Apparent Lack of Interaction Between Dimethyl Sulfoxide (DMSO) and a Variety of Drugs.* (29961)

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The unusual solubility characteristics of dimethyl sulfoxide (DMSO) have long been recognized. DMSO is one of several dipolar aprotic solvents which share a number of unique solvation properties. These properties result in changes in dissociation of acids and bases and affect rates and mechanisms of reactions. DMSO also forms complexes with many metallic salts(1). These properties have found application in a number of synthetic reactions. DMSO has been used as a biological preservative to prevent freezing damage to living cells(2), and has also been shown to be a radioprotective agent in mice (3). Reports indicate that DMSO has very low toxicity(4). A number of new applications and uses of DMSO have recently been suggested. DMSO has also been reported to alter cellular permeability and affect drug penetration by acting as a "penetrant carrier"(5). Suggestions have been made that DMSO might act as a local analgesic, anti-inflammatory, antibacterial, diuretic, tranquilizer and offers a new and unique form of pharmacological mechanism(5). This is an attractive hypothesis, and has been the focus for much interest(6).

This paper reports the results of preliminary pharmacological tests that indicate that drugs dissolved in DMSO and administered systemically do not differ significantly in their lethality or cellular penetration. Our results also indicate that DMSO is without significant local anesthetic or analgesic action. Our data fail to support the hypothesis that DMSO administered systemically can alter pharmacological action or drug penetration.

Methods. Twenty-one-day LD_{50} values were calculated by the method of Litchfield and Wilcoxon(7) after a single injection of a drug to male Swiss albino mice. Drugs were administered orally and intravenously (i.v.) at a concentration so that a mouse received 0.01 ml of solution per gram of body weight. At least 4 dose levels of each drug (10 animals/dose) were used. Drugs were dissolved in either 1) saline, or 2) DMSO and 3 volumes of saline added. In another study mice were injected with the drug dissolved in saline with and without DMSO (2.5 g/kg) administered consecutively. Acute i.v. and oral LD_{50} values were also determined for DMSO using adult Swiss albino mice.

Local anesthetic activity was tested utilizing spinal and corneal anesthesia tests in adult New Zealand rabbits. Analgetic action was assessed in rats with the Randall-Selitto test(8). Cardiac electrical activity was recorded from mongrel dogs and Rhesus monkeys anesthetized with barbiturate utilizing a Burdick EK-3 electrocardiograph.

Penetration of para-aminohippuric acid carboxyl-C^{14} (PAH) (specific activity of 1.05 mc/mM) to various tissues was investigated. Drug penetration to various tissues and the cerebrospinal fluid (CSF) was studied after continuous i.v. administration. PAH was administered by constant infusion to anesthetized dogs for 4 hours. The drug was either dissolved in saline or DMSO, and brought to final volume with saline. The dose of DMSO was 2.5 g/kg administered over a 4-hour period as about a 5% solution. CSF was sampled at the cisterna magna at 30-minute intervals and small pieces (15-25 mg) of tissues were dissected at the end of each experiment. Radioactivity was determined with a Packard Tri-Carb Model 3002 liquid scintillation counter. PAH was determined in CSF by counting an aliquot of the fluid, and was determined in tissues after a weighed sample was digested in KOH as previously described by Rall *et al*(9).

Hexobarbital sleeping times were determined after acute, concomitant, and chronic

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TABLE I. LD_{50} of Various Drugs When Dissolved in Saline or 25% DMSO.

Compound	LD_{50} in saline, mg/kg	Slope	LD_{50} in 25% DMSO, mg/kg	Slope
Chlorpromazine HCl	47 (39–56)*	1.41	49 (40–60)	1.48
Curare	.140 (.132–.148)	1.09	.170 (.158–.182)	1.12
Insulin	6300 (5680–6690)†	1.23	5800 (5040–6670)†	1.38
Morphine SO_4	203 (185–223)	1.16	195 (176–216)	1.23
Ouabain	2.2 (1.5–3.3)	1.57	2.0 (1.4–2.9)	1.53
Penicillin G	1,250,000 (950,000–1,640,000)†	1.70	1,150,000 (1,050,000–1,270,000)†	1.17
Pentobarbital sod.	91 (71–117)	1.34	98 (75–128)	1.36
BCNU‡	45 (39–52)	1.26	52 (48–56)	1.09
Vincristine	3.6 (3.0–4.2)	1.22	3.5 (3.2–3.7)	1.20
Aspirin (orally)	1300 (1130–1500)	1.17	1250 (1110–1410)	1.15
Penicillin (orally)§	>10,000,000†	—	>10,000,000†	—

* LD_{50} 95% confidence limits in parentheses. † Units/kg. ‡ 1,3-bis(2-chloroethyl)-1-nitrosourea. § Highest practical dosage level in required volume.

DMSO treatment of mice. Hexobarbital 100 mg/kg was administered intraperitoneally (i.p.) and the duration of loss of righting reflex was recorded.

Results and discussion. The 21-day LD_{50} value with confidence limits calculated after a single dose of DMSO administered orally was 18.5 (14.7-23.3) g/kg. The LD_{50} of DMSO after i.v. administration was 8.10 (3.79-11.6) g/kg. These results are in agreement with those previously reported and indicate a low degree of acute toxicity. DMSO was significantly more toxic after i.v. administration. These differences in toxicity might imply a delayed absorption after oral administration which is in contrast to the rapid membrane penetration claimed for the agent.

Doses of various drugs calculated to be lethal to 50% of mice are presented in Table I. It can be seen that DMSO had no significant effect on increasing the lethality of any drug studied (DMSO appears to have decreased the lethality of curare and BCNU). This was true whether the drug was first dissolved in DMSO before injection or administered concomitantly with DMSO. The drugs investigated were selected because they are, with the exception of the antineoplastic agents, commonly employed clinically. If one were to assume that DMSO increased tissue penetration and drug distribution it would follow that in many cases DMSO would cause increased drug level to reach the target organ and perhaps alter toxicity. On the other hand, however, DMSO may cause a more universal distribution of the agent, and actually result in a decreased amount of the drug reaching the site of action.

DMSO did not appear to increase the oral absorption of 2 drugs studied. DMSO had no effect on toxicity of aspirin or benzylpenicillin when these drugs were administered by either route. This would seem to indicate that DMSO did not significantly increase oral absorption of the drugs mentioned. Benzylpenicillin is of interest because of its low toxicity after systematic administration in contrast to lethal convulsions produced after intrathecal administration(10). However, DMSO did not alter lethality of penicillin after oral or i.v. administration. These data do not suggest an increased penetration of penicillin to the central nervous system when administered with DMSO. These experimen-

tal results are not indicative of the presence of a "penetrant carrier."

In an effort to evaluate the local anesthetic action of DMSO, 2 local anesthetic tests were utilized. DMSO in concentrations as high as 50% v/v with saline failed to anesthetize the cornea of albino rabbits after instillation. Also, 0.1 ml of 50% v/v DMSO failed to result in any spinal blockade after intrathecal administration to adult rabbits. Low concentrations of butacaine hydrochloride render the cornea anesthetized which allows one to touch it directly without any reflex responses, and results in paralysis of the hindlimbs of rabbits due to a reversible spinal blockade after intrathecal injection. DMSO was also without significant analgetic effect as determined by the Randall-Selitto paw method. There was no difference in pain threshold, skin temperature or circumference of injected paw between control and animals treated locally or systemically with DMSO. Injection i.v. of 50% DMSO in doses as high as one g/kg failed to alter the electrocardiogram of anesthetized dogs or monkeys observed for 30 minutes after DMSO administration. Agents capable of altering membrane permeability might be expected to alter electrical activity as measured by the EKG.

PAH-C^{14} was dissolved in DMSO and diluted with saline for infusion i.v. to 2 mongrel dogs which were lightly anesthetized with pentobarbital. DMSO was administered over a 4-hour period at a total dose of 2.5 g/kg. Two control dogs received the same dose of PAH-C^{14} dissolved in saline. The experiment was continued for 4 hours during which time the CSF was sampled every 30 minutes *via* the cisterna magna. At the end of the experiments the animals were killed, and small pieces of liver, brain, muscle, and kidney were dissected, blotted dry, weighed, and digested with KOH in a counting vial and radioactivity determined. PAH in tissue or CSF, expressed as per cent of total PAH in plasma during steady state, from experimental animals treated with DMSO, did not differ significantly from control animals (Table II). PAH is representative of a number of drugs which are weak organic acids. These preliminary results seem to indicate that DMSO did not alter the penetration of this polar, highly water soluble molecule to the CSF, brain, liver, muscle, or kidney.

TABLE II. Tissue Concentration of PAH in Control and DMSO Treated Dogs.

	Drug solvent	
	DMSO	Saline
Cerebrospinal fluid	2.1	1.8
Brain cortex	9.1	12.2
Skeletal muscle	17.2	15.4
Liver	58.8	55.1
Kidney	507.0	414.0

Values are means of 2 experiments and represent % of total plasma PAH activity in tissue.

In another attempt to assess penetration of a drug to the central nervous system induction times after phenobarbital were determined. The time in minutes from injection to loss of righting reflex was recorded as induction time. Phenobarbital was administered i.p. to mice at a dose of 300 mg/kg. There was no significant difference between induction time when phenobarbital sodium was dissolved in DMSO and brought to total volume with saline (25% DMSO) or dissolved in saline. These results would seem to indicate that DMSO had little effect on the penetration to the CNS of phenobarbital.

DMSO administered subcutaneously as a 25% solution with saline, 2.5 g/kg, one hour, 24 hours, 7 days, or daily for 7 days prior to hexobarbital did not alter hexobarbital sleeping times. These experiments were intended to investigate any changes which might occur in tissue absorption, distribution or metabolism of the drug due to the presence of DMSO. This rather high dose of DMSO failed to indicate any change in these parameters.

DMSO was tested for its effect on the water-chloroform partition of benzyl-C^{14}-penicillin and PAH. It is generally assumed that membrane penetration correlates well with the lipid solubility of an agent. Drugs dissolved in concentrations of DMSO as high as 2%, higher than the concentration that could be extrapolated to the *in vivo* situation, did not reveal any change in partition between aqueous and lipid phase. The aqueous phase was 0.1 M phosphate buffer, pH 7.4, and the lipid layer was chloroform. An increase in lipoid solubility (chloroform) might be expected if DMSO were to result in these polar

compounds passing through membranes more easily. Our tests in this regard were negative.

DMSO is, however, a very effective solvent for a number of agents which are almost insoluble in polar solvents. For instance, 3,4-benzypyrene and 3-methylcholanthrene were readily solubilized in 100% DMSO. DMSO was also an effective solvent for highly insoluble antineoplastic agents and pesticides.

Our observations with DMSO confirm the fact that DMSO is a very effective solvent with low toxicity and few pharmacological effects(11). These data do not suggest, however, that systemically administered DMSO alters drug penetration. No evidence was seen to indicate an increased oral absorption or increased penetration to the central nervous system in the cases studied. However, it would not be unreasonable to assume that a drug dissolved in 100% DMSO and applied topically might result in some increase in absorption.

Summary. The lethality of a number of drugs dissolved in DMSO or saline has been compared. In no case was there a significant increase in drug lethality when administered with DMSO. Preliminary tests failed to indicate that DMSO possesses local anesthetic or analgetic action. DMSO also failed to alter the CSF or tissue penetration of PAH. DMSO appears to be a very good solvent with little or no effect on pharmacological action.

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Continuous Cell Strains Derived from Human Atheromatous Lesions and their Viral Susceptibility.* (29962)

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This report is concerned with a study of human atheromatous lesions, carried out by growing tissue fragments in culture. From cells which grow out from such fragments, a number of continuous cell lines were established and they have been characterized by determining their susceptibility to a variety of viruses.

Materials and methods. A total of 60 atheromatous lesion specimens obtained during surgical operations for either abdominal or thoracic aneurysms were processed for growth in tissue culture. The specimens were received in sterile petri dishes and were processed within a few hours after surgical removal. For the primary cultivation of all specimens, the following procedure was used (1,2). The specimens were washed 3 times with Eagle's medium and then minced with scissors into pieces ranging from 1-2 mm in size. About 5-10 minced pieces suspended in a small amount of growth medium were squirted with a large-bore pipette onto the flat surface of a 1-oz bottle previously heated to about 45°C. Six 1-oz bottles of explants were made from each specimen. About 3 ml

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