Biocontrol Science, 1996, Vol. 1, No. 1, 25−32

Original

An Assessment of the Antimicrobial Activity of Commercially Available Disinfectants

TAKASHI AKAMATSU[1]*, MASAO MINEMOTO[1], MICHITAKA HIRONAGA[2], AND MASARU UYEDA[3]

[1]*Department of Pharmacy and* [2]*Central Clinical Laboratory, Kyushu Kousei-Nenkin Hospital, 2-1-1 Kishinoura, Yahata Nishi-ku Kitakyushu 806, Japan, and* [3]*Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan*

Received 25 April 1996/Accepted 22 July 1996

The antimicrobial activity of aqueous solutions of benzalkonium chloride (BAC), chlorhexidine digluconate (CHG), povidone-iodine (PVP-I), and ethanol (EtOH) were tested against a wide range of microorganisms, including hepatitis B virus (HBV) at the actual concentration employed at medical facilities. The chemical stability of these disinfectants in the presence of human serum was also investigated. BAC, CHG, PVP-I, and EtOH preparations showed potent bactericidal activity against nine vegetative bacteria tested and *Candida albicans*. Three strains of mycobacteria and four strains of spore forming fungi were more resistant to these disinfectants than vegetative bacteria. The spores of *Bacillus subtilis* were not killed by all four disinfectants even in 360 min at any concentration tested. However, the spores of *Clostridium sporogenes* and *Clostridium sphenoides* were killed by PVP-I within 30 min, though they were not killed by BAC, CHG, and EtOH even in 360 min. BAC, CHG, and PVP-I did not inactivate hepatitis B surface antigen (HBs-Ag) in 120 min at any concentration tested. Although HBs-Ag was inactivated by 75 and 95% (v/v) EtOH in 30 s, it was not inactivated by 35 and 55% (v/v) EtOH even in 120 min. The addition of human serum decreased the concentration of aqueous BAC and PVP-I solutions. The decrease of available iodine in aqueous 0.5% (w/v) PVP-I was particularly large ; namely, compared with that of the control, the concentration was decreased over 24 h by approximately 88 and 100% after the addition of 10 and 30% (v/v) human serum, respectively. In contrast, the concentration of aqueous CHG and EtOH solutions decreased little over 24 h after the addition of human serum.

Key words : Disinfectants/Antimicrobial activity/Chemical stability/Hepatitis B surface antigen.

INTRODUCTION

Disinfectants can be divided according to the scheme of Spaulding and Groschel (1974) into three categories : high-(e. g., glutaraldehyde), intermediate-(e. g., povidone-iodine, ethanol, and chlorine compounds), and low-level (e. g., benzalkonium chloride and chlorhexidine digluconate). Needless to say each disinfectant is different, and none is ideal for all purposes. The decision about which disinfectant to use should be based on a variety of considerations including its possible inactivation by organic matter as well as tissue irritation, corrosiveness, and the type of microorganism it is targeting.

To date, there are numerous publications (Bergan and Lystad, 1971 ; Scott et al., 1986 ; Smith, 1947) that refer to the antimicrobial properties of disinfectants against particular organisms. However, the methods used vary, and it is often difficult to compare results. We have, therefore, performed a series of studies (Akamatsu et al., 1995 ; 1996) regarding the antimicrobial activity of several disinfectants against identical strains of various types of microorganism by

*Corresponding author. Tel : +81-93-641-5111, Fax : +81-93-641-5139.

the same microbiocidal method to compare their activities, giving informative advice to infection control staff regarding when to use disinfectants and the appropriate selection.

In this paper, we investigated the potential of aqueous solutions of benzalkonium chloride (BAC), chlorhexidine digluconate (CHG), povidone-iodine (PVP-I), and ethanol (EtOH) by testing their antimicrobial activity and chemical stability in the presence of human serum in order to estimate the effectiveness of solutions used at medical facilities for disinfection purposes. In addition, the effectiveness of these disinfectants against hepatitis B virus (HBV) was examined.

MATERIALS AND METHODS

Disinfectants and neutralizer

BAC (Ezol®, Ebisu Yakuhin K. K., Osaka), CHG (5% (w/v) Hibitane® Concentration, Zeneca Yakuhin K. K., Osaka), PVP-I (Isodine® solution, Meiji Seika Kaisha, Ltd., Tokyo), and EtOH (Ethyl Alcohol 99.5% (v/v), Wako Pure Chemical Industries, Ltd., Osaka) were diluted with sterile distilled water to the specified concentrations to prepare the test solutions.

The following neutralizers were used : a mixture of 0.5% (w/v) lecithin, 1% (w/v) Lubrol W, and 1% (w/v) Tween 80 was used to neutralize BAC and CHG ; 1% (w/v) sodium thiosulfate was used to neutralize PVP-I. Neutralization by dilution with distilled water was used for EtOH. The neutralizer system was checked and verified before the test.

Test organisms and preparation of test suspension

The twenty standard strains and clinical isolates listed below were used : *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* KN 93-256, *Staphylococcus epidermidis* KN 93-188, *Enterococcus faecalis* KN 93-35, *Escherichia coli* ATCC 25922, *Escherichia coli* KN 93-152, *Pseudomonas aeruginosa* ATCC 27853, *Stenotrophomonas maltophilia* KN 93-17, *Acinetobacter* sp. KN 93-25, *Mycobacterium tuberculosis* KN 93-7, *Mycobacterium kansasii* KN 93-21, *Mycobacterium avium complex* KN 93-13, *Bacillus subtilis* ATCC 6633, *Clostridium sporogenes* ATCC 11437, *Clostridium sphenoides* ATCC 19403, *Candida albicans* ATCC 10231, *Mucor racemosus* KN 93-5, *Rhizopus nigricans* SN 32, *Aspergillus niger* ATCC 6275, *Aspergillus terreus* KN 93-11.

Suspensions of test organisms were prepared as described in a previous paper (Akamatsu et al., 1995). All test suspensions were washed twice and resuspended in sterile distilled water to obtain a concentration of approximately 10^8 colony forming units (cfu)/ml before testing.

Test Method

The inoculum (0.5 ml of a 10^8 cfu/ml suspension) was added to 5 ml of each test solution under test at 25℃. After different contact periods, 0.1 ml of this mixture was added to 5 ml of a neutralizer for a minimum of 5 min; thereafter 0.1 ml of the resultant solution was transferred to the respective medium described in a previous paper (Akamatsu et al., 1995). These samples were cultured in an incubator at 37 or 25 ℃, and kept under observation for 7 to 35 d according to the type of organism used. To study the effect of organic matter on antimicrobial activity, human serum at a final concentration of 10 or 30% (v/v) was added to the test solution. Antimicrobial activity was evaluated by the turbidity in the culture medium.The killing time was defined as the minimum time of exposure for the test solution to reach a condition in which no turbidity could be observed.

Inactivation of HBs-Ag

An amount of 0.1 ml HBs-Ag positive human serum was added to 0.9 ml of the test solution. After allowing the reaction to proceed at 25 ℃ for a specified time, the reaction was terminated by the addition of 2 ml of a neutralizer or 100 ml of distilled water. The antigenicity of HBs-Ag was measured by radioimmunoassay (Ausria Ⅱ; Dainabot Co., Ltd., Tokyo).

Stability in the presence of human serum

The stability of the solutions was determined by allowing a test solution containing 10 or 30% (v/v) human serum to stand at 25℃ for 24 h. As a control, 10 or 30% (v/v) distilled water was added to the test solution.

Determination of disinfectant solutions

Ⅰ) BAC : After addition of 2 ml of 6 N HCl and 20 ml of a mixture of chloroform : acetonitrile (95 : 5) to 10 ml of the test solution, the reaction mixture was shaken for 10 min. Next, the reaction mixture was centrifuged at 5000×*g* for 10 min, and 5 ml of the lower layer was filtered through a membrane filter with 0.2 μm pores (Toyo Roshi, PTFE) and diluted with 20 ml of a mixture of chloroform : acetonitrile (95 : 5). Then spectrophotometric analysis at 263.5 nm was performed using a Shimadzu double beam spectrophotometer UV-2200 A.

Ⅱ) CHG : Five ml of distilled water, 10 ml of acetate buffer (0.1 N acetic acid : 0.1 mol sodium acetate, 2 : 1, pH 4.4), 0.5 ml of 3 N HCl, and 20 ml of a mixture of ethanol and acetonitrile (1 : 1) were added

to 10 ml of the test solution and left standing for 10 min. Next, the reaction mixture was filtered through a membrane filter with 0.2 μm pores and diluted (15→100) with a mixture of ethanol and acetonitrile (1 : 1) before spectrophotometric analysis at 259 nm was performed using a Shimadzu double beam spectrophotometer UV-2200 A.

Ⅲ) PVP-I : Two ml or 20 ml of the test solution, each equivalent to about 10 mg of iodine, was added to distilled water to make a total volume of 50 ml. Then, the total available iodine was assayed by titration with 0.005 N sodium thiosulfate, determining the endpoint potentiometrically, using a platinum-cork electrode system (Potentiometric Automatic Titrater AT-310 ; Kyoto Electronics Co., Ltd., Kyoto).

Ⅳ) EtOH : Ten ml of the test solution was centrifuged at 3500×g for 15 min. Then, this solution was diluted with 2-propanol (1→25) and filtered through a 0.2 μm membrane filter . Gas chromatography was carried out using the following apparatus and conditions : gas chromatograph, Hitachi GC 263-50 ; detector, flame ionization detector ; column, 3 mm inner diameter×1.5 m length, glass column ; packing, Porapak Q 80-100 mesh ; column temperature, 130℃ ; injection temperature, 150℃ ; carrier gas (He) flow, 40 ml/min ; injection volume, 1 μl.

RESULTS

Antimicrobial activity against test organisms

Tables 1, 2, 3, and 4 show the antimicrobial efficacy of aqueous solutions of BAC, CHG, PVP-I, and EtOH against 20 different test organisms. In the absence of human serum, BAC, CHG, PVP-I, and EtOH preparations showed potent bactericidal activity against vegetative bacteria. Particularly, 75 and 95% (v/v) EtOH showed rapid activity against all nine bacteria in killing them in 15 s. Three strains of mycobacteria were more resistant to these four disinfectants than vegetative bacteria. Namely, BAC and CHG could not kill them even in 120 min. PVP-I killed them in 30 to 45 min, 15 to 30 min, and 15 to 30 min at the concentrations of 0.05, 0.5, and 5% (w/v), respectively. EtOH, however, showed good bactericidal activity against these three mycobacteria, killing them in 3 min, 30 s, and 30 s at the concentrations of 55, 75, and 95% (v

TABLE 1. Bactericidal activity of aqueous solutions of BAC, CHG, PVP-I, and EtOH against vegetative bacteria.

Organism	Concentration of human serum (%, v/v)	Killing time (min)											
		BAC (%, w/v)			CHG (%, w/v)			PVP-I (%, w/v)			EtOH (%, v/v)		
		0.05	0.2	0.5	0.05	0.2	0.5	0.05	0.5	5	55	75	95
S. aureus ATCC 25923	0	1	0.5	0.5	3	1	0.5	1	0.5	1	3	0.25	0.25
	10	1	0.5	0.5	3	1	0.5	45	5	1	3	0.25	–
	30	3	1	1	5	1	0.5	>120	30	1	5	–	–
S. aureus KN 93-256	0	1	0.5	0.5	5	3	1	1	0.5	1	5	0.25	0.25
	10	1	1	0.5	5	3	1	45	5	1	5	0.25	–
	30	3	1	1	5	3	1	>120	60	1	5	–	–
S. epidermidis KN 93-188	0	1	0.5	0.5	3	1	0.5	1	0.5	0.5	3	0.25	0.25
	10	1	0.5	0.5	3	1	0.5	30	3	0.5	3	0.25	–
	30	3	1	0.5	5	1	0.5	>120	45	1	3	–	–
E. faecalis KN 93-35	0	3	0.5	0.5	5	1	0.5	1	0.5	0.5	0.5	0.25	0.25
	10	3	0.5	0.5	5	1	0.5	60	15	0.5	0.5	0.25	–
	30	3	1	0.5	5	3	0.5	>120	45	1	1	–	–
E. coli ATCC 25922	0	3	0.5	0.5	1	0.5	0.5	1	0.5	0.5	0.5	0.25	0.25
	10	3	0.5	0.5	1	0.5	0.5	60	3	0.5	0.5	0.25	–
	30	5	3	0.5	1	0.5	0.5	>120	30	0.5	1	–	–
E. coli KN 93-152	0	3	1	0.5	3	0.5	0.5	1	0.5	0.5	0.5	0.25	0.25
	10	5	1	0.5	3	1	0.5	45	5	0.5	0.5	0.25	–
	30	5	3	1	3	1	0.5	>120	30	0.5	1	–	–
P. aeruginosa ATCC 27853	0	5	3	1	5	3	0.5	1	0.5	1	0.5	0.25	0.25
	10	5	3	1	5	3	0.5	60	5	1	0.5	0.25	–
	30	10	5	3	5	3	0.5	>120	45	3	1	–	–
S. maltophilia KN 93-17	0	5	3	1	3	1	0.5	1	0.5	1	0.5	0.25	0.25
	10	5	3	1	3	1	0.5	60	5	1	0.5	0.25	–
	30	10	5	3	3	1	0.5	>120	60	1	1	–	–
Acinetobacter sp. KN 93-25	0	3	1	1	3	1	0.5	1	1	0.5	0.5	0.25	0.25
	10	5	1	1	3	1	0.5	60	1	1	0.5	0.25	–
	30	10	3	1	3	1	0.5	>120	15	1	1	–	–

– : Not tested.

TABLE 2. Bactericidal activity of aqueous solutions of BAC, CHG, PVP-I, and EtOH against mycobacteria.

Organism	Concentration of human serum (%, v/v)	Killing time (min)											
		BAC (%, w/v)			CHG (%, w/v)			PVP-I (%, w/v)			EtOH (%, v/v)		
		0.05	0.2	0.5	0.05	0.2	0.5	0.05	0.5	5	55	75	95
M. tuberculosis KN 93-7	0	>120	>120	>120	>120	>120	>120	30	15	15	3	0.5	0.5
	10	>120	>120	>120	>120	>120	>120	60	60	15	3	0.5	–
	30	>120	>120	>120	>120	>120	>120	>120	>120	30	3	–	–
M. kansasii KN 93-21	0	>120	>120	>120	>120	>120	>120	45	45	30	3	0.5	0.5
	10	>120	>120	>120	>120	>120	>120	120	120	45	3	0.5	–
	30	>120	>120	>120	>120	>120	>120	>120	>120	45	5	–	–
M. avium complex KN 93-13	0	>120	>120	>120	>120	>120	>120	30	30	15	3	0.5	0.5
	10	>120	>120	>120	>120	>120	>120	120	120	15	3	0.5	–
	30	>120	>120	>120	>120	>120	>120	>120	>120	15	5	–	–

– : Not tested.

TABLE 3. Sporicidal activity of aqueous solutions of BAC, CHG, PVP-I, and EtOH.

Organism	Concentration of human serum (%, v/v)	Killing time (min)											
		BAC (%, w/v)			CHG (%, w/v)			PVP-I (%, w/v)			EtOH (%, v/v)		
		0.05	0.2	0.5	0.05	0.2	0.5	0.05	0.5	5	55	75	95
B. subtilis ATCC 6633	0	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360
	10	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360	–
	30	>360	>360	>360	>360	>360	>360	>360	>360	>360	>360	–	–
C. sporogenes ATCC 11437	0	>360	>360	>360	>360	>360	>360	15	10	15	>360	>360	>360
	10	>360	>360	>360	>360	>360	>360	>360	90	15	>360	>360	–
	30	>360	>360	>360	>360	>360	>360	>360	180	15	>360	–	–
C. sphenoides ATCC 19403	0	>360	>360	>360	>360	>360	>360	30	15	15	>360	>360	>360
	10	>360	>360	>360	>360	>360	>360	>360	90	15	>360	>360	>360
	30	>360	>360	>360	>360	>360	>360	>360	270	30	>360	–	–

– : Not tested.

TABLE 4. Fungicidal activity of aqueous solutions of BAC, CHG, PVP-I, and EtOH.

Organism	Concentration of human serum (%, v/v)	Killing time (min)											
		BAC (%, w/v)			CHG (%, w/v)			PVP-I (%, w/v)			EtOH (%, v/v)		
		0.05	0.2	0.5	0.05	0.2	0.5	0.05	0.5	5	55	75	95
C. albicans ATCC 10231	0	1	0.5	0.5	1	0.5	0.5	1	0.5	0.5	0.5	0.25	0.25
	10	1	1	0.5	1	0.5	0.5	30	10	0.5	0.5	0.25	–
	30	3	3	0.5	1	0.5	0.5	120	30	1	1	–	–
M. racemosus KN 93-5	0	>120	120	30	>120	120	60	60	30	30	45	15	15
	10	>120	120	30	>120	120	60	120	90	30	60	30	–
	30	>120	>120	120	>120	120	60	>120	>120	60	60	–	–
R. nigricans SN 32	0	>120	120	30	>120	>120	60	45	30	30	45	30	30
	10	>120	120	60	>120	>120	60	120	90	45	60	30	–
	30	>120	>120	120	>120	>120	60	>120	>120	45	60	–	–
A. niger ATCC 6275	0	>120	120	30	>120	>120	60	60	45	45	90	30	30
	10	>120	>120	30	>120	>120	60	>120	90	45	90	30	–
	30	>120	>120	60	>120	>120	60	>120	>120	45	90	–	–
A. terreus KN 93-11	0	>120	>120	30	>120	>120	60	60	30	30	60	30	30
	10	>120	>120	30	>120	>120	60	>120	90	45	60	30	–
	30	>120	>120	60	>120	>120	60	>120	>120	45	60	–	–

– : Not tested.

/v), respectively. The spores of *B. subtilis* had the highest resistance to BAC, CHG, PVP-I, and EtOH among the tested organisms. None of the four disinfectants could kill these spores even in 360 min at any concentration tested. The spores of *C. sporogenes* and *C. sphenoides* had a lower resistance to PVP-I than those of *B. subtilis*, and were killed within 15 to 30, 10 to 15, and 15 min at the concentrations of 0.05, 0.5, and 5% (w/v), respectively. BAC, CHG, and EtOH, however, could not kill these spores even in 360 min. Like vegetative bacteria, *C. albicans* was rapidly killed by all disinfectants. In contrast, spore forming fungi such as *M. racemosus*, *R. nigricans*, *A. niger*, and *A. terreus* were moderately resistant to BAC, CHG, PVP-I, and EtOH.

Although CHG and EtOH maintained their original levels of antimicrobial activity even in the presence of human serum, BAC and PVP-I showed reduced activity in the presence of 10 or 30% (v/v) human serum.

Inactivation of HBs-Ag

HBs-Ag positive serum (10,220 cpm) was treated with BAC, CHG, PVP-I, and EtOH at various concentrations (Figs. 1, 2, 3, and 4, respectively). Antigenicity was not reduced to the cut-off value (782.5 cpm), at which antigenicity was lost or reduced, after 120 min with BAC, CHG, and PVP-I at any concentration tested. In contrast, 75 and 95% (v/v) EtOH inactivated HBs-Ag in 30 s, though 35 and 55% (v/v) EtOH failed to do so even after 120 min.

Effect of human serum on the stability of BAC, CHG, PVP-I and EtOH

Tables 5 and 6 list the stability of test solutions of BAC, CHG, PVP-I, and EtOH at 25℃ in the presence of 10 and 30% (v/v) human serum, respectively. The concentration of these disinfectants in the control solutions was not changed over 24 h. Compared with the control, the addition of human serum decreased the concentration of aqueous BAC and PVP-I solutions. The decrease of available iodine in aqueous 0.5% (w/v) PVP-I was particularly large; namely, compared with that of the control, the concentration decreased over 24 h by approximately 88 and 100% after the addition of 10 and 30% (v/v) serum, respectively. In contrast, the concentration of aqueous CHG and EtOH solutions decreased little over 24 h after the addition of human serum.

DISCUSSION

In this study, we tested the in vitro antimicrobial activity of aqueous solutions of BAC, CHG, PVP-I and EtOH, and their subsequent chemical stability in the presence of human serum. The results revealed that BAC and CHG showed good activity against vegeta-

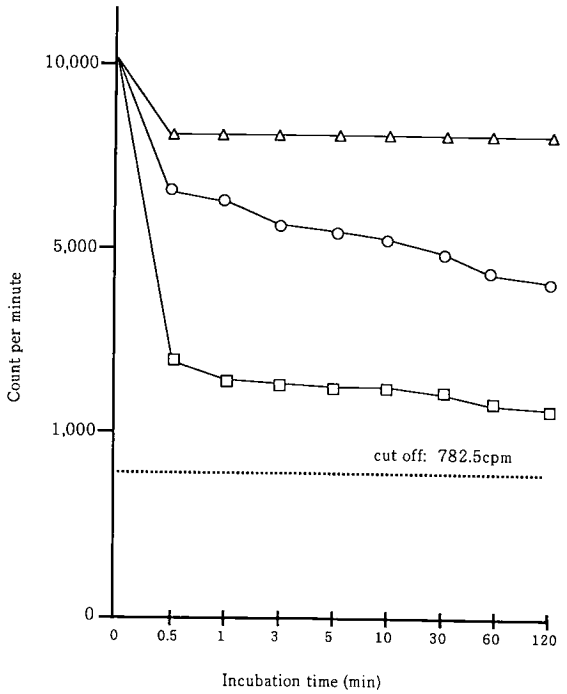

FIG. 1. Inactivation of HBs-Ag by aqueous solutions of BAC. HBs-Ag was measured by radioimmunoassay. : ○, 0.05% (w/v) ; □, 0.5% (w/v) ; △, control (physiological saline).

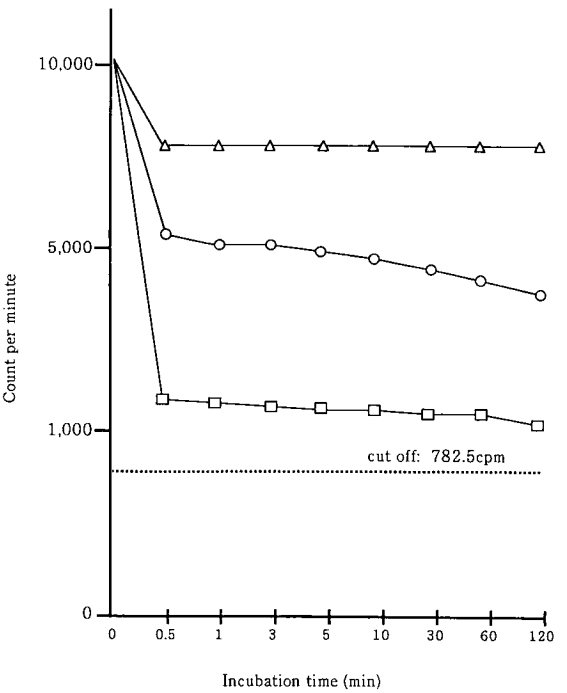

FIG. 2. Inactivation of HBs-Ag by aqueous solutions of CHG. HBs-Ag was measured by radioimmunoassay. : ○, 0.05% (w/v) ; □, 0.5% (w/v) ; △, control (physiological saline).

tive bacteria and fungi, though neither disinfectant killed mycobacteria and bacterial spores as pointed out in previous studies (Broadley et al., 1991 ; Russell, 1990). Regarding the bactericidal activity of BAC and CHG against vegetative bacteria, however, it should be remembered that some Gram-negative bacteria, such as *Pseudomonas* sp., *Acinetobacter* sp., and *Serratia marcescens* easily acquire resistance to aqueous solutions of both disinfectants (Bassett, 1970 ; Frankand Schaffner, 1976 ; Uyeda et al., 1982). In such cases, the resistant strains might easily be

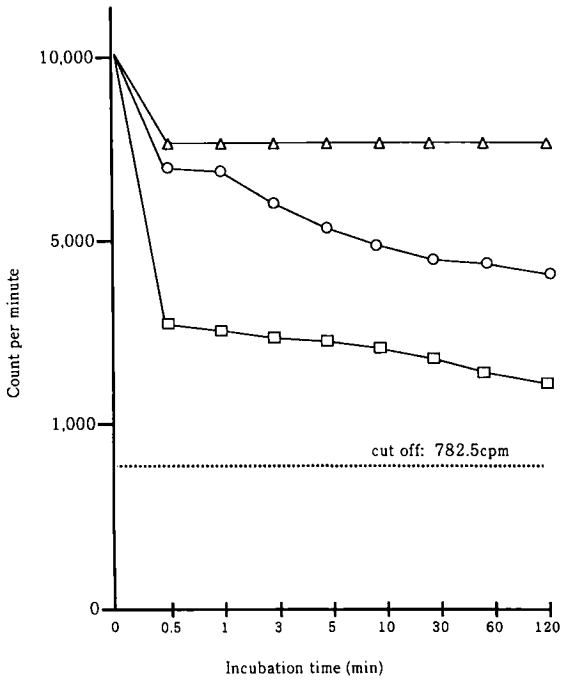

FIG. 3. Inactivation of HBs-Ag by aqueous solutions of PVP-I. HBs-Ag was measured by radioimmunoassay. : ○, 0.5% (w/v) ; □, 5% (w/v) ; △, control (physiological saline).

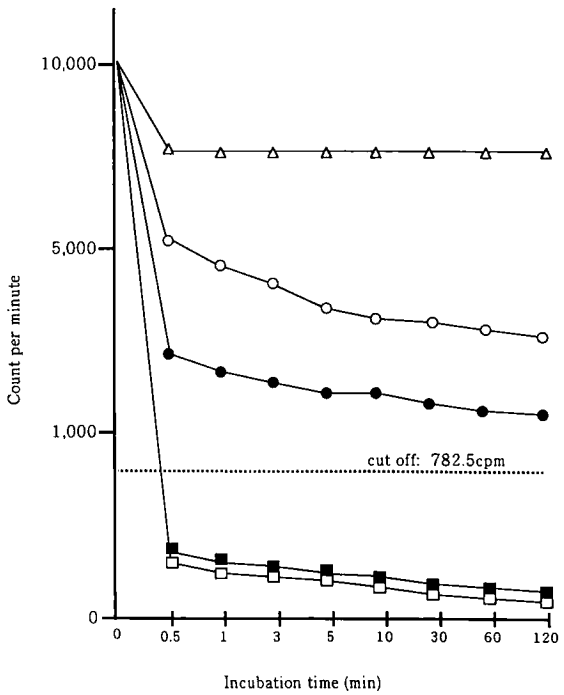

FIG. 4. Inactivation of HBs-Ag by aqueous solutions of EtOH. HBs-Ag was measured by radioimmunoassay. : ○, 35% (v/v) ; ●, 55% (v/v) ; □, 75% (v/v) ; ■, 95% (v/v) ; △, control (physiological saline).

TABLE 5. Effect of 10% (v/v) human serum on the stability of aqueous solutions of BAC, CHG, PVP-I, and EtOH at 25℃.

Time (h)	BAC				CHG				PVP-I				EtOH			
	0.05% (w/v)		0.5% (w/v)		0.05% (w/v)		0.5% (w/v)		0.5% (w/v)		5% (w/v)		35% (v/v)		55% (v/v)	
	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH
0	0.0485	8.0	0.471	7.6	0.0497	7.8	0.499	7.4	0.158	6.0	4.354	5.4	34.9	8.2	54.8	8.9
1	0.0485	7.6	0.468	7.7	0.0497	7.9	0.498	7.3	0.136	5.7	4.330	5.3	34.8	8.2	53.7	8.9
3	04.474	7.5	0.467	7.5	0.0491	7.9	0.497	7.3	0.111	5.6	4.290	5.3	34.8	8.2	53.5	8.8
6	0.0470	7.6	0.458	7.6	0.0489	7.9	0.494	7.5	0.093	5.6	4.279	5.2	34.8	8.1	53.4	8.8
24	0.0467	7.7	0.424	7.4	0.0486	7.9	0.494	7.5	0.065	5.4	4.227	5.2	34.7	8.2	53.4	8.9

TABLE 6. Effect of 30% (v/v) human serum on the stability of aqueous solutions of BAC, CHG, PVP-I, and EtOH at 25℃.

Time (h)	BAC				CHG				PVP−I				EtOH			
	0.05% (w/v)		0.5% (w/v)		0.05% (w/v)		0.5% (w/v)		0.5% (w/v)		5% (w/v)		35% (v/v)		55% (v/v)	
	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH	Concn	pH
0	0.0478	7.8	0.391	7.4	0.0498	7.9	0.498	7.6	0.049	6.7	3.578	5.9	34.9	7.9	54.9	8.3
1	0.0458	7.9	0.272	7.6	0.0495	8.0	0.498	7.6	0.037	6.6	3.465	5.8	34.6	7.9	54.9	8.3
3	04.448	7.8	0.267	7.4	0.0482	8.0	0.498	7.6	0.016	6.6	3.461	5.8	34.6	8.0	54.9	8.4
6	0.0418	7.8	0.259	7.6	0.0480	8.0	0.497	7.7	0	6.5	3.346	5.8	34.5	7.9	54.7	8.4
24	0.0357	7.8	0.238	7.6	0.0468	8.0	0.497	7.6	0	6.5	3.321	5.8	34.4	8.0	54.6	8.3

killed by the combination of BAC or CHG and diluted EtOH (e.g., 0.2%, w/v, CHG in 10%, v/v, EtOH) as reported by Oie et al. (1984). When BAC and CHG as low-level disinfectants are compared to PVP-I and EtOH as intermediate-level disinfectants, the latter had higher activity against vegetative bacteria, fungi, and mycobacteria than low-level disinfectants. In terms of sporicidal activity, neither PVP-I nor EtOH could kill the spores of *B. subtilis* which was considered to be the organism most resistant to chemical agents. However, the spores of *C. sporo-genes* and *C. sphenoides* were killed by PVP-I, though they were not killed by EtOH.

The presence of human serum remarkably reduced the antimicrobial activity of BAC and PVP-I, especially at low concentrations (0.05 and 0.5%, w/v) of PVP-I as reported in a previous study (Sheikh, 1986). Tables 5 and 6 clearly show that available iodine in aqueous PVP-I greatly decreased over 24 h after the addition of human serum. Although low concentration PVP-I solutions have been shown to have good antimicrobial activity (Berkelman, 1982) because the amount of free iodine increases to some extent as the solution is diluted, we must consider the possibility that the available iodine in aqueous PVP-I solutions is rapidly reduced in the presence of organic matter such as human serum.

The effects of BAC, CHG, PVP-I, and EtOH on HBV were tested using HBs-Ag positive human serum. Of course, the disappearance of the infectivity of HBV was not equivalent to the inactivation of HBs-Ag. We performed tests assuming that HBV had already lost its infectivity before it lost its HBs-Ag activity in treatment with BAC, CHG, PVP-I, and EtOH, as examined in previous studies with formalin (Buynak et al., 1976 ; Maupas, 1976). The results indicated that the anti-genicity of HBs-Ag positive human serum was not lost after treatment with BAC, CHG, and PVP-I, but was rapidly inactivated by 75 and 95% (v/v) EtOH. The World Health Organization Technical Report stipulates that sodium hypochlorite and glutaraldehyde are recommended for inactivation of HBV infectivity (WHO Scientific Group on Viral Hepatitis, 1973). Yet, Kobayashi et al. (1984) showed that the HBV infectivity was lost on treatment with 80% (v/v) EtOH, and Bond et al. (1983) also showed that it was lost on treatment with 70% (v/v) isopropyl alcohol or an aqueous solution (1 : 213) of PVP-I using the direct chimpanzee inoculation method. These results suggest that EtOH can be considered an effective chemical against HBV. Regarding the viricidal activity against HBV, it is probably correct to state that PVP-I is effective against HBV in actual use at medical facilities, though our results differ from those of Bond et al. More definitive experiments are needed before the exact activity of PVP-I against HBV is known.

On the basis of these results, we confirmed that disinfection with BAC or CHG is appropriate for general disinfection: between-patient processing of certain noncritical instruments or devices, or for routine cleaning of hospital environments. Neither disinfectant should be used if bacterial spores, mycobacteria, or HBV are a concern. The disadvantage of PVP-I as a disinfectant is considered to be a rapidly decreased activity in the presence of organic matter, though, in its absence, PVP-I has higher activity against various organisms than BAC or CHG. Regarding the antimicrobial activity of EtOH, it is equally active to glutaraldehyde as a high-level disinfectant against vegetative bacteria, mycobacteria, fungi, and even against HBV (Akamatsu et al., 1996). Conceivably, its ineffectiveness against bacterial spores makes EtOH an intermediate-level disinfectant rather than a high-level one.

Finally, in conclusion, it should be strongly suggested that disinfectants must appropriately be selected and used in consideration of the type of microorganism, time of exposure, amount of organic matter on the item to be disinfected, and so on, because failures in disinfection using chemical agents have resulted in outbreaks of infections (Casewell and Phillips, 1977 ; Webb and Vall-Spinosa, 1975) among hospitalized patients.

REFERENCES

Akamatsu, T., Tabata, K., Hironaga, M., and Uyeda, M. (1995) Antimicrobial activity and chemical stability of sodium dichloroisocyanurate. *Jpn. J. Toxicol. Environ. Health*, **41**, 134-141.

Bassett, D. C. J., Stokes, K. J., and Thomas, W. R. G. (1970) Wound infection with *Pseudomonas multivorans*. A water-borne contaminant of disinfectant solutions. *Lancet*, **1**, 1188-1191.

Bergan, T., and Lystad, A. (1971) Disinfectant evaluation by a capacity use-dilution test. *J. Appl. Bacteriol.*, **34**, 741-750.

Berkelman, R. L., Holland, B. W., and Anderson, R. L. (1982) Increased bactericidal activity of preparations of povidone-iodine solutions. *J. Clin. Microbiol.*, **15**, 635-639.

Bond W. W., Favero, M. S., Petersen, N. J., and Ebert, J. W. (1983) Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. *J. Clin. Microbiol.*, **18**, 535-538.

Broadley, S. J., Jenkins, P. A., Furr, J. R., and Russell, A. D. (1991) Anti-mycobacterial activity of

biocides. *Lett. Appl. Microbiol.*, **13**, 118-122.
Buynak, E. B., Roehm, R. R., Tytell, A. A., Bertland II, A. V., Lampson, G. P., and Hilleman, M. R. (1976) Development and chimpanzee testing of a vaccine against human hepatitis B. *Proc. Soc. Exp. Biol. Med.*, **151**, 694-700.
Casewell, M., and Phillips, I. (1977) Hands as route of transmission for *Klebsiella* species. *Br. Med. J.*, **2**, 1315-1317.
Frank, M. J., and Schaffner, W. (1976) Contaminated aqueous benzalkonium chloride. An unnecessary hospital infection hazard. *JAMA*, **236**, 2418-2419.
Kobayashi, H., Tsuzuki, M., Koshimizu, K., Toyama, H., Yoshihara, N., Shikata, T., Abe, K., Mizuno, K., Otomo, N., and Oda, T. (1984) Susceptibility of hepatitis B virus to disinfectants or heat. *J. Clin. Microbiol.*, **20**, 214-216.
Maupas, G., Goudeau, A., Coursaget, P., and Drucker, J. (1976) Immunisation against hepatitis B in man. *Lancet*, **1**, 1367-1370.
Oie, S., and Koshiro, A. (1984) Combined bactericidal effect of chlorhexidine and diluted ethanol. *Yakugaku Zasshi*, **104**, 780-785.
Russell, A. D. (1990) Bacterial spores and chemical sporicidal agents. *Clin. Microbiol. Rev.*, **3**, 99-119.
Scott, E. M., Gorman, S. P.,McGrath, S. J. (1986) An assessment of the fungicidal activity of antimicrobial agents used for hard-surface and skin disinfection. *J. Hosp. Clin. Pharm.*, **11**, 199-205.
Sheikh, W. (1986) Comparative antibacterial efficacy of hibiclens and betadine in the presence of pus derived from human wounds. *Curr. Ther. Res.*, **40**, 1096-1102.
Smith, C. R. (1947) Alcohol as a disinfectant against the tubercle *Bacillus*. *Public Health Rep.*, **62**, 1285-1295.
Spaulding, E. H., and Groschel, D. H. M. (1974) Hospital disinfectants and antiseptics. In *Mannual of Clinical Microbiology, 2nd edition*, 852-857.
Uyeda, M., Sakata, M., Shibata, M., and Hitoshi, T. (1982) Some properties of chlorhexidine-resistant strain of *Serratia marcescens*. *Chemotherapy*, **30**, 286-290.
Webb, S. F., and Vall-Spinosa, A. (1975) Outbreak of *Serratia marcescens* associated with the flexible fiberbronchoscope. *Chest*, **68**, 703-705.
WHO Scientific Group on Viral Hepatitis (1973) *WHO Technical Rep. Ser.*, **512**, 47-48.