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Comparative Sporicidal Effects of Liquid Chemical Agents

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We compared the effectiveness of glutaraldehyde, formaldehyde, hydrogen peroxide, peracetic acid, cupric ascorbate (plus a sublethal amount of hydrogen peroxide), sodium hypochlorite, and phenol to inactivate *Bacillus subtilis* spores under various conditions. Each chemical agent was distinctly affected by pH, storage time after activation, dilution, and temperature. Only three of the preparations (hypochlorite, peracetic acid, and cupric ascorbate) studied here inactivated more than 99.9% of the spore load after a 30-min incubation at 20°C at concentrations generally used to decontaminate medical devices. Under similar conditions, glutaraldehyde inactivated approximately 90%, and hydrogen peroxide, formaldehyde, and phenol produced little killing of spores in suspension. By kinetic analysis at different temperatures, we calculated the rate of spore inactivation (k) and the activation energy of spore killing (ΔE) for each chemical agent. Rates of spore inactivation had a similar ΔE value of approximately 20 kcal/mol (ca. 83.68 kJ/mol) for every substance tested. The variation among k values allowed a quantitative comparison of liquid germicidal agents.

Sterilants and disinfectants are considered to be medical devices designed to protect patients from serious infections that could result if contaminated instruments are used to treat them (33). The use of aqueous glutaraldehyde, hydrogen peroxide, and aqueous formaldehyde has been recommended for sterilization (12, 20, 34, 35). These substances plus chlorine and iodine compounds have been suggested for use in high-level disinfection. Peracetic acid and metal-based formulations have also shown high-level microbicidal activity (5, 27, 30).

Eight hundred fifty thousand implant- and device-related infections occur every year in the United States (9). As a result of reviewing information relating to these infections and deaths and the effectiveness of disinfectants, the U.S. General Accounting Office concluded in 1990 that "although scientific controversies cloud the issue. . . , up to 20% of disinfectants on the market may be ineffective" (32).

A relatively large body of information on individual disinfecting and sterilizing substances has been accumulated (reviewed in reference 4). However, assessing the relative activities of different disinfectants remains difficult. Sporicidal activity is commonly used to evaluate disinfecting and sterilizing agents (3), but many factors, including spore preparation, chemical agent, and testing methodology, alter sporicidal results (13). Slight methodological differences can substantially alter the measured activities of biocides (14, 17). Most data available on the microbicidal activities of chemical agents have been obtained with different species, strains, or crops of spores and under different testing conditions (temperature, exposure time, etc.). Sporicidal data obtained by qualitative methods further hinder a quantitative comparison of microbicidal activities and the subsequent selection of chemical agents most adequate for a particular application.

In the *Guidelines on Sterilization and Disinfection Methods Effective against Human Immunodeficiency Virus* (35), the World Health Organization defines "sterilization" as the destruction of all microbes, including spores of *Bacillus subtilis*. Accordingly, the goal of this study was to compare the activities of several microbicidal agents under similar conditions against spores of *B. subtilis*. Our findings should assist medical personnel and scientists in selecting the best sporicidal agent for a given germicidal product.

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MATERIALS AND METHODS

Bacteria. Spores of *B. subtilis* subsp. *globigii* (ATCC 9372) were purchased from American Sterilizer Co. (Erie, Pa.). According to the manufacturer, a population of 1.7×10^9 spores per ml survive dry heat at 160°C for 11 min but is killed after 27 min of exposure. The manufacturer indicated that these spores survived 16 min but were killed after a 38-min exposure to ethylene oxide (600 mg/liter at 54°C). The reported D value for dry-heat killing was 2.6 min, and the D value for ethylene oxide killing was 3.7 min. We stained spores with trypan blue and examined them microscopically to confirm spore morphology. No vegetative cells (rods) were observed during the counting of 1,000 spores. Spores were centrifuged, resuspended in 10 times the original volume of deionized and glass-distilled autoclave-sterile water, aliquoted in polypropylene vials, and stored at −75°C until use. Spores stored in this way maintained the original number of viable organisms for up to 2 months. Spores were exposed for various periods to either deionized, glass-distilled, autoclave-sterile water (controls) or hydrochloric acid (2.5 N), neutralized with ice-cold Luria-Bertani (LB) broth (Advanced Biotechnology Inc., Columbia, Md.) and titrated on broth-agar (LB broth [Miller-Difco, Detroit, Mich.] and Agar Select [Gibco-BRL, Paisley, Scotland]) plates 100 mm in diameter. Survival of spores exposed to acid for 5 and 10 min was 100 and 88% that of water-treated controls, respectively.

Chemical agents. Five different glutaraldehyde preparations were tested. Fifty percent glutaraldehyde (wt/vol) (molecular biology grade) was purchased from Sigma Chemical Co. (catalog number [cat.] G-7651, lot 63H5016; St. Louis, Mo.), shipped on dry ice, maintained frozen, and used within a week of being received. Seventy percent glutaraldehyde (wt/vol) (cat. 20105, lot 660; Ladd Research Industries, Burlington, Vt.) and 50% glutaraldehyde (wt/vol) (cat. 18431, lot 921104; Ted Pella, Redding, Calif.) in glass-sealed ampules were stored frozen and used the same day in which the glass seal was broken. All glutaraldehydes were alkalinized with sodium bicarbonate (0.05 M final concentration in the spore treatment mixture, pH 9.3), with the exception of experiments designed to assess the effect of pH. A commercial disinfectant (containing 2% [wt/vol] glutaraldehyde and an alkaline activator) and a technical-grade glutaraldehyde were also tested, but because of low-level sporicidal activity, neither was used for the experiments shown below.

Formaldehyde (37.2% [wt/vol]) was purchased from Mallinckrodt Chemicals (cat. 5016, lot 5016KMKZ; Paris, Ky.) and from Aldrich Chemical Co. (cat. 25,254-9, lots 0471772 and 039OLF; Milwaukee, Wis.). Sodium hypochlorite (5.3% [wt/vol], as available chlorine) was purchased from Aldrich Chemical Co. (cat. 23,930-5). A technical-grade sodium hypochlorite showed little sporicidal effect, and it was not used in this study. Peracetic acid (32% [wt/vol]) was purchased from Aldrich Chemical Co. (cat. 26,933-6, lot 00816LF). Molecular biology-grade ultra pure phenol was purchased from BRL Life Technologies (cat. 5509 UA, lot 71209; Gaithersburg, Md.) and was received frozen and maintained at −20°C until use.

Cupric chloride ($CuCl_2 \cdot 2H_2O$) was purchased from Mallinckrodt Chemical

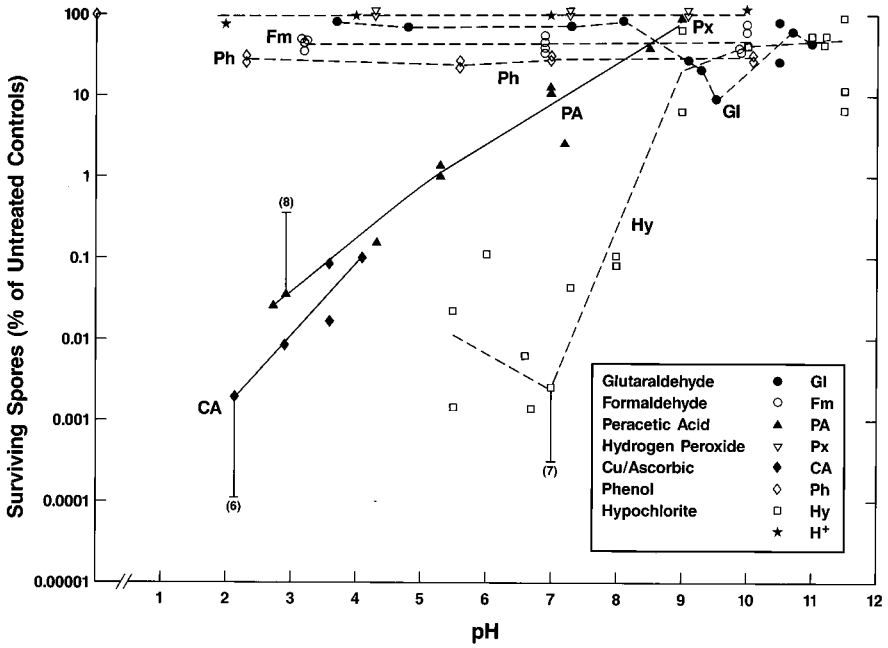

FIG. 1. Approximately 10^8 spores were exposed to 5% phenol, 10% hydrogen peroxide, 8% formaldehyde, 0.5% cupric ions (as cupric chloride)–0.1% ascorbic acid–0.003% hydrogen peroxide, 0.05% sodium hypochlorite, 0.03% peracetic acid, and 2% glutaraldehyde, respectively, as described in Materials and Methods. The resistance of spores exposed to buffers described in Materials and Methods at different pHs in the absence of microbicidal agents is shown for comparison (★). Exposure was for 30 min at 20°C. Individual symbols represent the results of independent experiments. Spores surviving PA at pH 3.4, CA at pH 2.9, and Hy at pH 7 are represented as the mean of the number of independent experiments represented in parentheses ± the standard deviation.

(cat. 4824, lot 4824KHBA-R), and Aldrich Chemical Co. (cat. 20,314-9, lot 10913TF). Ascorbic acid was purchased from Sigma Chemical Co. (cat. A-0278, lot 40H0752) and Aldrich Chemical Co. (cat. 25,556-4, lot 00218HF). Hydrogen peroxide (30% [wt/vol]) was purchased from Sigma Chemical Co. (cat. H-1009, lot 32H3446) and Aldrich Chemical Co. (cat. 21,676-3, lot B609730KF). Deionized distilled water and reagents low in heavy metals were used (29).

The reagents used to study the effect of pH on sporicidal activity were calibrating buffers (pH 2.00, KCl-HCl; pH 4.00, KH [phthalate]; pH 7.00, Na_2HPO_3-KH_2PO_3; and pH 10.00, $NaHCO_3$-Na_2CO_3), all with an accuracy of ±0.01 pH unit, from Baxter Diagnostics Inc. (Deerfield, Ill.). Metrepak buffers (pHs 11.00 and 12.00 ± 0.05) were from MicroEssential Laboratories (Brooklyn, N.Y.).

Fixed-time assays to determine effects of pH and disinfectant concentration. Fifty microliters of spore suspension, containing 1.6×10^9 to 2.2×10^9 CFU/ml were added, without touching the tube walls, to the bottom of a 1.5-ml Eppendorf conical plastic tube (PGC Scientifics, Gaithersburg, Md.) and brought to 20°C in a water bath. Fifty microliters of chemical agent at twice the studied final concentration was added to the spore suspension and mixed with a vortex-type mixer. Incubation proceeded precisely for 30 min at 20°C before the addition of 0.9 ml of ice-cold LB broth. No survival was obtained when spores treated with hypochlorite or peracetic acid were directly seeded over agar plates. Survival was evident, however, when spores treated with hypochlorite or peracetic acid were washed by centrifugation (5 min at 15,000 rpm, Microfuge model 5414; Brinkman Instruments Inc., Westbury, N.Y.) and resuspended by vortexing in new LB broth (1 ml).

The spores were serially diluted 1/10 with water, and 0.1 ml of each dilution was spread on LB broth (Miller-Difco) agar plates 100 mm in diameter. Between 90 and 120 bacterial colonies were typically present in 0.1 ml of the untreated control diluted 10^{-5} (exposed to water). The assay allowed bacterial survival to a level as low as 0.00001% that of the untreated controls (10^7-fold reduction) to be measured.

Kinetic assays. The effects of time and temperature on spore inactivation by different disinfectants was determined in 1.5-ml plastic tubes. To 310 μl of spores (1.6×10^9 to 2.2×10^9 CFU/ml) was added the disinfectant (310 μl) at twice the concentration under study. Immediately thereafter, a 100-μl aliquot from the mixture was withdrawn. This was considered to be the zero-time sample; the actual contact time of spores with disinfectants in the zero-time sample was less than 2 min before dilution in ice-cold LB broth. At various additional preestablished times, 100-μl aliquots were withdrawn from each reaction mixture tube. Each aliquot was immediately diluted to 1 ml with ice-cold media and kept in ice until the last time point was obtained. Surviving spores were then titrated as described above.

RESULTS

Effect of pH on sporicidal activity. The survival of spores incubated with several germicidal agents at different pHs is shown in Fig. 1. Treatment with formaldehyde (8%), phenol (5%), or peroxide (10%) resulted in a less than 90% inactivation of the spores throughout the pH range studied. Peracetic acid retained little sporicidal activity at neutral or alkaline pH. Copper-ascorbate was studied in acid conditions through pH 4, above which precipitation became evident. Hypochlorite was inactive at alkaline pH, showing a maximum level of efficacy around neutral pH. Glutaraldehyde was sensitive to pH, with sporicidal activity measurable within a narrow range centered near pH 9. The control experiment whereby spores were exposed only to various pH conditions showed that spore survival was not affected by pH in the absence of disinfectants (Fig. 1).

Comparative effects of concentrations. The effect of concentration on the sporicidal activity of each disinfectant can be seen in Fig. 2. Glutaraldehyde, hypochlorite, copper ascorbate, and peracetic acid were tested at the optimum pH determined above. Phenol, hydrogen peroxide, and formaldehyde were tested at pH 5.2, 4.0, and 3.2, respectively.

Five additional commercial germicidal formulations intended for hospital and institutional use were tested: (i) a sanitizing agent and germicide labeled to contain 10.00% alkyl dimethyl benzyl ammonium chlorides, (ii) a cleaning disinfectant with 11.00% alkyl dimethyl benzyl ammonium chlorides plus 1.20% didecyl dimethyl ammonium chloride, (iii) a health care personnel handwash containing 1% chloroxylenol, (iv) a disinfectant-detergent (with 7.24% *o*-benzyl-*p*-chlorophenol, 4.04% isopropyl alcohol, 2.23% *o*-phenylphenol, and an undisclosed proportion of biodegradable detergents), and (v) a ger-

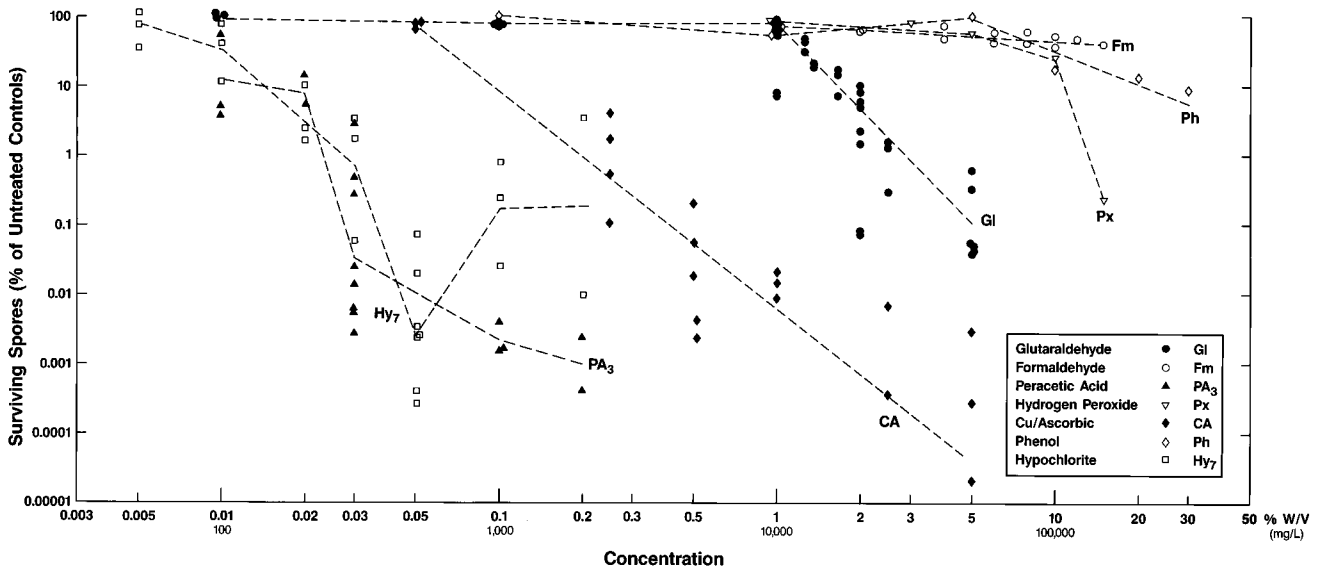

FIG. 2. The survival of spores treated with various disinfectants for 30 min in comparison to survival of spores in water is displayed as a function of concentration. The concentration of cupric ions in a mixture with ascorbic acid and hydrogen peroxide 5:1:0.03 (CA) is indicated on the abscissa. Symbols represent the results of independent experiments. W/V, weight per volume.

micidal detergent (with 9.65% sodium *o*-phenylphenate and 8.34% *p*-tertiary amylphenate). All five formulations did not reduce the number of spores after a 30-min treatment at the respective recommended concentrations (data not shown).

Ageing of activated disinfectants. Disinfectants were diluted to the concentration indicated in the legend of Fig. 3 in sterile distilled water, adjusted to the optimum pH, and tested immediately. At various time intervals thereafter, each disinfectant solution was retested under identical conditions; the remaining sporicidal activity is depicted in Fig. 3. The decay of sporicidal activity with time fitted the equation $\log_{10}$ (percent survival) = $C + k_dT$, where C is a constant, k_d is the decay rate constant, and T is the time elapsed since the chemical agent was activated by adjusting it to optimum pH (glutaraldehyde, peracetic acid, or hypochlorite) or since the ingredients were mixed (cupric salt and ascorbic acid). The values of the decay rate constant (per day) were as follows: glutaraldehyde, 0.30 ± 0.07 (regression coefficient $r = 0.86$); hypochlorite, 0.89 ± 0.19 (r =

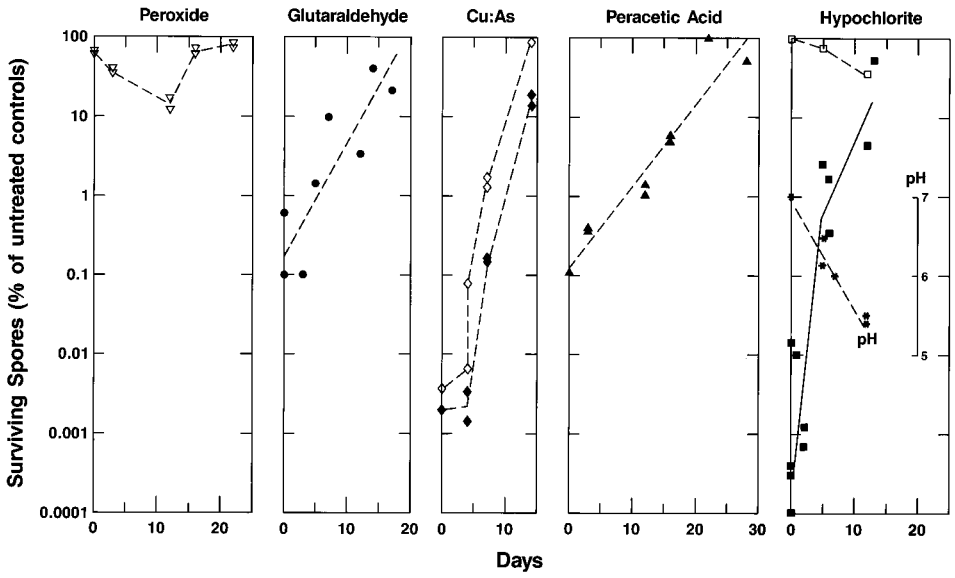

FIG. 3. The decreases in levels of sporicidal activity of germicidal agents indicated in the figure are plotted as a function of the time elapsed from (i) opening a liquid-filled glass sealed container and adjusting the pH (glutaraldehyde), (ii) the dissolution of copper and ascorbate in water (filled diamonds) or the dissolution of copper and ascorbate in water and the mixing of the solutions (open diamonds), and (iii) opening a sealed analytical-grade substance (peroxide, peracetic acid, and hypochlorite) and adjusting it to optimum pH. The shifts in pH (asterisks) of sodium hypochlorite (filled squares) was measured at various times after an adjustment to pH 7. The activity of hypochlorite at pH 11 as a function of time was also studied (open squares). The final concentration of each chemical agent was 10% hydrogen peroxide, 2% glutaraldehyde, 0.5% Cu(II)–0.1% ascorbic acid–0.003% hydrogen peroxide, 0.03% peracetic acid, and 0.05% sodium hypochlorite. Exposure of spores to the agent was for 30 min at 20°C. Symbols represent the results of independent experiments.

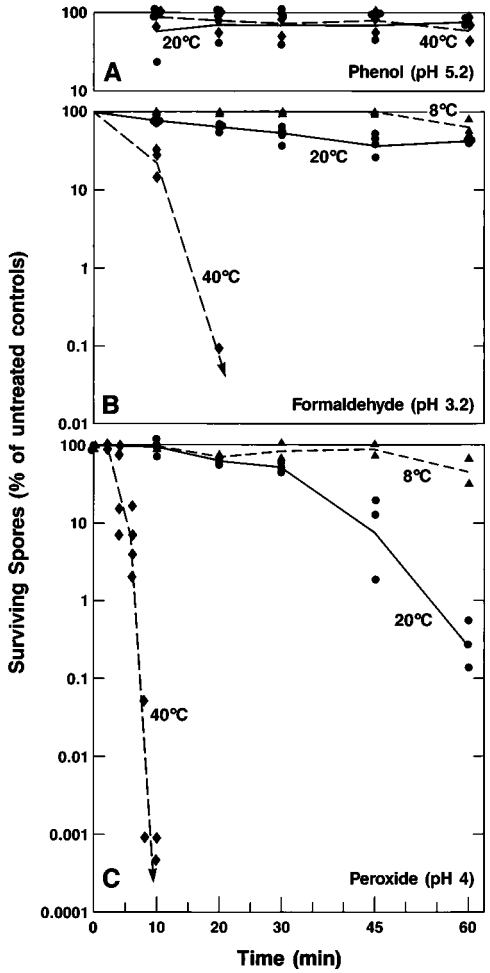

FIG. 4. Sporicidal kinetics of 5% phenol, pH 5.2 (A), 8% formaldehyde, pH 3.2 (B), and 10% hydrogen peroxide, pH 4 (C), respectively, at three different temperatures. Symbols represent the results of independent experiments.

0.85); peracetic acid, 0.24 ± 0.03 (r = 0.95); and cupric ascorbate, 0.74 ± 0.10 (r = 0.96).

The storage time that resulted in a 50% reduction of the spore inactivation level produced by a freshly prepared solution of chemical agent was calculated as $S_{50} = \ln 2/k_d$ (21, 26). Storage times that resulted in a 50% reduction of sporicidal activity were 2.3, 0.8, 2.9, and 0.9 days for glutaraldehyde, hypochlorite, peracetic acid, and cupric ascorbate, respectively.

Low-level sporicidal activity precluded calculation of the decay rate constants or S_{50} values for hydrogen peroxide, formaldehyde, or phenol.

Sporicidal activity was maintained when cupric chloride and ascorbic acid that had been stored dry in their respective bottles for more than a year were dissolved and tested.

Effect of temperature. The rates of spore inactivation were studied at three different temperatures, and the results obtained are depicted in Fig. 4 to 8.

The kinetics of spore killing by sodium hypochlorite, pH 7, appeared complex (Fig. 6). We investigated whether this response was due to a heterogeneous population of spores that included some that were highly resistant to treatment. In additional experiments, spores were exposed to hypochlorite for 30 min at 20°C, diluted in water, and pelleted down by centrifugation. The supernatant with the diluted hypochlorite was discarded. The surviving spores were resuspended in water and treated for a second time with new hypochlorite under conditions identical to those described above. The spore suspension was similarly sensitive to the first and second hypochlorite treatments (data not shown), indicating that our preparation does not include an additional population of highly resistant spores.

The data shown in Fig. 4 through 8 were analyzed by multivariate-linear regression for their best fit to N-order models (19, 24). The kinetic order of each sporicidal reaction is shown in Table 1. Nineteen of twenty-one sets of data fit a first-rate kinetic model (with an N-order close to 1). Hypochlorite, pH 11, at 40°C and peracetic acid at 20°C did not yield a good fit, but they fit a first-order kinetic model at the other two temperatures. Hence, the inactivation kinetics of these two agents was assumed to be a first-order reaction within the range of temperature studied.

Once the first-order kinetic model was established, the death rate constants at 8, 20, and 40°C (k_8, k_{20}, and k_{40}, respectively) were calculated with commercially available software (Excel; Microsoft Corporation, Bothell, Wash.) and according to the equation ln survival = $a - kt$, where a is a constant (y axis intersect), t is time, and k is the reaction rate and the slope of the straight-line function per min. The spore half-life ($t_{1/2}$) under different treatments was calculated as $t_{1/2} = \ln 2/k$ (21, 26). For relatively fast and slow reactions, a better fit of the data and more precise calculation of k were obtained by iterative computer-assisted analysis of equally weighted multilinear regression (19, 24). The values of k for each chemical agent are shown in Table 1. The activation energy of spore killing (ΔE) was estimated by Arrhenius's equation (21, 26) $\ln(k_1/k_2) = (-\Delta E/R)(1/T_1 - 1/T_2)$ and it is also shown in Table 1.

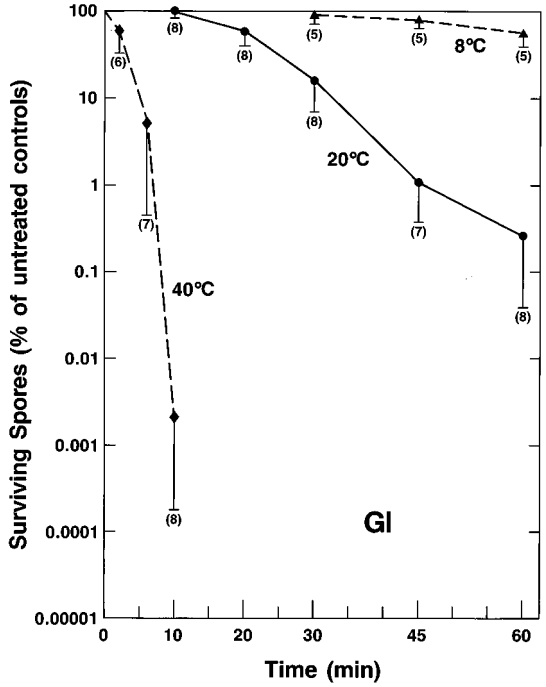

FIG. 5. Kinetics of spore inactivation by 2% glutaraldehyde (Gl) at pH 9.3 (0.05 M sodium bicarbonate) at three temperatures, 8, 20, and 40°C. Symbols represent averages and bars indicate standard deviations in the numbers of independent experiments represented in parentheses.

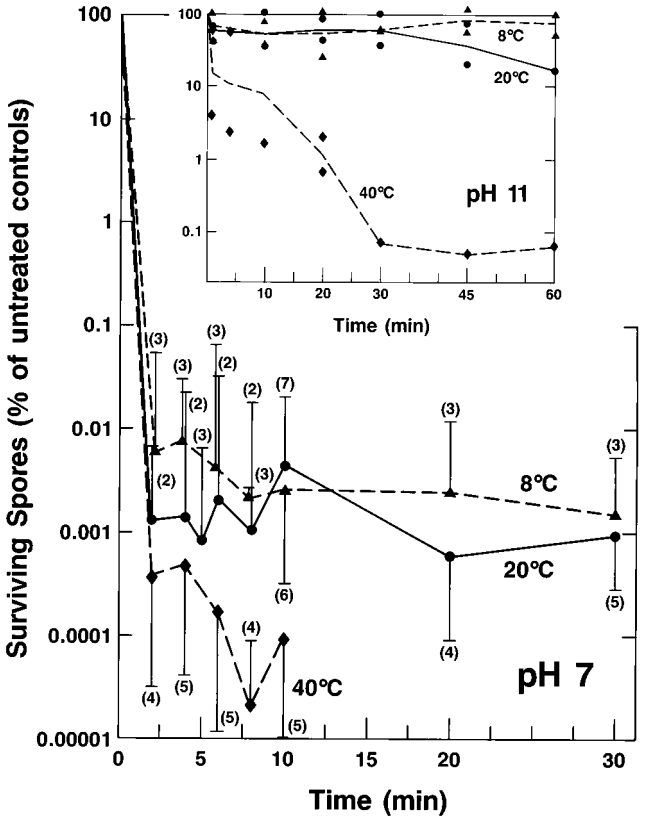

FIG. 6. Kinetics of spore inactivation by 0.05% sodium hypochlorite at neutral pH compared with activity at pH 11 (inset) at 8, 20, and 40°C. Results of independent experiments are shown at pH 11 (inset). Averages ± standard deviations in the parenthetically listed numbers of independent experiments are shown at pH 7.

DISCUSSION

Although phenol no longer plays a significant role as an antibacterial agent, this substance is the standard of microbicidal activity in the AOAC International phenol coefficient methods (3), and hence, it was included in our study. The fact that phenol did not inactivate spores at all three temperatures studied should preclude the use of phenol coefficient methods for the evaluation of sporicidal activity. A more active reagent that could be stored for a long period of time in solid form such as the copper-ascorbate formulation described in this paper should be a more practical sporicidal reference reagent.

Cupric ions in the presence of ascorbic acid and a small amount of peroxide resulted in a $10^{4.5}$-fold decrease in spores after 30 min at room temperature. The addition of a small amount of hydrogen peroxide to copper-ascorbate resulted in smaller variance and hence better reproducibility of survival data. This effect is likely associated with a more constant level of oxygen in the reaction mixture as a result of the presence of even minor amounts of hydrogen peroxide. We believe that copper-mediated killing involves free radicals that can bind and damage the DNA of microorganisms (28, 29). The sporicidal effect of cupric ascorbate is reduced within a few days. However, the cupric salt and ascorbic acid can be kept dry almost indefinitely.

Peracetic acid has been shown to be lethal to bacteria, yeast, and viruses at concentrations near or below 300 ppm (0.03%) (2, 23). We observed that peracetic acid (0.03%) resulted in an approximately $10^{3.5}$-fold decrease in the spores present after a 30-min exposure at 20°C. A decrease in levels of sporicidal activity with storage paralleled the reported rate of hydrolysis of peracetic acid (16). In agreement with previous studies (2), we observed that the sporicidal efficacy of peracetic acid depended strongly on pH. Elevated concentrations may extend sporicidal activity levels toward higher pH, but such practice should be carefully considered since 1% peracetic acid is a potent tumor promotor (6).

In agreement with previous findings (31), 8% formaldehyde showed less sporicidal activity than 2% glutaraldehyde. Our findings also agree with a previous report indicating that 10% hydrogen peroxide at room temperature is ineffective and that high concentration (35%) and temperature (80°C) are required for the destruction of spores (5).

In our experiments, glutaraldehyde yielded the least consistent results. Instability, lot and brand variations, and high pH and ionic strength sensitivity made reproducible results difficult to obtain. The relatively low level of sporicidal activity of glutaraldehyde obtained with our test conditions is consistent with previous observations. Dyas and Das (10) found that *B. subtilis* subsp. *globigii* survived a 2-h treatment with 2% glutaraldehyde. Boucher (7) found that a 10-h treatment was necessary for a complete spore kill.

We studied glutaraldehyde at 2%, pH 9.3, and at an ionic strength of 0.05 M (sodium bicarbonate). The apparent level of sporicidal activity of glutaraldehyde increased at higher ionic strength (up to a 1,000-fold increase at 1 M ionic strength [data not shown]). However, this effect of salt likely reflects an increased sensitivity level of the bacteria to glutaraldehyde rather than an effect on the chemical agent (14, 22, 31). A relatively rapid precipitation of 2% glutaraldehyde (within 3 h in 1 M sodium bicarbonate) and a salty residue after washing should preclude the use of an ionic strength much higher than that studied in this work.

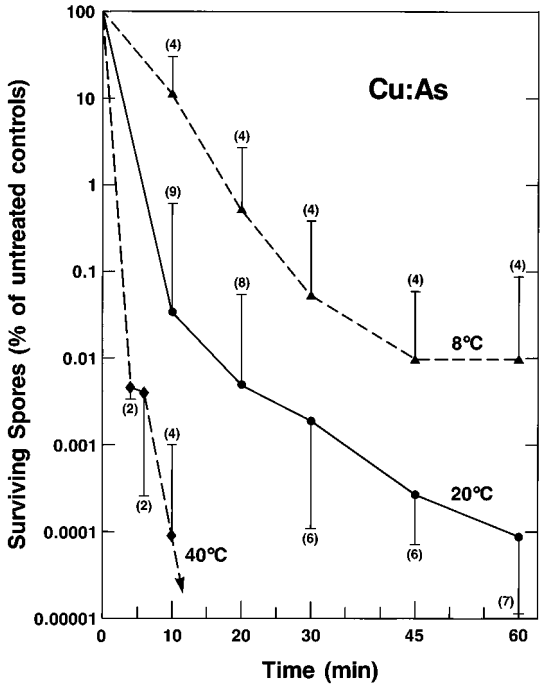

FIG. 7. Kinetics of spore inactivation by a mixture (Cu:As) of 0.5% Cu(II) as cupric chloride, 0.1% ascorbic acid, and 0.003% hydrogen peroxide (pH 2.9) at three temperatures, 8, 20, and 40°C. Averages ± standard deviations from the parenthetically listed numbers of independent experiments are shown.

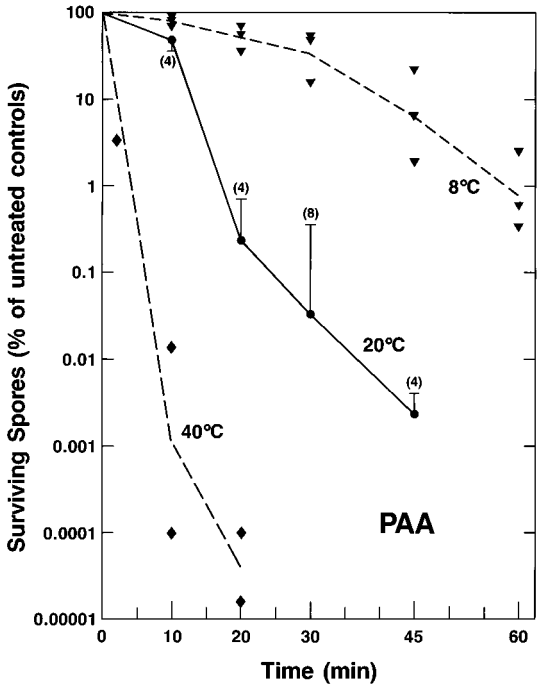

FIG. 8. Spores surviving different time exposures to 0.03% peracetic acid (PAA), pH 3.4, at three temperatures. Symbols represent the results of independent experiments at 8 or 40°C or averages ± standard deviations obtained from the parenthetically listed numbers of independent experiments at 20°C.

Under our experimental conditions (pH 9.3, 0.05 M sodium bicarbonate), a 2-h exposure to 2% glutaraldehyde at 20°C was required to reduce the colony-forming ability of *B. subtilis* subsp. *globigii* spores 10^6-fold (data not shown). Our data demonstrate that 2% glutaraldehyde is inadequate for killing spores in 30 min, the period recommended by the World Health Organization for decontamination of medical devices used for AIDS patients (35).

We observed that sodium hypochlorite at neutral pH is the agent that more rapidly inactivates a suspension of spores of *B. subtilis*; however, at this pH, it is also the most unstable among the substances tested. The rapid inactivation of spores by hypochlorite agrees with the previous observations of Babb et al. (1).

It has been previously established that pH has a great influence on the antimicrobial activity of chlorine in solution (11). Hypochlorite at pH 11 was nearly ineffective at either 20 or 8°C and showed a moderate spore inactivation at 40°C (Fig. 6, inset) after 30 min. The low level of activity of hypochlorite at alkaline pH is important, since commercial sodium hypochlorite is obtained at pH 12. Using hypochlorite without adjusting its pH could result in an overestimation of its sporicidal effectiveness. The extremely rapid inactivating rate of hypochlorite made the calculations of k and ΔE imprecise.

Although germicidal agents inactivated spores at different rates, the value of activation energy of spore killing (ΔE) was approximately constant and near 20 kcal/mol (83.68 kJ/mol). This finding suggests that the limiting step of spore killing could be common to different germicidal agents.

The ranking of the studied substances based upon the levels of sporicidal activity shown in Fig. 2 is as follows: hypochlorite ≅ peracetic acid > copper-ascorbate > glutaraldehyde > peroxide > phenol ≅ formaldehyde. Differential sensitivity to pH, ageing, and temperature greatly affect this ranking order.

TABLE 1. Kinetic[a] parameters of sporicidal reactions at three temperatures

Germicide	40°C				20°C				8°C				ΔE (kcal/mol) (kJ/mol)
	Order of reaction ± SD[b]	Spore $t_{1/2}$	Reaction rate[c]	n[d]	Order of reaction ± SD	Spore $t_{1/2}$	Reaction rate	n	Order of reaction ± SD	Spore $t_{1/2}$	Reaction rate	n	
Glutaraldehyde (2%, alkaline)	0.90 ± 0.01	1.3 min	0.53 ± 0.11	27	0.99 ± 0.02	22 min	0.03 ± 0.01	40	0.987 ± 0.001	63 min	0.011 ± 0.003	16	20 ± 6 (83.68 ± 25.104)
Hypochlorite (0.05%, pH 7)	0.99 ± 0.64	ca. 1.5 μs	ca. 3×10^7	15	0.99 ± 0.34	ca. 0.03 s	ca. 1.16×10^3	28	1.01 ± 0.04	9 s	4.65 ± 0.33	25	ND[e]
Hypochlorite (0.05%, pH 11)	2.61 ± 0.89	4.8 min	0.14 ± 0.02	13	1.01 ± 0.05	28 min	0.025 ± 0.010	6	0.992 ± 0.005	130 min	0.005 ± 0.010	11	19 ± 3 (79.496 ± 12.552)
Peracetic acid (0.03%, acid)	1.010 ± 0.001	24 s	1.721 ± 0.001	7	Too many iterations	3.6 min	0.19 ± 0.02	25	0.99 ± 0.05	23 min	0.03 ± 0.01	14	22 ± 3 (92.048 ± 12.552)
Copper-ascorbic acid (0.5–0.1%)	0.99 ± 0.51	3 s	ca. 15	10	1.01 ± 0.01	1.2 min	0.58 ± 0.03	37	0.09 ± 0.03	4.7 min	0.15 ± 0.02	21	25 ± 5 (104.6 ± 20.92)
Peroxide (10%)	0.99 ± 0.04	55 s	0.75 ± 0.19	16	0.99 ± 0.05	ca. 25 min	ca. 0.028	16	0.86 ± 0.01	92 min	0.007 ± 0.004	6	24 ± 6 (100.416 ± 25.104)
Formaldehyde (8%)	0.85 ± 0.14	5.1 min	0.14 ± 0.02	8	0.99 ± 0.01	52 min	0.013 ± 0.002	21	0.993 ± 0.001	172 min	0.004 ± 0.003	11	19 ± 2 (79.496 ± 8.368)

[a] The kinetics of spore death can be described by the Arrhenius equation (21, 26) $k_t = A^{-E/RT}$, where k_t is the reaction rate constant at temperature T (in kelvins), E is the activation energy necessary to kill a mole of spores, A is a constant, and R is the gas constant (1.988 cal/(molK) [8.3177992 J/{molK}]).

[b] The order of the reaction reflects the best fit of the data.

[c] The reaction rate (k_t min^{-1}) is obtained by fitting data to the equation ln survival = $a - kt$.

[d] The number of datum points analyzed.

[e] ND, not determined.

According to their spore inactivation reaction rates (k in Table 1), chemicals could be classified into three groups. Phenol and the sanitizer-germicides we tested had k values small enough to make sporicidal studies with these substances impractical. A second group of agents could include substances with $k_{20} < 0.1$, $k_{40} < 1.0$, and $k_8 < 0.02$, respectively. This group showing intermediate levels of activity includes both aldehydes, formaldehyde, and glutaraldehyde, as well as hydrogen peroxide and hypochlorite at pH 11. Hypochlorite at neutral pH, copper-ascorbate, and peracetic acid could be placed in a group of agents with high-level sporicidal activity in which k_{20} is larger than 0.1, k_{40} is larger than 1.0, and k_8 is larger than 0.02. Although supporting data should be generated with other organisms, it appears that k values can be a good measure of microbicidal efficacy.

Considering the Arrhenius equation (see Results) and using the ΔE of spore killing and the value of k for a substance at a temperature shown in Table 1, the value of k and the spore $t_{1/2}$ can be estimated for any other temperature. Considering the rate of decay and the rate of spore killing, the survival of spores in the presence of each chemical agent could be estimated for a given exposure period, at a particular temperature, and at various days postactivation. The predictive values of the kinetic equations and data presented here should help to characterize, compare, develop, and evaluate disinfecting agents, particularly when limited data are available.

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