Alkalinized Glutaraldehyde, a New Antimicrobial Agent

By P. M. BORICK, F. H. DONDERSHINE, and V. L. CHANDLER†

A high degree of microbiological activity was demonstrated when aqueous glutaraldehyde solutions were alkalinized with the appropriate buffer. The potent bactericidal, tuberculocidal, fungicidal, sporicidal, and virucidal activity of alkalinized glutaraldehyde was not affected by the presence of serum and retained activity for a minimum of 2 weeks. Acidic glutaraldehyde solutions, stored at room temperature, are highly stable. Alkalinized glutaraldehyde solutions show a significant change in pH and loss of glutaraldehyde after 2 weeks. They have a mild odor and exhibit a relatively low order of toxicity as they are only slightly irritating to the skin when not removed. No damage to lensed instruments was observed with 2 per cent solutions.

THE IMPORTANT ROLE played by the environment in the spread of disease has emphasized the need for effective chemical disinfectants. The use of delicate instruments and equipment which cannot be heat-sterilized makes an extensive microbiological testing program essential in searching for potent antimicrobial agents (1). Adequate disinfection may not be achieved by agents destroying only bacterial forms. Antimicrobials intended for universal application should possess practical bactericidal, tuberculocidal, fungicidal, sporicidal, and virucidal activity as well. They should not be harmful to individuals or cause damage to instruments (6).

This is a report on alkalinized glutaraldehyde solutions[1] which meet most of these requirements.

EXPERIMENTAL

Test Agent.—The dialdehyde, glutaraldehyde (Union Carbide Chemical Co., N. Y.), undergoes typical aldehydic reactions to form acetals, cyanohydrin, oximes, and hydrazones. Oxidation results in glutaric acid, while reduction produces 1,5-pentanediol. Aqueous glutaraldehyde solutions are stable for at least 2 years while still acidic with little change in pH. Alkalinized glutaraldehyde solutions show a significant change in pH after 2 weeks.

Glutaraldehyde—$OCH(CH_2)_3CHO$—Antimicrobial action was demonstrated upon the addition of 0.3% sodium bicarbonate to 2% aqueous glutaraldehyde solutions; this alkalinization was necessary to elicit activity (5). When alkalinized, 2% aqueous glutaraldehyde solutions continued to show microbicidal activity for a minimum of 2 weeks at room temperature. These solutions were nonflammable and of low volatility. Glutaraldehyde concentrations were confirmed by the bisulfite iodometric technique of Siggia (7).

Test Organisms.—The following test organisms were used:

(*a*) Nonsporeforming bacteria: *Staphylococcus aureus*, American Type Culture Collection (ATCC) 6538; *S. aureus*, penicillin-resistant strain (Hunterdon Medical Center, Flemington, N. J.); *Streptococcus pyogenes*, ATCC 12384; *Diplococcus pneumoniae*, Type III (W. J. Nungester, University of Michigan); *Escherichia coli*, ATCC 6880; *Pseudomonas aeruginosa*, ATCC 10145; *Proteus vulgaris*, ATCC 6380; *Klebsiella pneumoniae*, ATCC 132; *Mycobacterium tuberculosis*, ATCC 7690.

(*b*) Sporeforming bacteria: *Bacillus globigii*, *Bacillus subtilis*, *Clostridium tetani*, *Clostridium perfringens* (L. S. Ortenzio, U. S. Department of Agriculture).

(*c*) Fungal strain: *Trichophyton interdigitale*, ATCC 640.

(*d*) Viruses: Poliomyelitis virus, Types I and II; Coxsackie virus, B-1; Echo 6; Herpes simplex; Vaccinia; Influenza A-2, Asian; Adeno virus, type 2; mouse hepatitis virus, MHV-3 (M. Klein, Temple University).

The bactericidal action of alkalinized 2% aqueous glutaraldehyde solutions was determined by the modified use-dilution method of Ortenzio and Stuart (4). This technique employs polished stainless steel cylinders (penicylinders) as carriers of the inoculum. After determining that each test organism had a uniform resistance to phenol, the inoculum was dried onto the penicylinders. The contaminated penicylinders were immersed in 10 ml. of the test agent and, after predetermined exposures, transferred to 40 ml. of eugonbroth (BBL) for incubation at 37° for 48 hours. All negative broth tubes were challenged for bacteriostasis by reinoculation.

Testing of alkalinized glutaraldehyde solutions on a human strain of *M. tuberculosis* was accomplished by exposing approximately 10,000 tubercle bacilli/ml. of saline to the test solution for 10 minutes at 30° (8). Samples of this mixture were used to inoculate Lowenstein-Jensen slants and inject the inguinal glands of guinea pigs. The slants were incubated for 4 weeks at 37°. The animals were sacrificed after 6 weeks, and all involved organs were examined for tubercle lesions.

Sporicidal tests were performed according to the method of the Association of Official Agricultural Chemists (5). This method contains features not found in earlier procedures. These are the inclusion of 2.5 *N* HCl as a standard reference solution to insure that the spores meet a prescribed resistance, porcelain penicylinders (Fisher Scientific Co., N. Y.) as inoculum carriers, and maintenance of 20° as the testing temperature. Each porous penicylinder holds approximately 1.3×10^6 spores. The test method and the stasis challenge were conducted as previously described. Incubation was in fluid thioglycollate broth (BBL) for 7 days at 37°.

Fungicidal action of aqueous, alkalinized glutaraldehyde solutions was assessed by exposing conidial suspensions of *T. interdigitale* in accordance with Association of Official Agricultural Chemists pro-

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† Deceased.

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[1] Marketed as Cidex Solution by Arbrook Division, Ethicon, Inc., U. S. pat. 3,016,328.

TABLE I.—BACTERICIDAL ACTIVITY OF AQUEOUS, ALKALINIZED 2% GLUTARALDEHYDE SOLUTIONS AT 20° C.

	Immediate Challenge	Challenged 2 Wks. After Activation	Challenged 4 Wks. After Activation
No. of samples tested	11	11	5
Mean pH	8.3	7.9	7.7
Mean % glutaraldehyde	2.02	1.71	1.50
	—Killing Time, Min.—		
S. aureus	<1	<1	<1
S. aureus, penicillin resistant	<1	<1	<1
Str. pyogenes	<1	<1	<1
D. pneumoniae	<1	<1	<1
E. coli	<1	<1	<1
Ps. aeruginosa	<1	<1	<1
P. vulgaris	<1	<1	<1
K. pneumoniae	<1	<1	<1

TABLE II.—TUBERCULOCIDAL ACTIVITY OF AQUEOUS, ALKALINIZED 2% GLUTARALDEHYDE SOLUTIONS AT 30° C.

	M. tuberculosis growth	
	Lowenstein-Jensen	Guinea Pig
2% Aqueous glutaraldehyde	−[a]	−
Aqueous control	+[b]	+
Saline control	+	+

[a] −, No growth and absence of lesions. [b] +, Growth and presence of lesions.

cedures (3). Subcultures were incubated at 25° for 10 days.

Antiviral activity of alkalinized glutaraldehyde solutions was determined according to the method reported by Klein (2).

RESULTS

Table I presents the bactericidal testing results of aqueous, alkalinized 2% glutaraldehyde solutions against Gram-positive and Gram-negative, nonsporeforming microorganisms. According to use-dilution techniques, the originally observed level of bactericidal activity appears to be maintained for 4 weeks after alkalinization and at pH 7.7 to 8.3 and at glutaraldehyde concentrations of 1.50 to 2.02%.

Table II describes the tuberculocidal activity of aqueous, alkalinized glutaraldehyde solutions against *M. tuberculosis* after 10 minutes exposure at 30°.

Table III hows the results of the same lots of aqueous, alkalinized 2% glutaraldehyde solutions when tested against the spores of aerobic and anaerobic bacteria. According to AOAC-20° testing, the originally observed level of sporicidal activity appears to be maintained for 8 weeks after activation at pH 8.3 to 7.7 and glutaraldehyde concentrations of 2.02 to 1.39%. It is interesting to note the change in pH and the loss of glutaraldehyde during these tests.

When preparations of alkalinized glutaraldehyde were tested against conidial suspensions (5×10^6/ml.) of *T. interdigitale* (Table IV), an exposure of 30 seconds did not yield viable spores.

Aqueous, alkalinized glutaraldehyde solutions prevented the multiplication of all viruses tested in less than 10-minute exposure at the 2% level (Table V). The susceptibility of the mouse hepatitis virus to glutaraldehyde does not necessarily infer a similar susceptibility to human hepatitis virus. However, since this is one of the few experimental hepatitis viruses which could be tested, the results are significant. Viability controls were positive for all strains tested.

The ability of antimicrobial agents to maintain their activity in the presence of organic material is extremely important, and alkalinized glutaraldehyde was tested with increasing concentrations of sterile bovine serum (Table VI). It is noteworthy that the serum was neither precipitated nor coagulated by the activated microbicide, and that all of the species tested appeared to be sensitive to alkalinized glutaraldehyde at the original levels previously noted regardless of the presence of organic matter.

The oral LD_{50} in mice was determined in our laboratories to be approximately 352 mg./Kg. of glutaraldehyde. Toxicological studies indicate that contact, as well as vapors, were nonirritating to the skin of laboratory animals or human subjects (9).

DISCUSSION

The addition of slight amounts of sodium bicarbonate to glutaraldehyde with its concomitant rise of pH resulted in broad antimicrobial activity accompanied by no detectable deleterious effects on the tissues of test animals. Both the aqueous and alcoholic preparations showed considerable antimicrobial activity (5) when this was done. It was felt that the aqueous preparation deserved greater attention since it possessed predictably a lower irritant and inflammability index than the alcoholic preparation. In addition, it is characterized by a lower volatility and will not exert solvent damage to adhesives in lenses, instruments, plastics, rubber, or other material.

The findings presented here strongly support the view that alkalinized glutaraldehyde is a highly effective germicidal agent. It exerts its activities

TABLE III.—SPORICIDAL ACTION OF AQUEOUS, ALKALINIZED 2% GLUTARALDEHYDE SOLUTIONS AT 20° C.

	Immediate	Post Activation Challenge 2 wks.	4 wks.	6 wks.	8 wks.
No. of samples tested	11	11	5	4	2
Mean pH	8.3	7.9	7.7	7.7	7.7
Mean % glutaraldehyde	2.02	1.79	1.50	1.40	1.39
			Killing Time, Hr.		
B. globigii	<3	<3	<3	<3	<3
B. subtilis	<3	<3	<3	<3	<3
Cl. tetani	<3	<3	<3	<3	<3
Cl. perfringens	<3	<3	<3	<3	<3

TABLE IV.—FUNGICIDAL ACTION OF AQUEOUS ALKALINIZED 2% GLUTARALDEHYDE SOLUTIONS AT ROOM TEMPERATURE

No. of samples tested	5
Mean pH	7.8
Mean % glutaraldehyde	1.41
	Killing Time, Min.
T. interdigitale	<0.5
Phenol 1:60, control	3–5

TABLE V.—VIRUCIDAL ACTIVITY OF AQUEOUS, ALKALINIZED 2% GLUTARALDEHYDE SOLUTIONS

Virus	Activity
Poliomyelitis, types I & II Echo type 6 Coxsackie B-1 Herpes simplex Vaccinia Influenza A-2, Asian Adeno type 2 Mouse hepatitis (MHV3)	complete inhibition in <10 min.

TABLE VI.—INFLUENCE OF SERUM ON THE MICROBICIDAL ACTION OF AQUEOUS, ALKALINIZED 2% GLUTARALDEHYDE

	Bovine Serum Concn., %		
	5	10	20
Mean pH			
Mean % glutaraldehyde			
Vegetative Bacteria	Killing Time, Min.		
S. aureus	<1	<1	<1
S. aureus, penicillin-resistant	<1	<1	<1
Str. pyogenes	<1	<1	<1
D. pneumoniae	<1	<1	<1
E. coli	<1	<1	<1
Ps. aeruginosa	<1	<1	<1
P. vulgaris	<1	<1	<1
K. pneumoniae	<1	<1	<1
Spores	Killing Time, Hr.		
B. globigii	<3	<3	<3
B. subtilis	<3	<3	<3
Cl. tetani	<3	<3	<3
Cl. perfringens	<3	<3	<3
Fungi	Killing Time, Min.		
T. interdigitale	<0.5	<0.5	<0.5

against Gram-positive and Gram-negative bacteria; it is very efficient in preventing germination of aerobic and anaerobic bacterial spore suspensions. Despite the contention that aqueous solutions of germicidal agents usually do not exert a tuberculocidal effect *in vitro*, the antimycobacterial activity demonstrated would exclude alkalinized glutaraldehyde from this category. While antifungal activity was tested with only one filamentous species, the speed with which conidia were eradicated suggests an efficient fungicidal agent. Since a high degree of microbicidal activity was demonstrable with alkalinized glutaraldehyde, one cannot deny the very broad antimicrobial capabilities possessed by this molecule. Its use in medical and commercial preparations for disinfection would seem appropriate.

SUMMARY AND CONCLUSIONS

The findings presented support the conclusion that aqueous, alkalinized 2% glutaraldehyde solution is a highly effective microbicidal agent. This broad spectrum antimicrobial killed Gram-positive and Gram-negative bacteria as well as tubercle bacilli within the minimum exposure time of the test. Alkalinized glutaraldehyde solutions killed aerobic and anaerobic spores in addition to the conidia of a common dermatophyte. Aqueous alkalinized 2% glutaraldehyde solutions were shown to be potent virucidal agents as well. The *in vitro* activity of this microbicide was not affected by the presence of bovine serum in the concentrations used.

Acidic glutaraldehyde solutions were stable for at least 2 years, while alkalinized 2% solutions retained full antimicrobial activity for 2 weeks. However, a diminution of glutaraldehyde concentration with changes in pH occurred with time when alkalinized glutaraldehyde solutions were tested during this period.

Aqueous glutaraldehyde solutions have a mild odor, low volatility, are nonflammable, and will not damage lensed instruments or equipment.

REFERENCES

(1) Borick, P. M., and Bratt, M., *J. Appl. Microbiol.*, **9**, 475(1961).
(2) Klein, M., personal communication, 1963.
(3) "Official Methods of Analysis," 9th ed., Association of Official Agricultural Chemists, Washington, D. C., 1960, p. 67.
(4) Ortenzio, L. S., and Stuart, L. S., *J. Assoc. Off. Agr. Chemists*, **44**, 416(1961).
(5) Pepper, R. E., and Chandler, V. L., *J. Appl. Microbiol.*, **11**, 384(1963).
(6) Reddish, G. F., "Antiseptics, Disinfectants, Fungicides and Chemical and Physical Sterilization," Lea and Febiger, Philadelphia, Pa., 1959, p. 619.
(7) Siggia, S., "Quantitative Organic Analysis Via Functional Groups," John Wiley & Sons, Inc., New York, N. Y., 1959, p. 10.
(8) Shelansky, M., personal communication, 1961.
(9) Stonehill, A. A., Krop, S., and Borick, P. M., *Am. J. Hosp. Pharm.*, **20**, 458(1963).