on dermal permeability were also studied *(10,19)*. Kligman *(10)* found that the clearance time from the dermis for fluorescein intradermally injected into the skin of the back decreased in the presence of DMSO, the decrease being greater with increasing concentrations of DMSO. The apparent delayed clearance was not ascribable to the erythemogenic and whealing action of DMSO, but to its effect in depressing the movement of water-soluble materials through the dermis.

VII. EFFECT OF DMSO ON VARIOUS EXPERIMENTAL CUTANEOUS REACTIONS

Görög and Kovacs *(20)* have evaluated the influence of topically applied DMSO on experimentally produced lesions of contact dermatitis and allergic eczema in guinea pigs, as well as of experimental skin calcifications in rats. They concluded that these induced lesions were "efficiently inhibited" by the local application of 70% DMSO. They further suggested that since DMSO is an excellent solvent for many of the compounds currently employed in dermatology, further research is necessary to show whether their combination will afford an additive beneficial effect.

VIII. CLINICAL STUDIES ON THE DERMATOLOGICAL EFFECTIVENESS OF DMSO ALONE AND AS A CARRIER AGENT FOR DERMATOTHERAPEUTIC AGENTS

Few clinical reports have, so far, appeared in the literature pertaining to the topical use of DMSO alone or in combination with known chemotherapeutic agents for the treatment of dermatological disorders. Results from these sparse investigations do not allow a definite

conclusion as to the therapeutic effectiveness of DMSO, either alone or as a vehicle for known chemotherapeutic agents currently used in the treatment of skin disorders. The number of patients studied has not only been small, but the studies have often lacked appropriate experimental design, e.g., double-blind, paired comparison, specific criteria for the grading of therapeutic responses, statistical analysis of data, and so forth.

In a series of 174 private patients, Ayres and Mihan *(9)* studied 32 dermatological entities for their response to the topical application of 90% DMSO alone and in combination with 0.25% fluocinolone acetonide. The investigations extended over a period of approximately 1 yr. The dermatoses studied were acne vulgaris, alopecia areata and totalis, chronic benign familial pemphigus, clavus, dermatitis herpetiformis, dermatitis venenata, Dupuytren's contracture, epidermolysis bullosa, granuloma annulare, granuloma "follicular," herpes simplex, herpes zoster, post-herpes zoster neuritis, "knuckle pad," lichen planus, lichen schlerosus et atrophicus, lichen simplex chronicus, lupus erythematosis (discoid), molluscum contagiosum, morphea, necrobiosis lipoidica diabeticorum, necrotizing angiitis, perioral dermatitis, Peyronie's disease, poikiloderma vasculare atrophicans, psoriasis, pustular eruption of the palms and soles (pustular psoriasis), systemic scleroderma, synovial cysts of the skin, tinea pedis, stasis ulcer, verrucus vulgaris and plana.

All of the patients were instructed to apply sparingly the test solution, contained in an unlabeled bottle, with a cotton-tipped applicator to the entire affected area two to three times in succession. The applications were made twice daily unless local discomfort of the skin (such as burning or dryness) developed, in which case the topical applications were reduced to once daily and a steroid cream used as needed.

The results are depicted in Table 1. In nearly all of the cases studied the therapeutic response observed to the topical application of fluocinolone acetonide in DMSO did not occur until several weeks, or even several months, after administration of therapy. No final conclusions were made, since the number of cases followed was not only unusually small, but the periods of observation were often too short. Likewise, the investigations proved difficult to conduct on a double-blind basis. When bilateral paired comparisons were initially made in the few cases of psoriasis and lichen planus and in the one case each of dermatitis herpetiformis, poikiloderma atrophicans vasculare, and chronic benign

TABLE 1

Response of Skin Diseases to the Topical Application of DMSO Alone or in Combination with 0.025% Fluocinolone Acetonide[a]

Dermatosis	Clinical response, number of patients				
	Excellent	Good	Moderate	Poor	Total
Acne vulgaris (cystic)	4	–	–	2	6
Alopecia areata and totalis	1	1	–	2	4
Chronic benign familial pemphigus	1	–	–	–	1
Clavus (corns)[b]	3	2	2	–	7
Dermatitis herpetiformis	1	–	–	–	1
Dermatitis venenata (paint)	1	–	–	–	1
Dupuytren's contracture[b]	–	1	1	3	5
Epidermolysis bullosa	–	–	–	1	1
"Follicular granuloma" (nose)	1	–	–	–	1
Granuloma annulare	3	1	1	–	5
Herpes simplex	1	–	2	1	4
Herpes zoster	22	3	1	1	27
Post-herpes zoster neuritis	6	4	1	–	11
"Knuckle pad"	1	–	–	–	1
Lichen sclerosus et atrophicus	1	–	1	–	2
Lichen planus	3	1	–	2	6
Lichen simplex chronicus	6	1	–	2	9
Lupus erythematosis (discoid)	3	–	1	–	4
Molluscum contagiosum[b]	1	–	–	–	1
Morphea[b]	1	–	–	–	1
Necrobiosis lipoidica diabeticorum	–	–	1	–	1
Necrotizing angiitis (finger)	1	–	–	–	1
Perioral dermatitis	2	–	1	1	4
Peyronie's disease	–	–	–	2	2
Poikiloderma vasculare atrophicans	1	–	–	–	1
Psoriasis	5	4	3	8	20
Pustular psoriasis	–	–	1	2	3
Scleroderma, systemic	–	–	3	–	3
Synovial cyst of skin	–	–	–	1	1
Tinea pedis	1	1	2	6	10
Ulcer, stasis (ankle)	–	–	–	1	1
Verrucus vulgaris	6	–	5	18	29

[a] Ayres and Mihan (*9*).
[b] Treated with DMSO alone.

pemphigus, the procedure was not continued, even though the investigators realized that DMSO in combination with fluocinolone acetonide was "markedly superior" to DMSO alone.

Engel *(21,22)* studied 36 patients with the following dermatoses (the number of cases are indicated in parentheses): psoriasis (18), atopic

eczema (1), lichen simplex (2), keloid (6), scleroderma (5), lichen amyloidosis (1), and keloid acne (4). DMSO alone, in concentrations from 40 to 80%, was applied to the lesions with a cotton-tipped applicator or by direct immersion of the involved area 2 or 3 times daily. All 18 patients with psoriasis demonstrated no improvement or their condition became worse with continued application of DMSO. The author claimed no beneficial effect from the use of DMSO in localized neurodermatitis or lichen simplex chronicus and in hyperkeratosis palmaris et plantaris. Dermatoses that showed only varying degrees of improvement included traumatic and burn keloids, hypertrophic scars, atopic dermatitis, and lichen amyloidosis. The disorder that showed the most benefit from topical DMSO treatment was scleroderma, especially of the phalangeal sites involved.

Scherbel *et al. (4),* in a series of 42 patients with "systemic sclerosis," showed that cutaneous manifestations of the disease were decidedly less intense in some of the patients who received topical DMSO therapy. The concentrations of DMSO used varied from 30 to 100% and the total dose administered topically to the entire body ranged from 60 to 100 daily for periods of 2–3 weeks. In most instances long-term therapy from 1 to 2 years was required to achieve significant cutaneous improvement. Actual softening of the skin and subcutaneous tissue did occur, with the return to near normal of the joint motion and skin pliability.

The major histological change noted was the development of intensive staining of the collagen with Mowry's acid mucopolysaccharide stain. The pretreatment controls, however, contained only sparse amounts of acid mucopolysaccharide, even less than in normal skin. One biopsy at the end of 16 months of treatment revealed a zone of collagen in the upper corium, between the epidermis and the underlying sclerodermatous collagen, which had the appearance of normal wavy collagen and contained normal elastic fibers. It was presumed that this zone of collagen in the DMSO-treated skin was undergoing degradation and return to the mucopolysaccharide phase. The theory was further supported by observation that the average increase of urinary hydroxyproline during the first month of treatment was greater than 50% above the average pretreatment urinary output, usually persisted for 2 or 3 months, and then declined to the pretreatment level. Ziff and associates *(23)* had previously reported that there was no significant difference in excretion of total urinary hydroxyproline in normal adults and in adults with rheumatoid arthritis and collagen diseases, including progressive systemic sclerosis.

Scherbel and co-workers *(4)* reported dramatic improvement of ischemic ulcers of the digits in 11 out of 12 patients with scleroderma

treated with topical DMSO. The ulcers healed within 1–3 weeks in 6 of the patients, and within 3–6 weeks in 3 others. In 3 patients the ulcers did not respond at all to topical treatment. However, when immersion of the hands in DMSO was substituted, the ulcers in 2 of these patients healed within 1 week. The remaining patient discontinued the treatment because of discomfort. In subsequent patients, using the immersion technique with a 50% DMSO solution, none of the ischemic ulcers of the digits were resistant to DMSO therapy. Healing of the ulcers has been attributed to the increased nutrition which apparently resulted upon relief of vascular spasms.

Topical cutaneous application of 90% DMSO to subcutaneous fibrosis lesions, such as those that occasionally result following cobalt therapy, not only caused their regression but also increased the urinary excretion of hydroxyproline *(24)*.

Brechner and co-workers *(25)* demonstrated that when concentrations of tetracaine base from 5 to 33% were prepared in solutions of 100% DMSO and applied topically under occlusive wrap for 30 min to intact human skin, intense anesthesia of the site resulted. With DMSO alone, surface anesthesia of the skin was not produced. Kligman *(11)* stated that DMSO "can scarcely be considered to have potent analgesic effects" on the skin.

In a preliminary study Yeats *(26)* reported on the therapeutic effectiveness of antileprosy drugs contained in DMSO for the topical treatment of the skin lesions of leprosy. Each of three groups of eight patients with tuberculoid changes of the skin and other associated lesions was treated with either dapsone, *p*-aminosalicylic acid, or isoniazid in 70% DMSO as a skin wash. All of the patients were given dapsone orally. The majority of patients showed rapid improvement of their skin lesions, with diminution in size. Because there was no control study with DMSO alone, the authors considered that the improvement was probably attributable to the DMSO rather than to the drugs themselves.

The use of thiabendazole in DMSO for the topical treatment of creeping eruption was evaluated in a double-blind study by Katz and Hood *(27)*. Lesions of varying severity in 25 patients were treated with the topical application of 2% thiabendazole in 90% DMSO or with 90% DMSO alone (10–40 drops being applied to each treatment area twice daily). By the end of the second week of therapy, all the areas treated with thiabendazole in DMSO had been cleared of at least 50% of the lesions. By the end of 2 weeks, 74% of the treated sites were completely

clear of lesions. In contrast, only 31% of the treated areas had cleared using DMSO alone. By the third week of treatment, 89% of the sites treated with thiabendazole in DMSO were completely free of lesions, in contrast to only 44% of the areas treated with DMSO alone. The response of this dermatosis to topical treatment with thiabendazole in DMSO paralleled the results for oral therapy.

Goldman *et al. (8)* reported that DMSO used as a vehicle for 5-fluorouracil (5-FU) increased its effectiveness as a cytotoxic agent for the topical treatment of keratoacanthoma, superficial basal cell carcinoma, and Bowen's disease. Significant results were not observed in a small series of patients treated topically for warts with 1–5% podophyllin in DMSO, for early lesions of severe herpes simplex with 5-iodo-2′-deoxyuridine (5-IDU) in DMSO, for warts and molluscum contagiosum using 1% *N*-methylisatin β-thiosemicarbazone in DMSO, for tinea pedis and capitis using 3% microcrystalline griseofulvin in 90% DMSO, or for psoriasis or alopecia areata with DMSO alone. MacCallum and Juel-Jensen *(28)* conducted a double-blind controlled study on 16 patients with herpes simplex infection of the face using 5% idoxuridine with and without DMSO. The average 9-day duration of the lesions was reduced by 6 days with idoxuridine in DMSO, by 3 days without DMSO, and by 4 days with DMSO alone.

Smith *et al. (29)* reported a most interesting case involving a 7 year-old negro male whose nourishment was maintained by the percutaneous absorption of nutrients dissolved in DMSO. The patient had showed progressive deterioration and difficulty in maintaining adequate nutrition because of complications following surgery for correction of a midgut volvulus and gangrene of the small bowel. Five months after surgery, recourse to intravenous feedings was necessary. Owing to progressive difficulties in finding veins, the authors decided to use DMSO in an attempt to supply essential nutrients through the skin. Electrolytes, carbohydrate, fat, and protein supplements as well as vitamins were dissolved in equal parts of 100% DMSO and topically applied to the skin daily. At the onset of therapy the patient weighed 32¾ pounds (15 kg). After 13 days of topical administration per os along with a regular diet, his weight increased 2¼ pounds (1 kg). After 24 days his total weight had increased to 38½ pounds. The authors stated that with the increase in blood glucose levels, increase in urinary amino acids, and weight gain, there was little doubt that the nutrients had crossed the skin barrier in usable forms. Although the child died of acute bronchopneumona, they commented that without the topical

administration of the supplemental feedings in DMSO the patient would have died sooner (although it cannot be proved).

IX. COMMENT

It seems likely that DMSO in itself does not possess remedial properties for most dermatological disorders. In contrast, prospects for the use of DMSO as a vehicle for the transport of dermatological medications into and through intact skin, and for their deposit within the skin, remain hopeful. Aside from the transient local irritation of the skin without apparent irreversible damage, DMSO remains second to water in its ability to enhance percutaneous penetration.

Further research will be required to determine the advantages of DMSO as a vehicle for therapeutic agents in inflammatory dermatoses and in skin diseases such as fungal infections, pyodermas, and acnes, and particularly its usefulness to form a depot in the horny layer for prophylactic agents such as sun screens, insect repellents, and antifungal and antibacterial drugs.

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Chapter 14

DMSO Bibliography

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B. Chemistry

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Chemistry Addendum

1. Anonymous, Highlights from Current Literature, *Chem. Ind.* (March 27, 1965).
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