THE EFFECT OF DIMETHYL SULFOXIDE (DMSO) ON ANTIBIOTIC SENSITIVITY OF A GROUP OF MEDICALLY IMPORTANT MICROORGANISMS: PRELIMINARY REPORT

Glenn E. Pottz, James H. Rampey and
Furmandean Benjamin
Department of Microbiology, Greenville General Hospital System, Greenville, S. C.

Early success in the use of dimethyl sulfoxide (DMSO) as a penetrant carrier in our work with acid-fast staining (Pottz, 1964) has suggested that this characteristic might be investigated further. The use of DMSO in the modification of various staining procedures; its effect in the antigen-antibody reaction; and its effect on morphology and growth characteristics of microorganisms is underway in this as well as other laboratories.

The ability of dimethyl sulfoxide to act as a penetrant carrier has been noted by several researchers. Its action as a penetrant carrier with reversible effect on the biological membrane was early observed by Jacob and coworkers (Jacob, 1964*a*). Membranes treated with dimethyl sulfoxide were rendered porous to compounds generally considered to be nondialyzable: penetration of normally dialyzable ions and compounds was shown to be increased (Jacob, 1964*b*). This group of researchers further report its use in a 15 per cent concentration as a penetrant carrier when mixed with heparin, insulin, sulfadiazine and other compounds. The ability of dimethyl sulfoxide to act as a vehicle to aid penetration of antibiotics through the human skin and into various parts of the body has been investigated (Rosenbaum, 1965). Inhancement of penetration through plant and animal membranes has been unequivocally demonstrated (Kligman, 1965*a*).

The role of dimethyl sulfoxide as a bacteriostatic or bactericidal agent has been investigated by several researchers. DMSO has been reported as bacteriostatic against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas* strains in 10 per cent concentration and against the tubercle bacillus in a 5 per cent concentration (Jacob, 1964*a*). The minimal inhibitory concentration (MIC) of dimethyl sulfoxide was determined for isolates of *Staphylococcus aureus, Staphylococcus aureus* variety *albus, beta hemolytic Streptococci, Corynebacterium* species (normal skin residents), *Alcaligenes faecalis, Escherichia coli,* and *Proteus* species and found to be 20 per cent in each case (Kligman, 1965*b*). Bactericidal concentration was found to be 50 per cent for *Staphylococcus aureus* and 30 to 40 per cent for the remainder of these microorganisms by the same workers. Investigations made in this laboratory indicate that bacteriostatic concentrations quoted by Jacob were essentially the same. Bactericidal concentrations required for *Staphylococcus aureus* were 30 per cent; the remainder as reported by Kligman (Rampey, 1965).

Dimethyl sulfoxide has been shown to be mildly antifungal and antibacterial. The effectiveness of dimethyl sulfoxide increases sharply above 70 per cent. It is probable that quite concentrated solutions, perhaps 80 per cent to 90 per cent, will be required if dimethyl sulfoxide is to be clinically useful in the control of pathogenic microorganisms. It is of further interest to note that DMSO has been found to be a stabilization agent in the preservation of the *Bacillus subtilis* phage (Yehle, 1965).

Introduction

The established characteristic of dimethyl sulfoxide 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, as bone marrow, brain, etc., underlines the importance of this question. Also, presence of DMSO in blood, urine, and other specimens might influence routine culture work and later antibiotic susceptibility testing. The added possibility that dimethyl sulfoxide acts as a bacteriostatic or bactericidal agent makes this question all the more interesting. It is the purpose of this preliminary study to investigate the effect of dimethyl sulfoxide in the in vitro susceptibility of a selected group of microorganisms to several of the more commonly used chemotherapeutic agents; to evaluate the bactericidal and bacteriostatic effect of DMSO when combined with the chemotherapeutic agent used; and the possibility of dimethyl sulfoxide neutralizing the penicillinase produced by a group of species of penicillin resistant *Staphylococcus aureus*.

Method of Study

Any study of the effects of dimethyl sulfoxide on the antibiotic susceptibility of a group of organisms requires a prior determination of the effects of the DMSO alone. Previously reported bacteriostatic characteristics of dimethyl sulfoxide (Kligman, 1965*a*, and Jacob, 1964*a*) were checked by standard tube dilution method. Tubes of brain heart infusion broth with concentrations of DMSO from 5 per cent to 80 per cent were inoculated with 18 hour broth cultures of the test organisms; incubated for 24 hours at 37.5°C; examined macroscopically; subcultured to brain heart blood agar plates; reincubated for 24 hours at 37.5°C and examined for evidence of growth. Results of this series of tests are shown in TABLE 1. To further delineate the bacteriostatic and bactericidal effects of dimethyl sulfoxide, a series of cultures of these microorganisms were exposed to varying concentrations of DMSO and colony counts were made. Stock cultures of the test organisms were grown for 24 hours at 37.5°C in trypticase soy broth; inoculated from this broth, a second series of trypticase soy broth were incubated for four 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 5.0 per cent, 10.0 per cent, 15.0 per cent, 20.0 per cent, 25.0 per cent and 30.0 per cent dimethyl sulfoxide and

TABLE 1
BACTERICIDAL AND BACTERIOSTATIC EFFECTS OF DIMETHYL SULFOXIDE (DMSO) ON A SELECTED GROUP OF MICROORGANISMS

Test Microorganism	Per Cent Concentration Dimethyl Sulfoxide Used									
	0	1	4	5	10	20	30	40	50	60
Escherichia coli	++++	++++	++++	++++	++++	+	-	-	-	-
Aerobacter cloacae	++++	++++	++++	++++	++++	+	-	-	-	-
Pseudomonas aeruginosa	++++	++++	++++	++++	++	-	-	-	-	-
Proteus vulgaris	++++	++++	++++	++++	++++	+	-	-	-	-
*Staphylococcus aureus**	++++	++++	++++	++++	++++	++	++	-	-	-
Streptococcus pyogenes†	++++	++++	++++	++++	++++	+	-	-	-	-
Streptococcus faecalis	++++	++++	++++	++++	++++	++	++	+	-	-
Salmonella schottmulleri	++++	++++	++++	++++	++	++	-	-	-	-
Diplococcus pneumoniae	++++	++++	++	-	-	-	-	-	-	-
Candida albicans‡	++++	++++	++++	++++	+	+	-	-	-	-

*Coagulase positive strains
†*Beta hemolytic Streptococci* group A
‡Isenberg V1 strain
++++Indicates positive growth in broth cultures and on blood agar subcultures
-Indicates lack of growth in broth cultures and on blood agar subcultures

incubated for 24 hours. 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 hours at 37.5°C, each plate was examined and colonies present were counted and recorded. Results of these colony count determinations are shown in TABLE 2.

The possibility of reaction between dimethyl sulfoxide and penicillinase elaborated by the penicillin-resistant *Staphylococcus aureus* strains was investigated by exposing sixteen strains of resistant organisms to 10 units of penicillin alone to establish resistance. A second series of broth cultures of the resistant *Staphylococci* were exposed to concentrations of DMSO varying from 0.5 per cent to 10.0 per cent to establish the nonbacteriostatic effect at these concentrations. A combination of 0.5 per cent to 10.0 per cent DMSO and penicillin, 1 unit to 40 units, was added to 18-hour broth cultures of the test organisms and then incubated for 24 hours at 37.5°C. A parallel series of broth cultures containing a concentration of 40 units of penicillinase per ml was included. Subcultures to brain heart blood agar plates were made, incubated at 37.5°C for 24 hours, and examined for growth or absence of growth to determine the possibility of reversal of resistance to penicillin of any of these

TABLE 2
BACTERIOSTATIC AND BACTERICIDAL EFFECT OF DIMETHYL SULFOXIDE BY PLATE COUNT METHOD

Test Micro-organism	Plate Count* on Exposure to Varying Concentration of DMSO							
	Control	5%	10%	15%	20%	25%	30%	40%
Pseudomonas aeruginosa	640	640+	380	1	0	0	0	0
Salmonella paratyphoid	1070	1070+	1070+	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
Canadida albicans	4	4	3	0	0	0	0	0
Streptococcus anginosus F	86	2700	2700	800	70	50	0	0
Streptococcus faecalis	7500	7500	7500	850	240	5	4	0
Escherichia coli	37550	37500	37500	9000	90	0	0	0

*All counts in millions as determined by serial dilution plate count method.

TABLE 3
EFFECT OF PENICILLIN AND DIMETHYL SULFOXIDE ON A GROUP OF PENICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Staphylococcus Strain*	Coagulase Test	Test Substance Used						
		Penicillin 10 Units	Penicillin 40 Units	DMSO 0.5%	DMSO 2.5%	DMSO 5.0%	DMSO 10.0%	Penicillinase 40 Units
DA-1329-5	+	+	+	+	+	+	+	+
DA-22-6	+	+	+	+	+	+	+	+
DA-599-6	+	+	+	+	+	+	+	+
DA-131-4	+	+	+	+	+	+	+	+
DA-60-5	+	+	+	+	+	+	+	+
131-R-25-1	+	+	+	+	+	+	+	+
DA-823-6	+	+	+	+	+	+	+	+
DA-822-6	+	+	+	+	+	+	+	+
DA-18-6	+	+	+	+	+	+	+	+
DA-14-6	+	+	+	+	+	+	+	+
DA-13-6	+	+	+	+	+	+	+	+
DA-12-6	+	+	+	+	+	+	+	+
DA-11-6	+	+	+	+	+	+	+	+
DA-10-6	+	+	+	+	+	+	+	+
DA-542-6	+	+	+	+	+	+	+	+
502a(Yw)	+	+	+	+	+	+	+	+

*All these strains of coagulase positive and penicillin-resistant *Staphylococcus aureus* were furnished for this study by Jay O. Cohen, Bacteriology Section, Communicable Disease Center, Atlanta, Ga.

TABLE 4
COMBINED DIMETHYL SULFOXIDE AND PENICILLIN ON
SUSCEPTIBILITY OF PROVEN PENICILLIN-RESISTANT *STAPHYLOCOCCI*

Staphylococcus Strain	Concentration of Penicillin and Dimethyl Sulfoxide Used						
	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%
Da-1329-5	++++	-	-	-	-	-	-
Da-22-6	++++	++++	++++	++++	++++	++++	++++
DA-599-6	++++	++++	++++	++++	++++	++++	++++
DA-131-4	++++	++++	++++	++++	++++	++++	++
DA-60-5	++++	++++	++++	++++	++++	++++	++++
131-R-25-1	++++	++++	++++	++++	++++	++++	++++
DA-823-6	++++	++++	++++	++++	++++	++++	++++
DA-822-6	++++	++++	++++	++++	++++	++++	++++
DA-18-6	++++	++++	++++	++++	++++	++++	++++
DA-14-6	++++	++++	++++	++++	++++	++++	++++
DA-13-6	++++	++++	++++	++++	++++	++++	++++
DA-12-6	++++	++++	++++	++++	++++	++++	++++
DA-11-6	++++	++++	++++	++++	++++	++++	++++
DA-10-6	++++	++++	++++	++++	++++	++++	++++
DA-542-6	++++	++	-	-	-	-	-
502a(Yw)	++++	++++	++++	++++	++++	++++	++++

++++ Indicates amount of growth.
- Indicated the absence of growth in broth and on blood agar subculture.

TABLE 5
NEUTRALIZATION OF PENICILLINASE BY DIMETHYL SULFOXIDE

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
GGH-137-5	-	+	+	+	+	+
GGH-210-5	-	+	+	+	+	+
GGH-212-5	-	+	+	+	+	+
GGH-019-6	-	+	+	+	+	+
GGH-119-6	-	+	+	+	+	+
GGH-152-6	-	+	+	+	+	+

+ Indicates good growth of test organism.
- Indicates no growth in broth or blood agar subcultures.

strains of *Staphylococci*. To determine the possibility of DMSO neutralizing penicillinase, a combination of 40 units of penicillinase and 5.0 per cent DMSO was added to broth cultures of penicillin-sensitive *Staphylococci*. After two hours of incubation at 37.5°C, penicillin in a final concentration of from 10 to 40 units was added and incubation was continued for 24 hours. Subcultures to brain heart blood agar plates were made and incubated at 37.5°C for 24 hours, then examined for presence or absence of growth of these penicillin-sensitive *Staphylococci*. Results of these three series of tests are found in TABLES 3, 4, and 5.

Utilizing the same methods outlined above, the combined effect of DMSO and erythromycin, DMSO and novobiocin, DMSO and streptomycin, DMSO and chloromycetin, and DMSO and dimethylchlorotetracycline was tested against these sixteen strains of penicillin-resistant *Staphylococcus aureus*.

The effect of dimethyl sulfoxide on the sensitivity of *Proteus volgaris, Aerobacter cloacae, Pseudomonas aeruginosa, Diplococcus pneumoniae, Streptococcus faecalis, Escherichia coli, beta hemolytic streptococcus,* and *Salmonella paratyphi* against penicillin, chloromycetin, tetracycline, novobiocin, erythromycin and streptomycin was tested. Concentration of dimethyl sulfoxide was varied from 0.5 per cent to 10.0 per cent. Concentration of the chemotherapeutic agents used was at the high and low concentration generally used in sensitivity testing. Twelve-hour broth cultures of the test organisms were used. To each broth culture, DMSO was added to a final concentration of 0.5 per cent, 2.5 per cent, 5.0 per cent, and 10.0 per cent. These tubes were set up

TABLE 6
EFFECT OF DIMETHYL SULFOXIDE ON ROUTINE SUSCEPTIBILITY TESTING WITH SIX COMMONLY USED CHEMOTHERAPEUTIC AGENTS

Test Microorganism	Penicillin 10 Units		Erythromycin 15 mcg		Chloromycetin 30 mcg		Novobiocin 15 mcg		Tetracycline 30 mcg		Dihydrostreptomycin 10 mcg	
	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 per cent.

in a series of four for each test organism. The first tube contained the test organism only; the second tube contained test organism, DMSO and low concentration of the test chemotherapeutic agent; the third tube contained the test organism, DMSO, and high concentration of the test chemotherapeutic agent; and the fourth tube contained the test organism and DMSO as a control on the dimethyl sulfoxide alone. Results of this series of tests are shown in TABLE 6.

Discussion

The bacteriostatic and bactericidal properties of dimethyl sulfoxide have been questioned by many. The series of experiments here had as its purpose to establish a baseline evaluation of the bacteriostatic and bactericidal properties of dimethyl sulfoxide on a group of microorganisms in order that the combined effect of this compound and chemotherapeutic agents on susceptibility testing could be determined. TABLE 1 shows the results of this series of experiments. Dimethyl sulfoxide exhibits bactericidal action against the test microorganisms at varying concentrations. The bactericidal concentration of DMSO for *Escherichia coli, Aerobacter cloacae, Proteus vulgaris, beta hemolytic streptococci group A, Salmonella paratyphi B,* and *Candida albicans* was determined to be 30 per cent; that for *Pseudomonas aeruginosa* was 10 per cent; for *Diplococcus pneumoniae* it was 5 per cent; while *Staphylococcus aureus* required a 40 per cent concentration and *Streptococcus faecalis* 50 per cent. TABLE 2 indicates that dimethyl sulfoxide is bacteriostatic at lower concentrations, between 5 per cent and 10 per cent, and bactericidal at higher concentrations. As the concentration is increased from 20 per cent to 80 per cent, the action as a bactericidal agent is greatly increased.

The mode of action of dimethyl sulfoxide in killing these microorganisms is unknown. This point was not an intention of the present study. 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. Four per cent concentration of DMSO added to young vigorous broth cultures of *Diplococcus pneumoniae* serve as an excellent solubility test. *Streptococci* are not soluble at this low concentration.

Earlier experiments in this laboratory indicated the possibility that a combination of penicillin and dimethyl sulfoxide would reverse the resistance of *Staphylococcus aureus* to penicillin. These early experiments were with *Staphylococcus* strains isolated in the course of routine clinical laboratory work. The assumption was made that this was most likely brought about by the neutralization of the penicillinase produced by the microorganisms.

An attempt to duplicate these findings by large-scale testing was attempted. Sixteen strains of proven penicillin-resistant *Staphylococcus aureus* were first obtained from the Communicable Disease Center, Atlanta, Ga. As indicated in TABLE 3, each of these sixteen strains was tested and found to be coagulase positive. In turn, each strain was tested against 10 units and 40 units of penicillin G and proven to be highly resistant. Growth of each of the sixteen

strains was uneffected by dimethyl sulfoxide in concentrations of 0.5 per cent, 2.5 per cent, 5.0 per cent and 10.0 per cent. Their growth was in no way restricted on exposure to 40 units of penicillinase.

In order to determine the effect of combined penicillin and dimethyl sulfoxide on this group of penicillin-resistant *Staphylococcus*, they were exposed to varying combinations of these two compounds. TABLE 4 shows the results obtained with each of these penicillin-resistant strains. Fourteen of these sixteen strains of resistant *Staphylococci* survived and multiplied without restriction in media containing penicillin varying from 1 unit to 40 units and DMSO in concentration from 0.5 per cent to 5.0 per cent. In the case of these fourteen strains, it must be assumed that no neutralization of the penicillinase or any degree of synergism between the penicillin and dimethyl sulfoxide took place. In the case of strains DA-1329-5 and DA-542-6, there appears to be a reversal of resistance of the microorganisms to penicillin. Cultures of these two strains were highly resistant to penicillin at the beginning of these experiments. However, subcultures from these two strains after exposure to penicillin and DMSO continued to show sensitivity to penicillin for several generations. It was assumed that this reversal was most likely chance mutation and not due to combined action of the penicillin and dimethyl sulfoxide.

The possible neutralization of penicillinase by DMSO was examined further by the series of experiments shown in TABLE 5. Three strains of penicillin-sensitive *Staphylococci* were first tested and found to be susceptible to 5 units of penicillin. Growth of these microorganisms was unrestricted in the presence of 40 units of penicillinase. Five per cent and 10 per cent dimethyl sulfoxide in broth cultures failed to limit the growth of these penicillin-sensitive strains. Penicillinase in the amount of 40 units per ml was added to protect these penicillin-sensitive *Staphylococci*. Combinations of 5.0 per cent DMSO and 5 units penicillin and 10 per cent DMSO and 20 units of penicillin were added. These penicillin-sensitive *Staphylococci* were protected by the penicillinase and showed unrestricted growth even in the presence of 20 units of penicillin. Subcultures from these treated cultures were again sensitive to 5 units of penicillin. This entire series was varied by first adding the penicillinase to broth cultures of the microorganisms and then adding the combined penicillin and DMSO; by adding the microorganisms to broth tubes already containing the penicillinase, penicillin and DMSO; and by adding the penicillinase, DMSO and penicillinase in that order to broth cultures of the test microorganisms. In each case, the growth of the microorganisms, protected by penicillinase, was unrestricted. This entire series appears to indicate that there is no degree of neutralization of penicillinase by dimethyl sulfoxide.

The recognized importance of chemotherapeutic susceptibility testing in clinical medicine raises the question of possible effect of dimethyl sulfoxide on this significant laboratory procedure. To investigate the possibility that DMSO might interfere with or enchance reactions in susceptibility testing, a study was made of this question using as test organisms *Salmonella paratyphi*,

Proteus vulgaris, Pseudomonas aeruginosa, Streptococcus faecalis, beta hemolytic Streptococcus group A, Diplococcus pneumoniae, Escherichia coli, and *Aerobacter cloacae.* High and low concentration levels of penicillin, erythromycin, chloromycetin, novobiocin, tetracycline, and dihydrostreptomycin were added to beef heart infusion broth cultures of the test microorganisms and incubated; the presence or absence of growth was checked on blood agar plates. A second series using low and high concentration discs of each of the chemotherapeutic agents was placed on beef heart infusion agar plates containing concentrations of dimethyl sulfoxide varying from 0.5 per cent to 10 per cent, 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 brain heart infusion agar plates, incubated, and examined for zones of inhibition. A summary of these three series of experiments is shown in TABLE 6. A study of this data shows conclusively that the presence of dimethyl sulfoxide to a concentration of 10 per cent 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 dimethyl sulfoxide that might be present in such specimens as urine, blood, body fluids, etc., or in blood bank blood used in media-making will not interfere with clinical laboratory susceptibility testing of microorganisms isolated in infection or disease.

The above series was repeated, using the sixteen strains of penicillin-resistant *Staphylococcus aureus*, and the results obtained were essentially the same. The presence of DMSO did not interfere with or enhance reactions of the chemotherapeutics used in susceptibility tests conducted.

Summary and Conclusions

(1) A historical review of the use of dimethyl sulfoxide as a penetrant carrier by Jacob, Kligman, Rosenbaum, Pottz and others is given.

(2) The possibility of dimethyl sulfoxide acting as a bacteriostatic and bactericidal agent is discussed. A series of experiments to prove the bacteriostatic and bactericidal properties is reported as a basis for a study of the influence of dimethyl sulfoxide on chemotherapeutic susceptibility testing.

(3) The influence of combined penicillin and dimethyl sulfoxide on sixteen strains of penicillin-resistant *Staphylococcus aureus* is investigated. DMSO exhibits no properties which lead to reversal of the penicillin resistance of this group of *Staphylococci.*

(4) A study showing that dimethyl sulfoxide exhibits no neutralizing effect on penicillinase is reported.

(5) The effect of combined dimethyl sulfoxide and commonly used chemotherapeutic agents on the susceptibility testing results of a group of patho-

genic microorganisms and sixteen strains of penicillin resistant *Staphylococcus aureus* is evaluated.

(6) There is no evidence that dimethyl sulfoxide, in concentrations likely to be encountered in specimens of blood, urine, body fluids, etc., or in media made from expired blood bank blood, either interferes with or enchances the action of the chemotherapeutic agents used in susceptibility testing.

(7) It is of interest that dimethyl sulfoxide in concentrations of 30 per cent and above appears to dissolve susceptible microorganisms. Four per cent concentration of DMSO serves as a solubility test for *Diplococcus pneumoniae*.

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